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FORMULATION AND EVALUATION OF ANTISEPTIC OINTMENT OF CURCUMA LONGA FOR THE TREATMENT OF LUMPY SKIN DISEASE

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ABSTRACT

Unfortunatly there are no specific drugs available for the treatment of lumpy skin disease.The only treatment available is supportive care of cattle. This can include Treatment of skin leisons using wounds care sprays and the use of antibiotics to prevent secondary skin infections. Anti-inflammatory pain killers can be used to keep up the apetite of affected animals. Intravenous fluid administration may be of benefit; however this may not be practical in the field. The lack of Treatment options for Lumpy skin disease virus emphasize the need of using effective vaccination for preventive disease. The aim of present study was to formulate an in-vitro study of antiseptic ointment of curcuma longa. It is an oily semisolid preparation, usually medicated that can be applied externally to the skin in order to heal, soothe or protect it. Antiseptic ointment system composed of Borax, Copper Sulphate, Catechu, Resin, Camphor, Turpentine Oil, Sesame Oil and Paraffin. Antiseptic ointment of Curcuma longa was prepared by using Fusion Method. Formulation of Curcuma Longa was evaluated such as pH, apparentviscosity, particlesize, rate of absorption, rate of penetration and content uniformity of antiseptic ointment. Formulation of Curcuma Longa Anti-Septic ointment by using fusion method was done successfully. All the formulations are dark brown in colour and characteristic odour. The whole study of antiseptic ointment of curcuma longa was revealed that the formulation F3 was best formulation.

KEYWORDS: Lumpy Skin Disease; Curcuma longa; Antibiotics; Borax; Catechu; Camphor etc.

INTRODUCTION

The skin is the first of the eleven body systems to be described. Each chapter from now on will cover one body system.

The skin, sometimes known as the Integumentary System is, in fact, the largest organ of the body. It has a complex structure, being composed of many different tissues. It performs many functions that are important in maintaining homeostasis in the body. Probably the most important of these functions is the control of body temperature. The skin also protects the body from physical damage and bacterial invasion. The skin has an array of sense organs which sense the external environment, and also cells which can make vitamin D in sunlight.

The skin is one of the first systems affected when an animal becomes sick so it is important for anyone working with animals to have a sound knowledge of the structure and functioning of the skin so they can quickly recognize signs of disease.

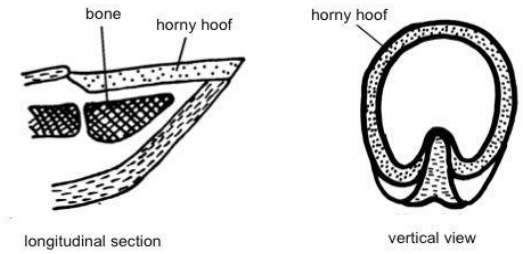
The skin comes in all kinds of textures and forms. There is the dry warty skin of toads and crocodiles, the wet slimy skin of fish and frogs, the hard shell of tortoises and the soft supple skin of snakes and humans. Mammalian skin is covered with hair, that of birds with feathers, and fish and reptiles have scales. Pigment in the skin, hairs or feathers can make the outer surface almost any color of the rainbow.

The epidermis is the layer that bubbles up when we have a blister and as we know from this experience, it has no blood or nerves in it. The cells at the base of the epidermis continually divide and push the cells above them upwards. As these cells move up they die and become the dry flaky scales that fall off the skin surface. The cells in the epidermis die because a special protein called keratin is deposited in them. Keratin is an extremely important substance for it makes the skin waterproof. Without it, land vertebrates like reptiles, birds, and mammals would, like frogs, be able to survive only in damp places.

Skin structures made of keratin.

Claws, Nails and Hoofs

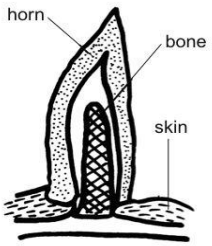
Reptiles, birds, and mammals all have nails or claws on the ends of their toes. They protect the end of the toe and may be used for grasping, grooming, digging or in defense. They are continually worn away and grow continuously from a growth layer at their base. Hoofs are found in sheep, cows, horses etc.



A horse's hoof

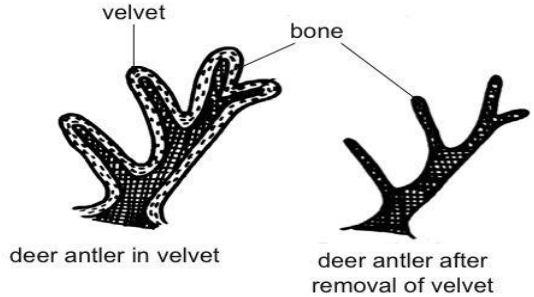
Horns And Antlers

True horns are made of keratin and are found in sheep, goats, and cattle. They are never branched and, once grown, are never shed. They consist of a core of bone arising in the dermis of the skin and are fused with the skull. The horn itself forms as a hollow cone-shaped sheath around the bone.



A horn

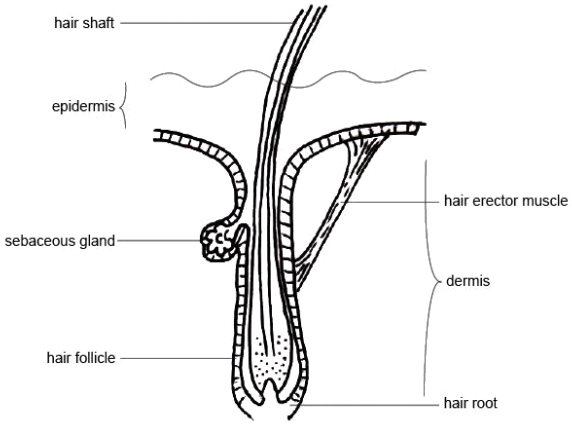
The antlers of male deer have quite a different structure. They are not formed in the epidermis and do not consist of keratin but are entire of bone. They are shed each year and are often branched, especially in older animals. When growing they are covered in the skin called velvet that forms the bone. Later the velvet is shed to leave the bony antler. The velvet is often removed artificially to be sold in Asia as a traditional medicine.



A deer antler

Hair

Hair is also made of keratin and develops in the epidermis. It covers the body of most mammals where it acts as an insulator and helps to regulate the temperature of the body. The color in hairs is formed from the same pigment, melanin that colors the skin. Coat color may help camouflage animals and sometimes acts to attract the opposite sex.

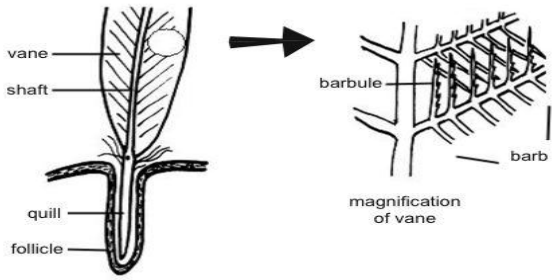


A hair

Hairs lie in a follicle and grow from a root that is well supplied with blood vessels. The hair itself consists of layers of dead keratin-containing cells and usually lies at a slant in the skin. A small bundle of smooth muscle fibres (the hair erector muscle) is attached to the side of each hair and when this contracts the hair stands on end. This increases the insulating power of the coat and is also used by some animals to make them seem larger when confronted by a foe or a competitor.

Feathers

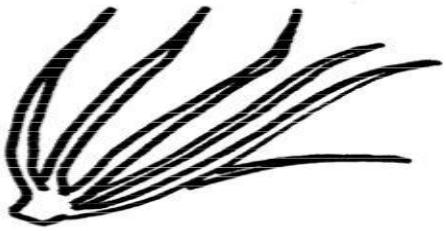
The lightness and stiffness of keratin is also a key to bird flight. In the form of feathers, it provides the large airfoils necessary for flapping and gliding flight. In another form, the light fluffy down feathers, also made of keratin, are some of the best natural insulators known. This superior insulation is necessary to help maintain the high body temperatures of birds.



A Contour Feather

Contour feathers are large feathers that cover the body, wings, and tail. They have an expanded vane that provides the smooth, continuous surface that is required

for effective flight. This surface is formed by barbs that extend out from the central shaft. If you look carefully at a feather you can see that on either side of each barb are thousands of barbules that lock together by a complex system of hooks and notches. if this arrangement becomes disrupted, the bird uses its beak to draw the barbs and barbules together again in anaction known as preening.



A Down Feather

A Pin Feather

Down feathers are the only feathers covering a chick and form the main insulation layer under the contour feathers of the adult. They have no shaft but consist of a spray of simple, slender branches.

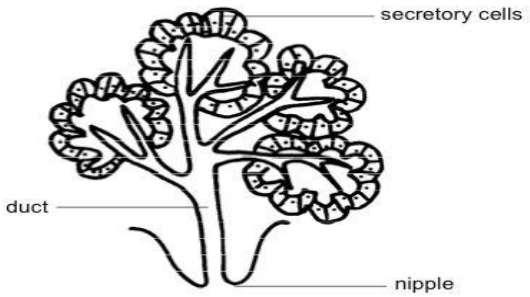
Pin feathers have a slender hair-like shaft often with a tiny tuft of barbs on the end. They are found between the other feathers and help tell a bird how its feathers are lying.

Skin Glands

Glands are organs that produce and secrete fluids. They are usually divided into two groups depending upon whether or not they have channels or ducts to carry their products away. Glands with ducts are called exocrine glands and include the glands found in the skin as well as the glands that produce digestive enzymes in the gut. Endocrine glands have no ducts and release their products (hormones) directly into the bloodstream. The pituitary and adrenal glands are examples of endocrine glands.

Most vertebrates have exocrine glands in the skin that produce a variety of secretions. The slime on the skin of fish and frogs is mucus produced by skin glands and some fish and frogs also produce poison from modified glands. In fact, the skin glands of some frogs produce the most poisonous chemicals known. Reptiles and birds have a dry skin with few glands. The preen gland, situated near the base of the bird's tail, produces oil to help keep the feathers in good condition. Mammals have an array of different skin glands. These include the wax producing, sweat, sebaceous and mammary glands.

- **Wax producing glands** are found in the ears.
- **Sebaceous glands** secrete an oily secretion into the hair follicle. This secretion, known as sebum, keeps the hair supple and helps prevent the growth of bacteria.
- **Sweat glands** consist of a coiled tube and a duct leading onto the skin surface. Sweat contains salt and waste products like urea and the evaporation of sweat on the skin surface is one of the major mechanisms for cooling the body of many mammals. Horses can sweat up to 30 litres of fluid a day during active exercise, but cats and dogs have few sweat glands and must cool themselves by panting. The scent in the sweat of many animals is used to mark territory or attract the opposite sex.
- **Mammary glands** are only present in mammals. They are thought to be modified sebaceous glands and are present in both sexes but are rarely active in males. The number of glands varies from species to species.



A Mammary Gland

The Skin and Sun

A moderate amount of UV in sunlight is necessary for the skin to form vitamin D. This vitamin prevents bone disorders like rickets to which animals reared indoors are susceptible.

Sunburn and Skin Cancer

Excess exposure to the sun can cause sunburn. This is common in humans, but light skinned animals like cats and pigs can also be sunburned, especially on the ears. Skin cancer can also result from excessive exposure to the sun.

The Dermis

The underlying layer of the skin, known as the dermis, is much thicker but much more uniform in structure than the epidermis. It is composed of loose connective tissue with a felted mass of collagen and elastic fibres. It is this part of the skin of cattle and pigs etc. that becomes commercial leather when treated, The dermis is well supplied with blood vessels, so cuts and burns that penetrate down into the dermis will bleed or cause serious fluid loss.

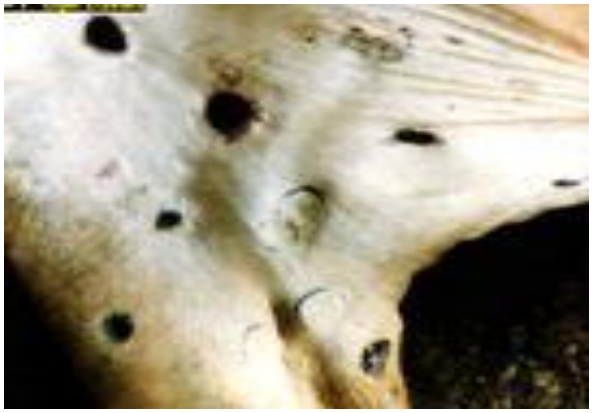
LUMPY SKIN DISEASE

Lumpy skin disease (LSD) is a devastating disease of cattle and buffalo caused by a capripox virus. Lumpy skin disease is an infectious, eruptive, occasionally fatal disease of cattle characterized by nodules on the skin and other parts of the body. Secondary bacterial infection often aggravates the condition. The virus is present in high concentrations in the skin nodules and scabs on affected animals and can be isolated from blood, saliva, ocular and nasal discharges and semen.

Lumpy skin disease virus can be found in blood for up to 21 days post-infection but shedding in semen may continue for at least 42 days post-infection.

Signs of LSD

Early Skin Leisons



Middle Skin Leisons

Late Skin Leisons

Healing Skin Leisons

Ointment

Any greasy or oily semisolid preparation, usually medicated, that can be applied externally to the skin in order to heal, soothe or protect it.

It is viscous semisolid preparation used topically on a variety of body surfaces.

Drug ingredients can be dissolved, emulsified or suspended in the ointment base.

Types of ointments

The various types of ointments are:

- Unmedicated ointments
- Medicated ointments

Unmedicated ointments

These ointments do not contain any drugs. They are useful as emollients, protectants. *Example:* Petroleum jelly.

Medicated ointments

These ointments contain drugs which show local or systemic effects.

These are of several sub-types:

- Dermatologic ointments
- Opthalmic ointments
- Rectal ointments
- Vaginal ointments
- Nasal ointments

Dermatologic ointments
These ointments are applied topically on the external skin. The ointment is applied to the affected area as a thin layer and spread evenly using gentle pressure with the fingertips. These are of three types.

1- Epidermis ointments
Example: Ketoconazole ointment.

2- Endodermic ointments
Example: Demodex ointment.

3- Diadermic ointments
Example: Nitroglycerine ointment.

Ophthalmic ointments
These are sterile preparations which are applied inside the lower eye lid.

***Example*:** Sulfacetamide sodium ointment.

Rectal ointments
These are the ointments to be applied to the perianal or within the anal canal.

Example: Benzocaine ointment.

Vaginal ointments
These ointments are applied to the vulvo-vaginal area or inside the vagina.

Example: Candicidin ointment.

Nasal ointments
These are used in the topical treatment of nasal mucosa. Drugs get absorbed into the general circulation through the rich blood supply of the nasal lining.

***Example*:** Ipratopium bromide ointment.

Advantages
- Handling of ointments is easier than bulky liquid dosage forms.
- They are chemically more stable than liquid dosage forms.
- They facilitate application directly to the affected body part and avoid exposure of other body parts to the drug.
- They prolong the contact time between the drug and affected area.
- The bioavailability of drugs administered as ointments is more since it prevents passage through liver.

Disadvantages
- They are bulkier than solid dosage forms.
- They are less stable than solid dosage forms.

Medicinal application of the ointment
- Ointments are used topically for several purposes, e.g., as protectants, antiseptics, emollients, ketorolytics and astringents.
- In the case of a protective ointment, it serves to protect the skin against moisture, air, sun rays and other external factors.
- It is necessary that the ointment neither penetrates the animal skin barriers nor facilitates the absorption of substances through this barrier.
- An antiseptic ointment is used to destroy or inhibit the growth of bacteria. Frequently bacterial infections are deeply seated; a base which has the capacity to either penetrate or dissolve and release the medication effectively is therefore desired.
- Ointments used for their emollient effect should be easy to apply, be non-greasy and effectively penetrate the skin.

Ointment bases
There are five classes or types of ointment bases which are differentiated on the basis of their physical composition.

These are
1- Oleaginous bases.
2- Absorption bases.
3- Water in oil emulsion bases.
4- Oil in water emulsion bases.
5- Water soluble or water miscible bases.

Oleaginous Base
- These bases are fats, fixed oils, hydrocarbon or silicons.
- They are anhydrous, greasy, non-washable does not absorb water and occlusive.
- They should not be applied to infected skin.
- They are used as protectants, emollients, vehicles for hydrolysable drugs.
- ***Example:*** White petrolatum, white ointment.

Absorption Base
- Oleaginous base + w/o surfactant.
- Anhydrous but hydrophilic ointment bases, they can absorb several times their weight of water to form water-in-oil emulsion.
- They are non-washable, not water soluble.
- They are also used as protectants, emollients, vehicles for aq. Solutions, solids and non-hydrolysable drugs.
- ***Example:*** Hydrophilic petrolatum, anhydrous lanolin

W/O emulsion Base
- These are anhydrous, hydrophilic, absorbs water and non water removable, with low thermal conductivity and occlusive.
- They have the same properties as the absorption bases.

- They are used as emollients, cleansing creams, vehicles for solid, liquid, or non-hydrolysable drugs.
- ***Examples:*** Cold cream type, hydrous lanolin, rose water ointment etc.

O/W emulsion Base

- These bases are anhydrous, water soluble, absorb water and water washable.
- They are either carbowaxes Polyethylene Glycols (PEGs) or hydrated gums (bentonite, gelatin, cellulose derivatives).
- They are used as drug vehicles.
- ***Examples:*** PEG Ointment, Polybase.

Water miscible Base

- These bases are anhydrous, water soluble, absorb water and water washable.
- They are either carbowaxes Polyethylene Glycols (PEGs) or hydrated gums (bentonite, gelatin, cellulose derivatives).
- They are used as drug vehicles.
- ***Examples:*** PEG Ointment.

MATERIALS AND METHODS

Curcuma longa, Borax, Copper Sulfate, Catechu, Resin, Camphor, Turpentine oil and Sesame oil were purchased from local market of Roorkee, Uttarakhand and Saharanpur, Uttar Pradesh and Paraffin were purchased from CDH (P) ltd.

METHODS

Extraction of drugs

Turmeric: Turmeric rhizome powder was extracted with 95% ethanol in soxhlet assembly until all the coloring matter is extracted. The obtained crude extract was concentrated to semi-solid brown coloured mass by evaporated ethanol.

Borax: The natural mineral mined from the ground or collected from evaporated deposits, is called Borax. When borax is processed, the purified chemical is Boric acid.

Catechu: Catechu is generally extracted from heart wood of Acacia catechu belonging to family Leguminosae. This is obtained by boiling chips of heartwood with water.

The chips are put into a pressure chamber called an autoclave. This chips are boiled with water, where pressure and temperature is maintained at 150^0 C. This leads to extraction of hot liquid.

The liquid extracted from autoclave is then put in an evaporator in which the concentration of liquid gets increased. The concentrated material gets crystallised through the cooling process.

The rectangular pieces are made and cut into biscuit like shape called katha. Katha are dried into a drying chamber for15-20days in cold air. The moisture get reduced in this chamber. The chamber includes hot air which hardened the katha and it gets ready to use.

Resin: Resin usually collected by causing minor damage to the tree by making a hole far enough into the trunk to puncture the vacuoles, to let sap exit the tree, known as tapping. This usually takes few days. Then, excess resin is collected.

Method of preparation of ointment

Antiseptic ointment of curcuma longa was prepared by fusion method in college laboratory. In this method, first dissolve turmeric, borax, copper sulphate, catechu, raal and both the oils together. Then, melt camphor and wax. Now, add all the contents with the melted substances and warm it to almost 60^0C temperature and stir thoroughly until cold.

Manufacturing formula

Ingredients	F1	F2	F3	F4	F5
Curcuma longa	20.00 gm	14.50 gm	13.50 gm	15.50 gm	13.50 gm
Borax	28.00 gm	26.00 gm	27.00 gm	28.00 gm	29.00 gm
Copper sulphate	2.50 gm	-	5.40 gm	-	5.50 gm
Acacia catechu	14.00 gm	15.50 gm	13.50 gm	13.00 gm	11.50 gm
Shorea robusta	16.50 gm	17.00 gm	13.50 gm	12.00 gm	13.00 gm
Cinnamonum camphera	-	10.00 gm	11.00 gm	15.50 gm	12.50 gm
Turpentine oil	5.50 ml	3.50 ml	2.50 ml	2.50 ml	2.0 ml
Sesamum indicum	0.10 ml	0.12 ml	0.12 ml	0.12 ml	0.12 ml
Paraffin Wax	13.50 gm	13.50 gm	13.50 gm	13.50 gm	13.00 gm

Methods for evaluation of ointment

1. Determination of organoleptic properties: This includes visual examination to identify the ointment by colour, separation, crystallization etc., in the final appearance of the product.

2. Content uniformity: The quantity of drug present in unit weight or volume of ointment or cream is determined by assay as standard method.

3. pH of ointments: When applicable, semisolid drug products should be tested for pH at the time of batch release and designated stability test time points for batch-to-batch monitoring. Because most semisolid dosage forms contain very limited quantities of water or aqueous phase, pH measurements must be warranted only as a quality control measure.

4. Impurities: The impurities arising from degradation of drug substance and during the manufacturing process of drug product should be assessed and controlled.

5. Non-irritancy Test: The bases used in the formulation of ointments may cause irritation or allergic reactions. The type of pharmacological action observed is noted. No visible reaction or erythema or intense erythema and vesicular erosion should occur.A good ointment base shows no visible reaction.

6. Rate of Penetration Test: The difference between the initial and the final weights of the preparation gives the amount of preparation penetrated through the skin and this when divided by the area and time period of application gives the rate of penetration of the preparation.

Weighed qty. of the preparation should be applied over selected area of the skin for a definite period of time.Then the preparation left over is collected and weighed.

7. Viscosity: In order to determine the viscosity of the formulation. It was kept at room temperature and an elevated temperature of 45^0C. For this, 50g of the ointment was kept at 45^0C in an oven. Viscosity of the ointment was measured after regular intervals of time for 1 month.

Rheological behaviour of a semisolid drug product may affect its application to treatment site and consistency of treatment and thus the delivered dose. Therefore, maintaining reproducibility of a product's flow behaviour at the time of release is an important product manufacturing control to maintain and demonstrate batch-to-batch consistency.

8. Spreadability Test: Spreadability was calculated using the spreadability apparatus made of wooden board with scale and two glass slides having two pans on both sides mounted on a pulley. Excess sample was placed between the two glass slides and 100g weight was placed on the glass slide for 5 min to compress the sample to a uniform thickness. Weight (250g) was added to the pan. The time in seconds required to separate the two slides was taken as a measure of spreadability.

9. Stability Testing: It was carried out by keeping 50g of ointment at 45^0C and another 50g at room temperature (37^0C). It was checked for any visual disturbances and phase separation from time to time over a period of 1 month.

RESULTS AND DISCUSSION

1. **Determination of organoleptic properties:** This includes visual examination to identify the ointment by colour, separation, crystallization etc., in the final appearance of the product. It was found that there is no crystals formed in the final formulation and the colour & odour of the all formulations are.

Physiochemical parameters	F1	F2	F3	F4	F5
Colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Odour	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics
Crystallization	No crystals appear	No crystals appear	No crystals appear	No crystals appear	No crystals appear

2. Content uniformity: The comparison of result with standard value are.

S. No.	Each 100gm contains	Result	Standard Value
1.	Curcuma longa	13.52gm	13.50gm
2.	Borax	27.15gm	27.00gm
3.	Copper sulphate	5.45gm	5.40gm
4.	Acacia catechu	13.65gm	13.50gm
5.	Shorea robusta	13.70gm	13.50gm
6.	Cinnamonum camphora	13.75gm	13.50gm
7.	Turpentine oil	2.50gm	2.50gm
8.	Sesamum indicum	0.121ml	0.12ml
9.	Wax	13.70gm	13.50gm

3. pH of ointments: The pH was obtained by pH meter

Physiochemical parameters	F1	F2	F3	F4	F5
pH	7.7	7.6	7.6	7.8	7.9

4. Impurities: There is no impurities arising from degradation of drug and during the manufacturing process of drug product.

5. Non-irritancy Test: As there is no visible reaction appears in all the 5 formulations. Hence, no irritant effect is observed.

6. Rate of Penetration Test: The rate of penetration of a semisolid dosage form is crucial in the onset and duration of action of the drug.The result obtained from all the 5 formulations are.

Physiochemical parameters	F1	F2	F3	F4	F5
Amount of ointment penetrate	1gm	2gm	1.5gm	0.75gm	1.2gm
Rate of penetration	Good	Average	Good	Not so good	Good

7. Viscosity: Rheological behaviour of a semisolid drug product may affect its application to treatment site and consistency of treatment and thus the delivered dose.
Therefore, result obtained for viscosity are.

Physiochemical parameters	F1	F2	F3	F4	F5
Viscosity	Less viscous	Virtuous viscosity	Stable	Stable	Low viscosity

8. Spreadability Test: The spreadability test are performed for all the 5 formulations and the result obtained for spreadability test are.

Physiochemical parameters	F1	F2	F3	F4	F5
Observation	5Sec	3Sec	3Sec	7Sec	8Sec
Spreadability	Average	Good	Good	Poor	Very poor

9. Stability Testing: Stability testing was performed for different formulations of antiseptic ointments and the result are as follows.

Physiochemical parameters	F1	F2	F3	F4	F5
Stability(37^{o}C, 45 ^{o}C)	Stable	Stable	Stable	Stable	Stable

CONCLUSION

With the development of modern medicine, several chemotherapeutic agents have been tried successfully for wound management in the last century. Those included several preparations of antibiotics, bacteriostatic drugs and microbicidal drugs. However, the allergic, toxic and microbial resistant effects of these chemicals have been encountered during their usage which made them unsafe for the human and animal lives. Moreover, this is an effort to save the human lives from the harmful effects of modern chemotherapeutic agents which used over a prolonged period of time for treatment of wounds in animals.

Antiseptic ointment of Curcuma longa were successfully prepared. On the basis of results of preliminary study, we found that the presumed excipients can be used for formulation development. There was no incompatibility between drug and excipients. The formulation F3 was found to be stable during the accelerated stability studies and selected as the best formulation for antiseptic ointment which is useful in the treatment of lumpy skin disease.

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