EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 7
267-275
Year: 2022

FORMULATION AND EVALUATION OF *FERULA ASAFOETIDA* OINTMENT IN THE TREATMENT OF ACNE VULGARIS

Shalumol Varghese*, Diya K. Babu, Jiya Johnson, Shafna, Shalu Joseph and S. Karthik

Elims College of Pharmacy, Thrissur.

***Corresponding Author: Shalumol Varghese**
Elims College of Pharmacy, Thrissur.

Article Received on 03/05/2022 **Article Revised on 23/05/2022** **Article Accepted on 13/06/2022**

ABSTRACT

The aim of the study was to formulate and evaluate asafoetida ointment, with an objective to treat acne. The study was carried out against *Staphylococcus epidermidis*, using 1% Clindamycin gel as standard. Asafoetida was extracted using various solvents and the anti-acne property of the extract was determined against *Staphylococcus epidermidis*, using Clindamycin as standard. A control test was done using the solvent to avoid any chances of interference in the anti-acne activity. The prepared extract was then incorporated into a fatty ointment base which was prepared by Fusion method. Then the ointment was evaluated for its parameters like drug content, spreadability, extrudability, pH, loss on drying, leakage test, washability, and Anti-bacterial activity. The ethyl acetate-asafoetida extract showed comparable zone of inhibition against *Staphylococcus epidermidis* as that of Clindamycin. The physicochemical parameters were evaluated and it was found that the ointment showed satisfactory results in terms of spreadability, extrudability, pH and washability. Asafoetida extract was found to have potential antimicrobial activity against microorganism. The ethyl acetate extract of asafoetida demonstrated more activity against *Staphylococcus epidermidis* than the other extracts and thus it is effective in the treatment of acne. The extract of asafoetida in ethyl acetate was formulated into an ointment for increasing their effectiveness and for their ease of application. As asafoetida is a natural substance, it exhibits fewer side effects when compared to other synthetic ingredients incorporated in ointments.

KEYWORDS: Asafoetida, Ethyl acetate extract, Ointment, Anti-acne activity, Clindamycin, Fusion.

INTRODUCTION

Asafoetida is an oleo-gum-resin obtained by incision from the living rhizome and root of *Ferula foetida Regel*, *F. rubricaulis Boiss*, and other species of Ferula.[1] Ferula asafoetida is a herbaceous, monoecious, perennial plant of UMBELLIFERAE family. Asafoetida is native to central Asia, eastern Iran to Afghanistan. Although not native to India, it has been used in Indian medicine and cookery for ages. It grows to two metre height with a circular mass of leaves. Asafoetida has a strong, alliaceous odour and bitter, acrid and alliaceous taste.[2]

Figure 1: Forms of ferula asafetida.

Acne is a hormone-associated disorder of the hair follicles and is characterized by excessive sebum production, comedones (blackheads), papules and postules (whiteheads).[1] Acne is a very common chronic inflammatory dermatosis found predominantly in adolescents in both sexes. The lesions are seen more

commonly on the face, upper chest and upper back.[2]

The clinical course of acne is influenced largely by genetic factors. However, transient relapses before menstrual periods often occur; various exogenous factors of physical and chemical nature may aggravate acne, and masculinizing conditions can also precipitate the disease. Remissions of acne are common after exposure to the sun.Acne vulgaris has a substantial impact on a patient's quality of life, affecting both self-esteem and psychosocial development.[3]

Ointments are homogeneous, viscous, semi-solid preparation, most commonly greasy and thick, with a high viscosity that is intended for external application to the skin or mucous membranes. They are often anhydrous and contain the medicament either dissolved or dispersed in the vehicle. All ointments consist of a base which act as a carrier for the medicaments.

MATERIALS AND METHOD

Table 1: List of materials and suppliers.

Sl. No	Materials	Suppliers
1	*Staphylococcus epidermidis*	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
2	Muller hinton agar plate	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
3	Wool fat	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
4	Hard paraffin	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings ,cochin 17,kerala
5	Cetostearyl alcohol	RON LAB chemicals ,distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
6	white soft paraffin	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
7	Methyl paraben	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
8	Propyl paraben	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
9	Methylated spirit	RON LAB chemicals ,distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
10	Ethyl acetate	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
11	Ethanol	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala
12	Chloroform	RON LAB chemicals, distributors of research lab-FINE CHEMICAL industries, ikkanat buildings, cochin 17,kerala

PLANT MATERIAL

Asafoetida was collected from local market. The powder was stored in an air-tight container for successive extraction.

APPARATUS

China dish, water bath, glass rod, mortar and pestle, ointment slab, 100 ml standard flask, Petri dish, 250 ml volumetric flask.

INSTRUMENTS

UV- VIS Spectrophotometer, electronic weighing machine, pH meter, hot air oven, ointment tube crimper, Autoclave.

PREPARATION OF EXTRACT

The crude form of asafoetida was crushed into fine powder with the help of mortar and pestle. Then 10g of crude form of asafoetida was weighed on weighing machine. It was dissolved in 100ml of ethyl acetate, Methylated spirit, and chloroform separately. The extract of asafoetida was prepared my maceration method. Then it was incubated for 24 hours and filtered.

MICROORGANISM USED

Staphylococcus epidermidis was cryopreserved at temperature below freezing. Freeze drying removes moisture from frozen samples by converting water into ice in a vacuum, thus arresting microbial metabolic process. Lyophilized form of strain was added into the small amount of LB broth. The broth was then incubated at 37^{o}C for 48 hours. Streaking was done on slant by taking a loop full of bacterial culture and allowed to incubate at 37^{o}C for 48 hours and then slants were stored at -4^{o}C for future use.

PREPARATION OF MHA PLATE

Suspend 38g of Muller Hinton agar powder in 1 litre of distilled water. Mix and dissolve them completely. The sterilization was done by autoclaving at 121^{o}C for 15

minutes. The MHA plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5 min.[4]

ANTIMICROBIAL ACTIVITY

Inoculum suspension was spread uniformly by using sterile swab on the MHA plate, and the inoculums were allowed to dry for 5 min. 70 microliters of extracts were loaded on 6 mm well, and then allowed to diffuse for 5 min. The whole procedure was carried out in aseptic laminar air flow chamber. The plates were kept for incubation at 37°C and allow it for growth of microorganism for 24 hr. At the end of incubation, inhibition zones formed around the disc were measured in millimetres.

FORMULATION OF OINTMENT BASE

Table 2: Formulation of ointment base.

SL no	Name of the ingredient	Quantity to be taken in grams
1	Wool fat	10.6
2	Cetosteryl alcohol	18.05
3	Hard paraffin	10.6
4	White soft paraffin	10.6
5	Methyl paraben	0.3
6	Propyl paraben	0.3

FORMULATION OF OINTMENT

Table 3: Formulation of ointment.

Formulation code	Prepared asafoetida extract(g)	Ointment base qs(g)
FC	10	50
FE	10	50
FM	10	50

PREPARATION OF OINTMENT

The ointment base was prepared using fusion method, followed by incorporating the medicament by trituration method.

Fusion method

This method is carried out in porcelain dish, which is heated on a water bath.

Melting done by addition of substances in the decreasing order of their melting point. To this melted mass, incorporate the medicament and stir thoroughly until the mass cools down and a homogenous ointment is formed.

If an aqueous liquid ingredient is required to be incorporated, that should also be heated to almost to the same temperature of the melted mass. Otherwise if temperature difference is there, waxy materials separate out, after mixing of two portions.

After mixing of two portions, stir the ingredients gently, until it becomes cool.

If foreign particles are visible after melting the ingredients, they are removed by decantation or straining.[5]

EVALUTION OF OINTMENTS

Organoleptic characteristics

Colour: visual observation

pH

2.5 gm ointment sample was taken in 100 ml dry beaker; 50 ml water was added to it. Beaker was heated on water bath maintained at about 100°C to 70^{0}c for 10 minutes. Cool to room temperature and then centrifuged at 3000 rpm for 10 minutes. The pH of water extracted was measured by using pH meter.

Drug content

Weigh 6g of each formulation were transferred in 250 ml of volumetric flask containing 20ml of alcohol and stirred for 30 minutes. The volume was made up to 100 ml and filtered. 1ml of the above solution was further diluted to 100 ml with alcohol and again 1ml of the above solution was further diluted to 10 ml with alcohol. The absorbance of solution was measured spectrophotometrically at 290 nm.[6]

For calibration, weigh 6g of asafoetida were transferred in 250 ml of volumetric flask containing 20ml of alcohol and stirred for 30 minutes. The volume was made up to 100 ml and filtered. 1ml of the above solution was further diluted to 100 ml with alcohol and again 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml of the above solution was further diluted to 10 ml with alcohol. The absorbance of solution was measured spectrophotometrically at 290 nm.

$a_1c_1=a_2c_2$

Where,

a_1= absorbance of formulation

a_2=absorbance of asafoetida extract

c_1=concentration of formulation

c_2=concentration of asafoetida extract

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 at two slides results better spreadability.
S=M*L/T

Where,
S: Spreadability
M: at fide to the upper slide
L: length of glass slide
T: time taken to separate

Extrudability
The formulation was filled in collapsible tube container. The extrudability was determined in terms of ointment required to extrude 0.5 cm of ribbon of ointment in 10 second[21].

Loss on drying
LOD was determined by placing the formulation in Petridish on water bath and dried for temperature 105^{o}C.

$$LOD = \frac{(\text{initial weight- final weight})*100}{\text{Final weight}}$$

Solubility
Solubility is determined in different solvents.

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

Leakage test
Select 10 tubes of ointment with seals applied when specified. Thoroughly clean and dry the exterior surfaces of each tube with an absorbent cloth. Place the tubes in a horizontal position on a sheet of absorbent blotting paper in an oven maintained at a temperature of 60 $\pm$ 3^{0}C for 8 hrs. The test was passed when there is no significant leakage occurred .If leakage is observed from one, but not more than one, of the tubes. Repeat the test with 20 additional tubes. The requirement is met if no leakage is observed from the first 10 tubes, or if leakage is observed from not more than one of 30 tubes test.[7]

Anti-bacterial activity
The extracts were tested for anti-bacterial activity in the well diffusion method by using standard procedure. The bacterial species used for the test were *Staphylococcus epidermidis*. All the stock cultures were obtained from MTCC lab. The microorganisms were grown overnight at 37˚C in nutrient broth (pH 7.4).[8]

Well diffusion method
The well diffusion method was used to screen the antimicrobial activity. The MHA plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5 min, inoculum suspension was spread uniformly by using sterile swab, and the inoculums were allowed to dry for 5 min. 70 microliters of extracts were loaded on 6 mm well, and then allowed to diffuse for 5 min, and the plates were kept for incubation at 37^{0}C for 24 hours. At the end of incubation, inhibition zones formed around the disc were measured in millimetres.[9]

RESULTS

Antimicrobial activity
Various extracts of *F.asafoetida* were prepared and screened for their antimicrobial activity. Three different asafoetida extract were selected and the study was carried against *Stayphylococcus epidermidis* to find out the effective solvent and concentration for Asafoetida with respect to that of 1% Clindamycin gel by comparison using zone of inhibition.

Figure 2: Zone of inhibition of choloroform asafoetida extract against *Staphylococcus epidermidis* (9mm).

Table 4: Diameter of zone of inhibition of chloroform extract against *Staphylococcus epidermidis*.

Test organism	Standard	Test	Control
Staphylococcus epidermidis	Clindamycin	Chloroform and asafoetida extract	Chloroform
	34mm	23mm	11mm

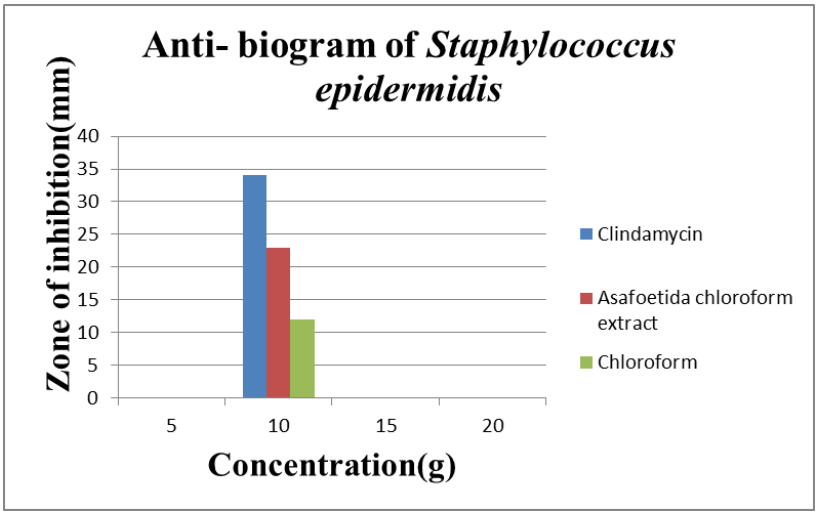

Figure 3: Anti-biogram of *S. epidermidis* in asafoetida-chloroform extract.

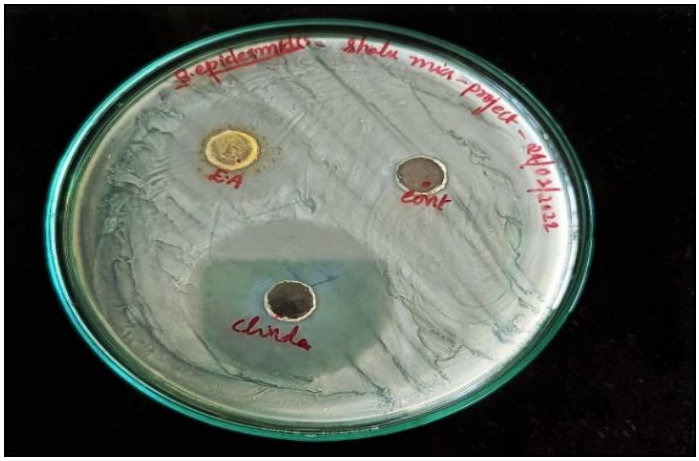

Figure 4: Zone of inhibition of Ethyl acetate asafoetida extract against *Staphylococcus epidermidis.*

Table 5: Diameter of zone of inhibition of Ethyl acetate extract against *Staphylococcus epidermidis.*

Test organism	Standard	Test	Control
Staphylococcus epidermidis	Clindamycin	Ethyl acetate and asafoetida extract	Ethyl acetate
	35mm	28mm	_

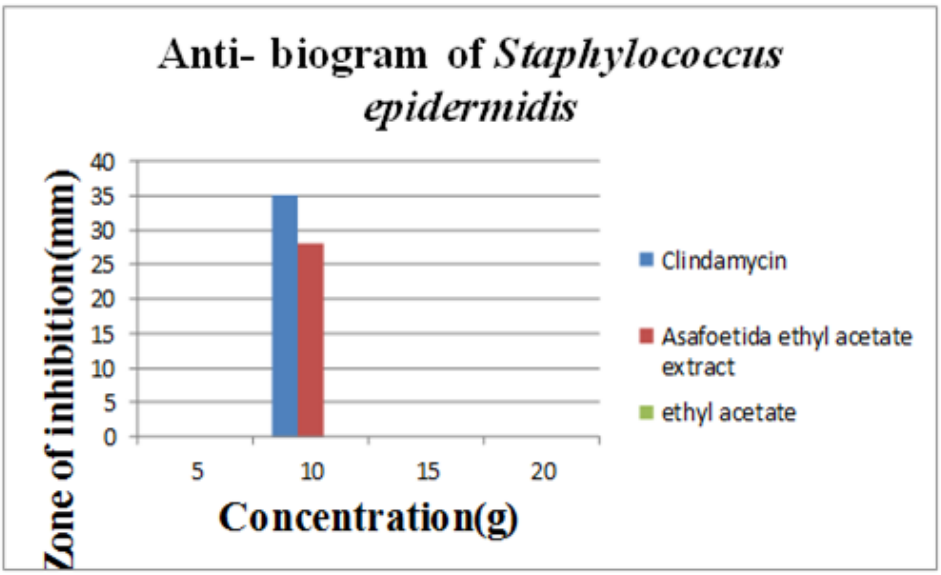

Figure 5: Anti-biogram of *S. epidermidis* in asafoetida ethyl acetate extract.

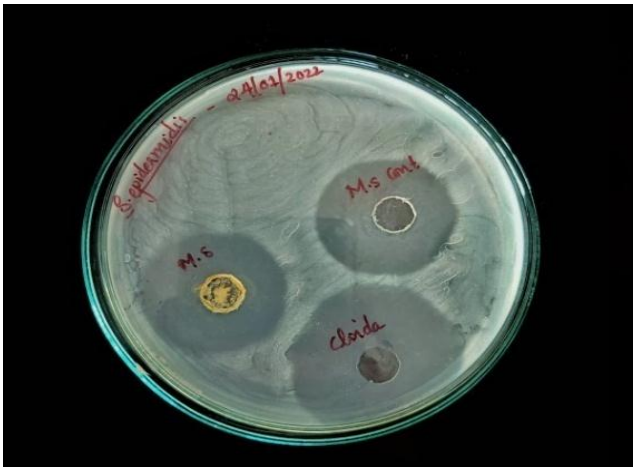

Figure 6: Zone of inhibition of Methylated spirit asafoetida extract against *Staphylococcus epidermidis.*

Table 6: Diameter of zone of inhibition of methylated spirit extract against *Staphylococcus epidermidis*

Test organism	Standard	Test	Control
Staphylococcus epidermidis	Clindamycin	Methylated spirit and asafoetida extract	Methylated spirit
	38mm	30mm	29mm

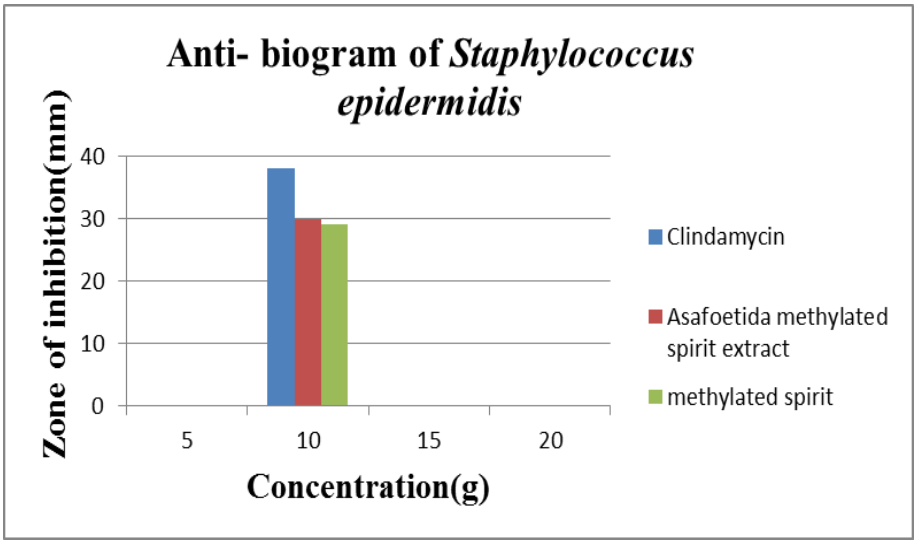

Figure 7: Anti-biogram of *S. epidermidis* in asafoetida methylated spirit extract.

All the extracts of *Ferula asafoetida* showed anti-acne activity against *Staphylococcus epidermidis* .Anti-acne activity of the extract was assessed by well diffusion method. The zone of inhibition is shown in Tables 4, 5 and 6. Chloroform and ethyl acetate extracts showed greater zones of inhibition in comparison to the metylated spirit extract (figure 2 and 4).

The choloroform extract of asafoetida showed anti-acne acitivity against *Staphylococcus epidermidis* with inhibition zones of 23mm compared to Clindamycin gel with zone of inhibition 34mm (table 4, figure 2).

The ethyl acetate extract of asafoetida showed anti acne acitivity against *Staphylococcus epidermidis* with inhibition zones of 28mm compared to Clindamycin gel with zone of inhibition 35 mm (table 5, figure 4).

The methylated spirit extract of asafoetida showed anti acne acitivity against *Staphylococcus epidermidis* with inhibition zones of 30mm compared to Clindamycin gel with zone of inhibition 38mm (table 6, figure 6).

Highest zone of inhibition was observed in case of ethyl acetate extract. Methylated spirit and its extract showed same activity against *staphylococcus epidermidis*.

Organoleptic characteristics

Colour and odour

Physical parameters like color and odour were examined by visual examination. The colour was found to be pale yellow and pungent odour.

pH

The pH of solution of ointment was found to be 5.1

Drug content

Table 7: Concentration v/s absorbance.

Concentration	Absorbance
0.5ml	0.603
1ml	1.204
1.5ml	1.812
2ml	2.410
2.5ml	3.012

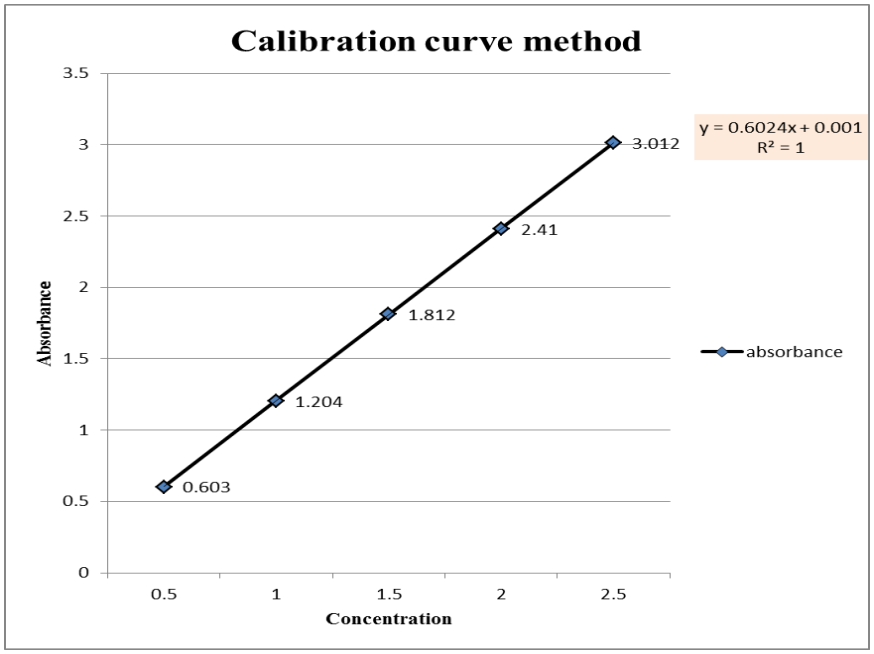

Figure 8: Calibration curve.

Absorbance of formulation (a_1) was found to be 1.24.
Drug content was determined by $a_1c_1=a_2c_2$
Drug content of formulation was found to be (c_1) =58.26µg/ml
Percentage purity was found to be = 97%$^{w}/_{w}$

Spreadability
It was found to be 6 seconds

Extrudability
The ointment was extruded through the nossle of ointment tube with minimal pushing.

Loss on drying
It was found to be 0.4%

Solubility
Soluble in Hot water and ethyl acetate
Slightly soluble in methylated spirit
Insoluble in water and chloroform

Washability
Formulation was applied on the skin can be washed with water and soap

Leakage test
No leakage was observed

Table 8: Results of evaluation parameters.

Physiochemical parameters	Observation
Color	Pale yellow
Odour	Pungent
pH	5.1
Drug content	58.26 µg /ml
Spreadability	6 sec
Extrudability	1gram
LOD	0.4%
Solubility	Soluble in ethyl acetate and hot water
Washability	Good
Non irritancy	Non irritant

DISCUSSION

Acne is a disorder resulting from the action of hormones and other substances on the skin's oil glands (sebaceous glands) and hair follicles. These factors lead to plugged pores and outbreaks of lesions commonly called pimples or zits. Acne lesions usually occur on the face, neck, back, chest, and shoulders.

Acne or pimples is a skin condition that usually affects adolescents or teenagers but may affect an individual at any age. Factors such as puberty, family trait, oily skin, hormonal changes, and also certain drugs such steroids, lithium responsible for acne. Almost everyone who experiences acne will develop the condition on their face, and a variety of treatment can be employed to reduce severity of the condition.

For people with mild acne, over-the-counter medication can be an effective treatment. For those with more severe cases, prescribed antibiotics and topical treatments are often used.

When used for acne, antimicrobials slow or stop the spread of bacteria and yeasts. Various types of antimicrobials have been found to reduce inflammatory lesions by up to 86%. However, it is thought that antimicrobials should be used less often or only for short periods of time to avoid bacterial resistances to medications developing.

Topical therapy is mainstay of dermatological treatment and holds many advantages including ease of use, relative lack of systemic side effects, and patient directed application .When the ointment is applied on the skin it directly enter the body.

There is a particularly strong medicine that tackles all of the causes of acne available, but due to the side effects patients choosing this course of medication must be regularly monitored by a healthcare professional. Side effects from this medication include rashes, diarrhoea and yeast infections like thrush. However, around 85% of patients are able to permanently clear their acne after a single course of this medication.

Natural treatments for acne are becoming more popular as they often pose fewer side effects and having better tolerance rates in patients. Asafoetida is natural amazing ingredient to promote skin health and glow. The powerful anti-inflammatory properties work well to lessen acne production and its antibacterial properties hinder the growth of pimples and the rashes. It improves the blood flow to the facial tissue and enhancing the skin radiance and glow.

The present study was done to prepare and evaluate the asafoetida ointment. For this the herbal extract were prepared by maceration method using chloroform, ethyl acetate and methylated spirit and screened for their antimicrobial activity. Then trituration method was done by incorporating asafoetida extract into the ointment base for preparing the ointment. The physiochemical properties of asafoetida ointment were studied using various evaluation methods.

CONCLUSIONS

The plant *Ferula asafoetida* is well known for its use as traditional system of medicine in Ayurveda. Asafoetida is also used for the treatment of acne, which is caused by gram positive bacteria *Staphylococcus epidermidis*. The present study has been undertaken to explore the anti-acne activity of asafoetida against microorganism.

For this purpose various extract of asafoetida with methylated spirit, chloroform and ethyl acetate were prepared and screened for their antimicrobial activity. The plant extract have great potential antimicrobial compound against microorganism. The ethyl acetate extract of asafoetida showed more activity against *Staphylococcus epidermidis* that the other extracts and thus it is effective for the treatment of acne.

The extract of asafoetida in ethyl acetate was formulated into ointment for increasing their effectiveness and for their ease of application.

As asafoetida is a natural substance, it has fewer side effects when compared to other chemical ingredients incorporated ointments.

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