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Formulation and Evaluation of Natural Anti Acne Cream Containing *Mimosa tenuiflora* Bark Extract

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ABSTRACT

Acne vulgaris is an extremely common skin disorder that affects virtually all individuals at least once during life. The incidence of acne peaks at teenage, but substantial numbers of men and women between 20-40 years of age were also affected by disorder. Acne is a disorder of the sebaceous follicles, which are specialized pilosebaceous units located on the face, chest and back. They consist of sebaceous glands associated with small hair follicles. Several factors contribute to the pathogenesis of acne, such as sebum, abnormal follicular differentiation, propionic-bacterium acnes, etc. Herbal cosmetics are now getting more important as their side effects are comparatively less than that of synthetic ones. Herbal medications are considered safer than allopathic medicines as allopathic medicines are associated with side effects such as contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling etc. Different plant parts such as leaves, stem bark, wood, rhizomes are used in cosmetics. In this study the anti-acne property of *Mimosa tenuiflora* bark extract was confirmed. The cream formulation was developed, which contains the effective concentration of *Mimosa tenuiflora* bark extract. The anti-acne property of developed formulation was evaluated by microorganism testing method of anti-bacterial activity. *Mimosa tenuiflora* bark extract containing creams possess a wide range of pharmacological properties especially as Antibacterial, Anti-inflammatory. The characteristics of cream in terms of spreadability, greasiness, tackiness, film forming, softening, comfortable and pleasant were analyzed by skin feel test. The results proved that the chosen formulation also having the effective anti-acne property. So, we can suggest that further investigation and in vivo studies will help in developing this formulation as marketed product.

1. INTRODUCTION

Acne vulgaris is an extremely common disorder of skin (pilocebaceous unit) that affects virtually all individuals at least once during life. The incidence of acne peaks atteenage, but substantial numbers of men & women between 20-30 years of age are also affected by the disorder.

Acne may be classified as comedonal, popular, pustular, cystic & nodular. Comedonal acne isnon-inflammatory & divided into two types: whiteheads & blackheads. White heads (closed comedo) present as fresh or white coloured, raised bumps whereas blackhead (open comedo) present as open pores containing dark coloured skin roughage consisting of melanin, sebum & follicular cells. Papules appear as red, solid, elevated lesions often less than 5mm in diameter. Puastules are circumscribed skin elevations containing purulent material. Cysts & nodules are solid, elevated lesions involving deeper dermal & subcutaneous tissue. Cysts are less than 5 mm in diameter whereas nodules exceed 5mm.

The pathogenesis of acne involves multiple physiological factors. These include follicular hyper-proliferation, increased sebum production due to higher androgen levels & colonization of micro-organism, Propioni-bacterium acnes & staphylococcus epidermis. Novel concept has emerged to help better understand its pathogenesis, these includes variation in target cell sensitivity, biological markers, neuroendocrine, genetic & environmental factors. Plenty of herbal as well as synthetic ingredients are reported to have remarkable beneficial effect on acne vulgaris.

Gel, Cream, Lotion, Face wash or cleanser, Face pack or mask. *Mimosa tenuiflora* (*Acacia hostilis*) Mart is reported to have very beneficial effect on acne due to anti-microbial, anti-inflammatory & anti-oxidant activities of different chemical constituents. Acne is caused by the following factors:

- Excessive oil production.
- Bacterial infection.
- Unhealthy eating habits or a sedentary lifestyle.
- Changes in hormones and stress.

- **Symptoms** :- Blackheads, Whiteheads, Pimples, Oily Skin, Scarring.
- **Complications** :- Anxiety, Reduced Self Esteem, Depression, Thoughts Of Suicide
- **Usual Onset** :- Puberty
- **Risk Factors** :- Genetics
- **Differential Diagnosis** :- Folliculitis, Rosacea, Hidradenitis Suppurativa, Miliaria
- **Treatment** :- Lifestyle Changes, Medications, Medical Procedure.

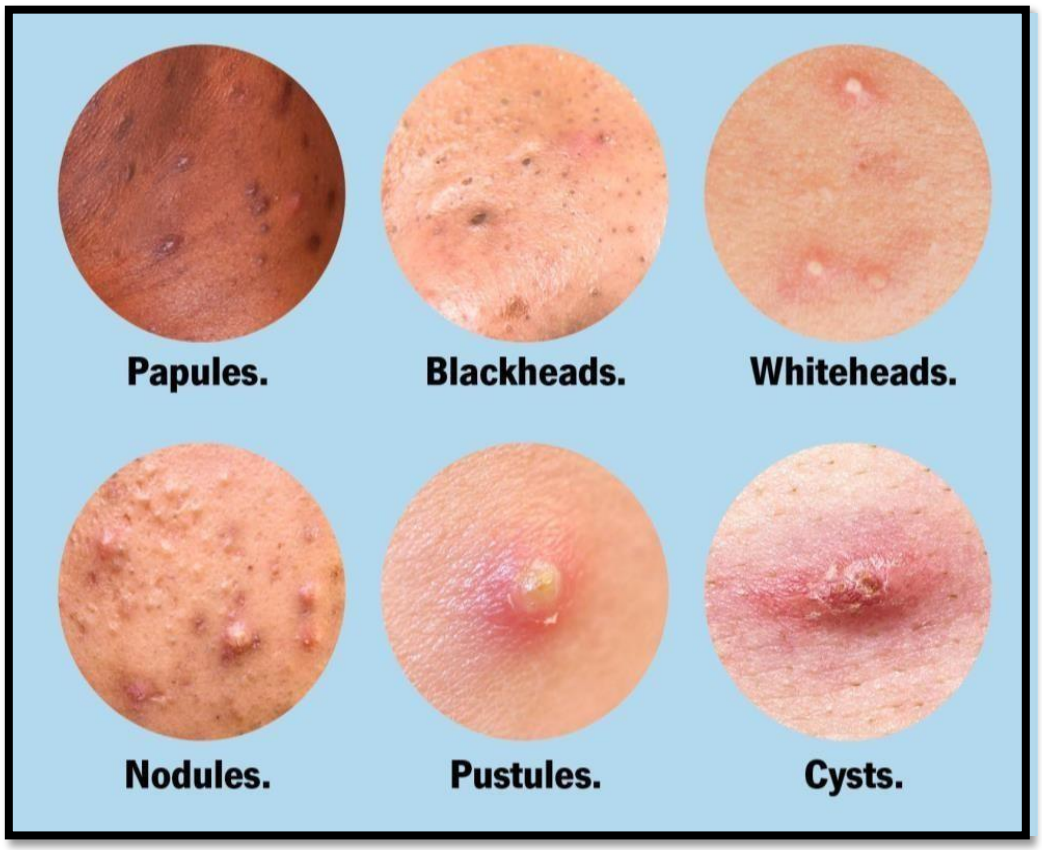

Fig no: 1:- Types of pimple

***MIMOSA TENUIFLORA*:**

Fig. no 2 :- *Mimosa tenuiflora*

Mimosa extract helps stimulate tissue repair, making it a choice of assets for damaged skin. It also has anti-bacteriostatic and helps repair acne lesions and tighten pores: this asset is therefore of particular interest to fight against acne.

Mimosa tenuiflora (Wild). Poiret is an American shrub, the bark of which is used in Mexican vernacular medicine for the treatment of burns and the prevention of inflammation (Grether, 1988). This material was used to alleviate the suffering of burn victims following the San Juanita natural gas explosion in 1982 and the 1985 earthquake. The results of these treatments have been observed by a great number of journalists and scientists world-wide: an analgesic effect lasting 2 or 3 h and a complete reconstitution of the epidermis within a few weeks. The death rate of patients suffering from very severe burns was also significantly reduced by the use of a powder of the bark of this tree. This plant attracted the attention of a set of multidisciplinary scientists, and we had the opportunity to participate in the chemical and biological investigation of this new material.

Botany

Mimosa tenuiflora (Willd.) Poiret, a spontaneous shrub distributed from Mexico to Venezuela and Brazil, belongs to the Mimosaceae -Fabaceae family.

Mimosa tenuiflora bears bi-pinnate leaves of 5-10 primary leaflet pairs and of 10 to 30

Secondary leaflet pairs, 3-6 mm long and 0.7-2 mm wide. White flowers are grouped in dense

heads 3-6.5 mm long. Fruits are lance-shaped, 2-4.5 mm long and 5-7 mm wide, with 2-6 articulations. The wall of the fruit is compressed between the seeds. The bark contains calcium oxalate crystals and large amounts of starch (Jiang, 1991a).

Chemistry

Three triterpenoid, saponins, mimonosides A (l), B (2) and C (3) and a mixture of three steroid saponins, campesterol-3-O-&D-glucopyranosyl (4), stigmasterol-3-O-P-D-glucopyranosyl (5) andfl-sitosterol-3-O-/3-D-glucopyranosyl (6) were isolated and identified from the bark of this plant (Jiang, 1991a-c). Compounds 1, 2, 3 and 4 are new natural substances. All these six saponins have been obtained for the first time from the genus Mimosa. For the extraction procedure, dried and powdered bark was treated by chloroform, ethyl acetate and methanol, respectively. The methanol extract was partitioned between n-butanol and water containing 1% sodium hydroxide. The butanol extract was chromatographed over Sephadex LH 20, Lichroprep RP-8 and silica gel columns, respectively, to afford 1, 2 and 3. The ethyl acetate extract was subjected to polyamide column chromatography and the saponin fraction was separated over a silica gel column to afforda mixture of 4, 5 and 6.

Biology

The new saponins isolated from this material were tested for cytotoxicity on lymphoma cells (Molt4 and RDM 4) and normal murine lymphocytes (thymocytes and splenocytes). It is known that murine thymocytes are composed of an average of 97% T-lymphocytes and of 3% B-lymphocytes and that murine splenocytes are composed of65% B-lymphocytes and of 35% T-lymphocytes (Bach, 1986). B- and T-lymphocytes are cells that can assure specific immunity. B-lymphocytes produce antibodies and T- lymphocytes, activated by antigens or non-specific mitogens, can produce many nonspecific mediators. Some mediators cooperate with B-lymphocytes to produce antibodies. Concanavaline A (Con A) predominantly stimulates the Tlymphocytes. It also stimulates the B-lymphocytes in the case of mixtures of B- and T-lymphocytes (e.g. murine splenocytes), while lipopolysaccharides (LPS) mainly stimulate B- lymphocytes.

2. PLANT PROFILE

Synonyms:-

- *Acacia hostilis* Mart
- *Acacia jurema* Mart
- *Acacia tenuiflora* Willd
- *Mimosa cabrera* H. Karst
- *Mimosa hostilis* (C.Mart.) Benth
- *Mimosa limana Rizzini MIMOSA TENUIFLORA*

Table no: 1. Plant Profile

Kingdom	Plantae
Family	Fabaceae
Subfamily	Caesalpinioideaeas
Order	Fabales
Genus	Mimosa
Species	*M. tenuiflora*
Clade	Tracheophyte, Angiosperms, Eudicots, Rosids , Mimosoid clade
Chemistry	tannins, saponins, alkaloids, lipids, phytosterols, glucosides, xylose, rhamnose, arabinose, lupeol, methoxychalcones, and kukulkanins .

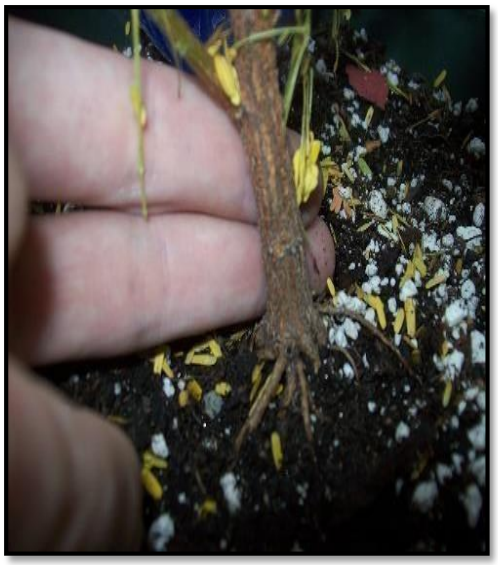

Fig no: 3. *Mimosa Tenuiflora* root

Fig no: 4. *Mimosa Tenuiflora* Plant

Fig no: 5. *M. tenuiflora* Bark Powder

Fig no: 6. *M.tenuiflora* bark finepowder

3. MATERIALS AND INSTRUMENTS

Table no 2: Materials

Sr. No.	Chemicals
1	Ethanol
2	Stearic acid
3	Cetyl alcohol
4	Glycerin
5	Triethanolamine
6	Distilled water
7	Methyl Paraben
8	Liquid paraffin
9	α-naphthol
10	Conc.sulphuric acid
11	Propyl paraben
12	Propylene glycol
13	Almond oil
14	Dragondroff's reagent
15	Copper sulphate
16	Potassium hydroxide
17	Acetic anhydride
18	Ferric chloride
19	Sodium picrate
20	Lead acetate
21	Peptone
22	Agar
23	Beef extract
24	Sodium chloride
25	Streptomycin
26	Dimethyl sulfoxide

3.1 INSTRUMENTS

Table No 3: Instruments

Sr. No.	Name of Instruments	Brand name
1	Analytical balance	Contech
2	Hot air oven	Tempo
3	Digital autoclave	ASI-254
4	B.O.D incubator	HMG
5	pH metre	Globe

3.2 ROLE OF INGREDIENTS

Table No: 4. Ingredients and its Role

INGREDIENTS	ROLE OF INGREDIENTS
Mimosa tenuiflora	Antibacterial, Anti-inflammatory, Antimicrobial
Stearic acid	Emulsifier
Cetyl alcohol	Stabilizer
Glycerin	Humectant
Triethanolamine	Neutralizer
Distilled water	Vehicle
Methyl Paraben	Preservative
Liquid paraffin	Lubricant

4. EXPERIMENTAL WORK AND RESULTS

A. Extraction method of maceration

1. Maceration. This is an extraction procedure in which coarsely powdered drug material which is root bark is placed inside a container; the menstrum is poured on top until completely covered the drug material.

2. The container is then closed and kept for at least three days. The content is stirred periodically, and if placed inside bottle it should be shaken time to time to insure complete extraction.

3. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, they separated from the menstuum by evaporation in an oven or on top of water bath. This method is convenient and very suitable for thermolabile plant material.

Fig no: 7. *M. tenuiflora* bark extract

Fig no: 8. *M. tenuiflora* bark extract

B. Phytochemical screening of different qualitative chemical tests

It can be performed for establishing profile of ethanol and aqueous extract for its chemical composition. The following tests were performed on extracts to detect various phytoconstituents present in them.

Detection of carbohydrate Molish Test

To 2 ml of filtrate, two drops of alcoholic solution of alpha naphthol are added, themixture is shaken well and 1 ml of concentrated sulphuric acid is added slowly alongthe sides of the tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

Detection of alkaloids Dragondroff's test

To a few ml of filtrate, 1 or 2 ml of Dragondroff's reagent are added. A prominent yellow precipitate indicates the test as positive.

Detection of saponinFoam test

The extract (50 mg) is diluted with distilled water and made up 20ml. the suspensionis shaken in graduated cylinder for 15 min, A 2 cm layer of foam indicates the presence of saponins.

Detection of proteinBiuret test

An aliquot of 2 ml filtrate is treated with one drop of 2% copper sulphate solution. To this, 1mlethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicates the presence of proteins.

Detection of steroids and triterpenoid Libermann-Burchard test

The extract (50mg) is dissolved in 2ml acetic anhydride. To this, one or two drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols.

Detection of glycosides Killer Killani test

To the test solution few drops of ferric chloride solution and concentratedsulphuric acid was added.

Baljet test

Sodium picrate was added to the test solution.Detection of tannin.

Ferric chloride solution test

To 1ml of the extract, ferric chloride solution was added.

Fig no: 9. Phytochemical Tests

Phytochemical screening of different qualitative chemical tests

Table no: 5. Phytochemical Test

Sr.No	Test	Observation	Result
1)	Detection of carbohydrate :- i) Molish test	Violet ring was not formed at the junction of two liquids.	Carbohydrates was absent
2)	Detection of Alkaloids :- i) Dragndroff's test	Formation of orange brown colored ppt.	Alkaloids was present.
3)	Detection of Saponins :- i) Foam test	Persistent foam formation.	Saponin was present.
4)	Detection of Protein :- i) Biuret test -	Not produce bluecolor.	Protein was absent.
5)	Detection of Steroids andTriterpenoids :- i) Libbermann - Burchard test	Formation of red colour and yellow colour at the lower layer.	Steroids and Triterpenoids was present.
6)	Detection of Glycosides :- i) Killer Killiani test – ii) Baljet test	No formation of two layers. No colour change occur from yellow to orange colour.	Glycosides were absent. Glycosides was absent.
7)	Detection of Tannins :- i) Ferric chloride solution test -	Formation of darkblue colour	Tannins was present.

8)	Detection of Flavonoids :- i) Lead acetate test -	Yellow coloured ppt was formed.	Flavonoids was present
9)	Detection of Fats and Oils :- i) Solubility test	Not soluble in ether, chloroform and benzene.	Not soluble in ether, chloroform and benzene.

4.1 PREPARATION OF CREAM

Table no 6: Ingredients for Preparation of Cream

Sr.No.	**Ingredient**	**Quantity (20%)**
1	Ethanol extract of *M.tenuiflora*	2
2	Stearic acid	4
3	Cetyl alcohol	1.6
4	Liquid Paraffin	1.6
5	Methyl paraben	2
6	Glycerin	2
7	Triethanolamine	0.15
8	Water	6.65

Preparation Method –

Procedure for preparation of cream

1) Oil in water (O/W) emulsion based cream was formulated.

2) The emulsifier (stearic acid) and other oil soluble components (cetyl alcohol, almond oil) were dissolved in the oil phase (part A) and heated to 75^0C.

3) The preservatives and other water soluble components (methyl paraben, propyl paraben, triethanolamine, propylene glycol, ethanol extract of *Mimosa tenuiflora*) were dissolved in the aqueous phase (Part B) and heated to 75℃.

4) After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until cooling of emulsifier took place.

Fig no 10: Formulation of Cream

Fig no 11: F1

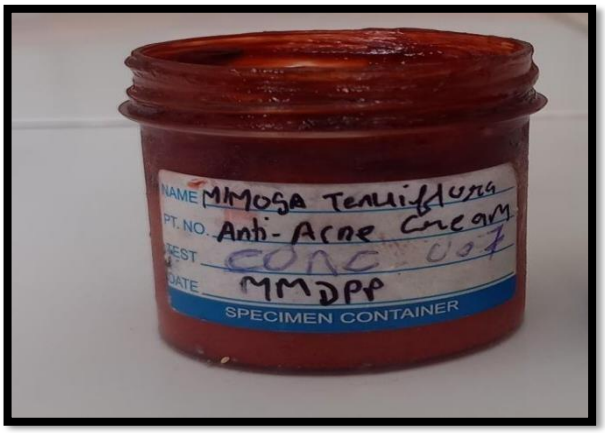

Fig no 12. F2

Fig no 13: F3

Fig no 14: F4

4.2 EVALUATION TEST

1) Determination of the type of Emulsion

A red dye was mixed with the cream. A few drops of the cream were placed on microscopicslide and observed under a microscope. If the disperse globules appear red the continual phase colorless, the cream is oil in water.

2) Determination of pH

The pH meter was calibrated employing a customary solution. About 0.5 g of the creamwas weighed and dissolved in 50 ml of water and its pH was measured.

3) Homogeneity

The formulation was tested for homogeneity by visual appearance and touch.

4) Appearance

The looks of the cream were judged by its color, pearlescence, and roughness, and graded.

5) After feel:

Emolliency, slipperiness, and also the quantity of residue left after the appliance of a collection amount of cream was checked.

6) Type of smear

After the appliance of the cream, the kind of film or smear formed on the skin was checked.

7) Washability

The ease of removal of the cream applied was examined by washing the applied dispensewith water.

8) Viscosity

Viscosity of the formulation was determined by Brookfield Viscometer at 100rpm and by using spindle no 7.

The sample 20 gm was placed in a beaker and was allowed to equilibrate for 5 min. Before measuring the dial reading using a T-D spindle (no 7) at 100rpm at speed, corresponding dial reading on the viscometer was noted the measurement where carried in triplicates at room temperature direct multiplication of a dial reading with factors is given in the Brookfield Viscometer catalogue give the viscosity in centipoises.

RESULT:

Table no: 7. Observation of evaluation parameters:

Parameters	**F1**	**F2**	**F3**	**F4**
Emulsion type	o/w	o/w	o/w	o/w
pH	6.9	6.2	6.27	6.5
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
Appearance	Semi-solid cream	Semi-solid cream	Semi-solid cream	Semi-solid cream
After feel	Emollient and Slipperiness	Emollient and Slipperiness	Emollient and Slipperiness	Emollient and Slipperiness
Type of smear	Non-greasy	Non-greasy	Non-greasy	Non-greasy
Washability	Easily washable	Easily washable	Easily washable	Easily washable
Viscosity	18557	6749	13467	15057
color	Creamy red	Creamy red	Creamy red	Creamy red

4.3. ANTIMICROBIAL ACTIVITY

*** Cup plate of cylinder plate method :-**

This method relies on the diffusion of an antibiotic from a verticle cavity or a cylinder through the solidified agar layer in a petri plate. The growth of test microorganism in observe to be inhibited in a circular area or zone around the cavity containing antibioticsolution.

*** Steps involved in cup plate method are given below,**

1. A liquefied assay medium (43-45^{0}c) is inoculated by the suspension of test microorganism.

2. This inoculated test culture medium poured and spread on sterile petri or preprepared agar plates.

3. Standard and test antibiotic solution of known concentration are prepared in appropriate solutions, which are then added to sterile cavities prepared on solid medium.

4. Uniform volume of sodium should be added to each cavity to fill them sufficientlyif papers discs are used, they should sterilize first, then dipped in standard or test solution and finally placed on medium surface.

5. The plates are allowed to stand at room temperature or at 4^0 c for 1-2 hours. This is the period of pre-incubation diffusion which minimizes the effect of variation time between the applications of different solutions.

6. All plates are then incubated at temperature 32-35^0 C for 18-24 hours.

7. The diameters or areas of circular inhibition zone produce by standard and test anti-biotic solution are accurately measured.

Fig no: 15. Effect of *M.tenuiflora* on S.aureus

Fig no: 16. Effect of *M.tenuiflora* on E. coli

Fig no : 17. Standard

RESULT:

Table no: 8. Table for Antimicrobial evaluations on S. aureus:

Sr.No	Test Culture	Positive control 0.1 ml for(1 mg/ml)	Bark extractof *M. tenuiflora*	Zone of inhibition in (mm)		
				DMSO	Positive control	Plant extract
1	*S. aureus*	Streptomycin	0.2 gm extract	-	1.4 cm	0.7 cm
2	*S. aureus*	Streptomycin	0.7gm extract	-	1.4 cm	0.9 cm
3	*S. aureus*	Streptomycin	0.27 gm extract	-	1.4 cm	1 cm
4	*S. aureus*	Streptomycin	0.8 gm cream	-	1.4 cm	0.5 cm

Table no: 9. Table for Antimicrobial evaluations on E.Coli:

Sr.No	Test Culture	Positive control 0.2 ml for(1 mg/ml)	Bark extract of*Mimosa tenuiflora*	Zone of inhibition in (mm)		
				DMSO	Positive control	Plant extract
1	*E.coli*	Streptomycin	0.5 gm extract	-	1.2 cm	0.4 cm
2	*E.coli*	Streptomycin	0.17gm extract	-	1.2 cm	0.6 cm
3	*E.coli*	Streptomycin	0.27 gm extract	-	1.2 cm	0.8 cm
4	*E.coli*	Streptomycin	0.8 gmcream	-	1.2 cm	0.4 cm

The *Mimosa tenuiflora* bark extract showed mild to moderate antibacterial activity against *Staphylococcus aureus and E.coli* .

5. DISCUSSION

The formulated anti-acne cream was evaluated for several physicochemical tests and theresults were shown above. The dye test was confirmed that all the formulations were o/w type of emulsion cream. The pH of the formulated cream was found to be in range 6-7 which is good and recommended pH for the skin. The formulated anti-acne cream was evaluated for several physicochemical tests and the results were shown in Table 7. The type of smear formed on the skin was not greasy after the application of all creams. The creams were easy to remove after application by washing with water. The formulations were able to produce uniform distribution of extracts in the cream. This was confirmed by visual examination and by touch. There were no changes in term of colour of the cream even it was kept for a long period of time. After feel test showed that the creams were emollient and slipperiness. Even though there is no change in a color reaction is observed when it was kept for a longer time in a room temperature which indicates the stability of the product.

The ethanolic extract of *Mimosa tenuiflora* bark showed the antibacterial activity against Escherichia coli as seen by the zone of inhibition ranges from 0.4 to 0.8 cm (Table no 9).The zone of inhibition against Staphylococcus aureus was 0.5 to 1 cm. All the extracts were showed a significant ($p<0.05$) zone of inhibition when increasing the concentration of the extract. However, the standard Streptomycin showed significantly increased zoneof inhibition against the entire tested organism when compared to tested extract.

6. CONCLUSION

Acne vulgaris is an extremely common skin disorder that affects virtually all individuals at least once during life. Acne can have important negative psychological consequences for the affected individual, including diminished self-esteem, social withdrawal due to embarrassment and depression. Herbal medications are considered safer than allopathic medicines as allopathic medicines are associated with side effects such as contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling etc. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than synthetic ones, so herbal anti-acne cream which is non-toxic, safe, effective and improves patient compliance by the utilization of herbal extracts would be highly acceptable. In this study the anti-acne property of each herbal extract was reconfirmed. The cream formulation was developed, which contains the effective concentration of poly herbal extract. The anti-

acne property of developed formulation was evaluated by in vitro method of anti-bacterial activity (broth dilution method and sub culturing method) The characteristics of cream in terms of spreadibility, greasiness,tackiness, film forming, softening, comfortable and pleasant was analyzed by skin feel test. The results proved that the chosen formulation also has the effective anti-acne property. So, we can suggest the further investigation and in vivo studies will help in developing this formulation as marketed product.

7. ACKNOWLEDGEMENT

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