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Evaluation and Development of Polyherbal Anti Acne Cream Containing Lemmon Grass Extract

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ABSTRACT
This project on Evaluation and Development of Polyherbal Anti Acne Cream Containing Lemmon Grass Extract by Using Liquid Paraffin**.** It is a very good attempt to formulate and evaluate the Polyherbal anti-acne cream along with the stability studies. Based on this studies, Polyherbal anti-acne cream prepared from the extract of Lemon Grass Extract, Stearic Acid, Borax, Liquid Paraffin, Cetyl Alcohol, Glycerin and Methyl Paraben. Formulation F5 Prepared from various ratio of the extract of Lemon Grass Extract (25ml), Stearic Acid (1.7gm), Borax (0.12gm), Liquid Paraffin (6ml), Cetyl Alcohol (14ml), Glycerin (2ml) and Methyl Paraben (2mg) showed significant good antibacterial activity on P. Acnes, Staphylococcus aureus and staphylococcus epidermis with no irritation. The polyherbal cream showed a synergistic effect as compared to individual extract with good stability. Thus the study result concludes that the formulated Polyherbal gel with extract of Lemon Grass Extract, Stearic Acid, Borax, Liquid Paraffin, Cetyl Alcohol, Glycerin and Methyl Paraben respectively can be used for the treatment of acne. The formulated Polyherbal Cream F5 shows better antibacterial activity with the zone of inhibition 36 mm for S. Epidermidis, Staphylococcus aureus, P. Acnes and 44mm for Staphylococcus epidermis. In addition to this, the Polyherbal cream showed a synergistic effect as compared to individual extract which can be useful for the treatment of local inflammation. Thus this topical formulation was suitable for the treatment of local anti-acne application and selecting for further tests. The duration of Stability studies of the formulation 5, there is no change in colour, but Variation of a pH, Viscosity, Spreadability and % Cumulative drug Release.

Keywords: Antibacterial Activity, Polyherbal Cream, Treatment of Local Inflammation, S. Epidermidis, Staphylococcus aureus, P. Acnes.

I. INTRODUCTION:

Acne is a skin condition that occurs when your hair follicles become plugged with oil and dead skin cells. It causes whiteheads, blackheads or pimples. Acne is most common among teenagers, though it affects people of all ages. Effective acne treatments are available, but acne can be persistent. The pimples and bumps heal slowly, and when one begins to go away, others seem to crop up. Depending on its severity, acne can cause emotional distress and scar the skin. The earlier you start treatment, the lower your risk of such problems. The pimples and knocks mend gradually, and when one starts to disappear, others appear to manifest. Contingent upon its seriousness, skin inflammation can cause enthusiastic misery and scar the skin. The previous you start treatment, the lower your danger of such issues. Skin break out vulgarize or just known as skin break out is a human skin sickness portrayed by skin with flaky red skin (Seborrhoea), zits and whiteheads (Comedones), pinheads (papules), Large papules (knobs) pimples and scarring [1].

Skin break out influences skin having thick sebaceous follicles in zones including face, chest and back skin inflammation might be of incendiary or non-provocative structures [2, 3].

Because of changes in Pilosebaceous units injuries are brought about by androgen incitement. Skin inflammation happens ordinarily during puberty, influencing around 80–90% of youngsters in the Western world and lower rate are accounted for in country social orders. Skin break out is normally brought about by increment in androgens level like testosterone chiefly during adolescence in both male and female [4]. Skin break out lessens after some time and will in general vanish over the age [5, 6].

The huge knobs called as growths and extreme incendiary skin break out called as Nodulocystic. The cystic skin break out happens on backside, crotch, armpit zone, hair follicles and

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sweat conduits and influences further skin tissue than basic skin break out [7, 8].

Skin inflammation causes scarring and mental impacts, for example, diminished confidence and in uncommon cases despondency or self destruction [9].

Reports demonstrated the frequency of self-destructive propensity in patients with skin break out as about 7.1%. Skin break out for the most part happens during youthfulness. The word skin break out alludes to the presence of papules, scars, comedones and pustules. The regular type of skin inflammation is known as skin inflammation vulgarize. Numerous adolescents experience the ill effects of this kind of skin inflammation. Skin inflammation vulgaris shows the presence of comedones. Skin inflammation rosacea is equivalent for Rosacea and a few people not have skin inflammation comedones related with their rosacea, consequently incline toward the term rosacea. Chloracne happens because of presentation to polyhalogenated mixes [10, 11].

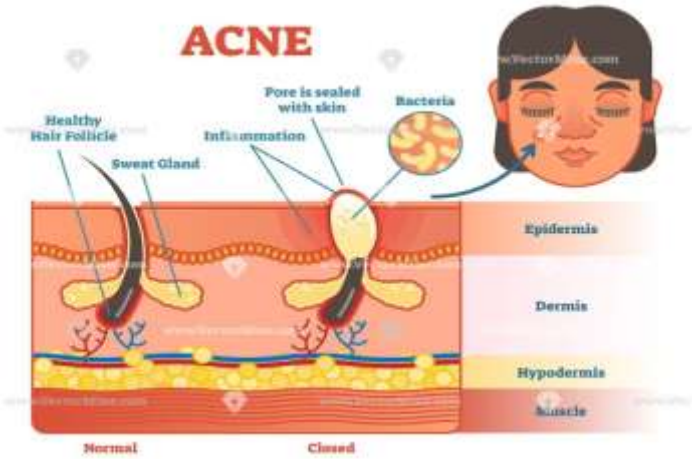

Fig.1. Changes in the skin Physiology due to Acne

II. MATERIALS AND METHODS:

Materials:

Table.1 List of Chemicals

S. No.	Chemicals	Brand
1	(Lemmon Grass Powder)	Farmer Mandawar Bijnor (U.P)
2	Stearic acid	Swami Enterprises Shiv Ram Park, Delhi
3	Cetyl alcohol	Vivek College of Pharmacy, Bijnor
4	Liquid paraffin	Maya Chemtech India Private Limited (Delhi)
5	Methylparaben	-
6	Borax	Vivek College of Pharmacy, Bijnor
7	Glycerin	-
8	Distilled H2O	-

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Table .2 Instruments Used in Research Work

S.No.	Instruments
1	Magnetic stirrer
2	pH meter
3	Digital balance
4	Heating plate
5	Viscometer
6	Oven
7	Incubator

Preformulation Study:

Identification of Lemmon Grass Leaves:

The whole plant, Lemmon Grass was collected from plants growing in Farmer Mandawar Bijnor Uttar Pradesh and dried at room temperature. The leaves were identified and authenticated by Dr. Vijai Malik, Head of Department of Botany, CCS University Meerut.

Physical Appearance:

The odor of palmarosa was pleasant odor with the rose like smell but the odor of citronella grass was a sweet smell.

Solubility Analysis:

The solubility of lemongrass Extract was analyzed by adding 1g of lemongrass oil sample into 10 ml of 70% ethanol and water respectively and the solubility was observed.

% LOD:

Air dried powdered lemongrass (2gm) were accurately weighed in a dried and tarred flat weighing bottle. The sample was dried to constant mass in a hot air oven at 105ºC. The percentage loss on drying was determined with respect to initial quantity of plant material taken.

Moisture Content:

Determination of moisture content A 2.0±0.5g of lemongrass powder were weighed into the crucible. The sample was dried using oven drying method (AOAC, 2000) in the conventional oven (BINDER, ED400, Germany) at 105 ± 2.0oC for 24 h. Moisture content of lemongrass powder was determined by the equation below;

MC % = Wt. of powder before drying (g) -Wt of powder after drying (g)/ Wt. of powder before drying (g) ×100

Scanning and Calibration Curve Preparation of lemongrass in Ethanol:

A std. stock sol. (100µg/ml) of lemongrass was pre-pared by accurately weighing 10mg of lemongrass and dissolved in 5ml of methanol in a 100ml volumetric flask. It was sonicated for 5mins & the volume was made up-to 100ml with CH3OH. From standard stock solution, lemongrass (10µg/ml) solution was prepared in methanol and UV-scan was taken between a wavelength range of 446nm and a wavelength showing maximum absorbance was selected as λmax for further analytical work.

FTIR Spectroscopy:

FTIR spectroscopy was led utilizing a Shimadzu FTIR 8400 Spectrophotometer (Shimadzu, Tokyo, Japan) and the range was recorded in the frequency district of 4000-400 cm-1. The technique comprised of scattering test (drug, stabilizer, actual blend) by KBr pellet strategy. The pellet was set in the light way and the range was recorded.

Method:

Lemmon Grass Leaves:

Fresh leaves were collected, washed and kept for drying. Paste the dried leaves are collected and grinded into fine powder.

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Fig.2 Lemmon Grass leaves Powder

Cold Maceration of Dried Lemmon Grass Leaves:

About 100 g of coarsely grounded leaves were steeped in hydro alcohol (1:1) for one week at ambient temperature and then filtered. This extraction was repeated with another fresh portion of hydro alcohol (1:1) for about one week. The hydro alcohol macerate extracts were combined and evaporated up to dryness, dark brown coloured product was obtained.

Fig.3 Lemmon Grass Extract

Photochemical Screening:

Detection of Alkaloids:

About 50mg of solvent free extracts was treated with 10ml of dilute hydrochloric acid and filtered. The filtrate was tested for the presence of alkaloids using the following tests.

Mayer's Test:

1ml of filtrate was taken into a test tube and added two drops of Mayer's reagent (potassium mercuric iodide solution) along the sides of the test tube and observed for white of creamy precipitate, which indicates the presence of alkaloids in the extract.

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Hager's Test:

To 1ml of filtrate, two drops of Hager's reagent (picric acid) was added and observed for prominent yellow precipitate, indicates positive test for the presence of alkaloids.

Dragendorff's Test:

To 1ml of filtrate, two drops of Dragendorff's reagent (potassium bismuth iodide solution) was added and observed for the formation of precipitate. Formation of prominent reddish brown precipitate indicates positive test for the presence of alkaloids.

Wagner's Test:

1ml filtrate was taken into a test tube, added two drops of Wagner's (potassium iodide solution) along the sides of the test tube and observed for reddish brown precipitate, which indicates the presence of alkaloids in the extract.

Detection of Proteins & Amino Acids:

Millon's Test:

To 2ml filtrate, 2 drops of Millon's reagent was added and observed for the formation of white precipitate, which indicates the presence of proteins.

Ninhydrine Test

To 3ml of filtrate, three drops of 5% Ninhydrine reagent was added and heated in boiling water bath for 10 mins & the formation of a characteristic purple colour indicates the presence of amino acids.

Biuret Test:

To 3ml filtrate, two drops of 4% NaOH was added and treated with two drops of 1% CuSO4 solution. Observed for formation of pink colour indicating the presence of proteins.

Detection of Steroids & Terpenoids:

Salkowski Test:

About 50mg of the extract, 2ml of chloroform and 2ml of concentrated sulphuric acid were added and shaken well. Then observed the coloration of chloroform and acid layers. Appearance of chloroform layer as red in colour and acid layer as greenish yellow fluorescence indicates the presence of steroids.

Liebermann Burchard Test:

About 50 mg of the extract was dissolved in 2ml of acetic anhydride in a test tube, added 2ml chloroform, heated to boiling and cooled. Then 1ml of concentrated sulphuric acid was added along the sides of the test tube and observed for the formation of colour at the junction. Formation of red, pink or violet colour at the junction of the liquids indicates the presence of steroidal tri-terpenoids.

Detection of Phenolic Compounds & Tannins:

Ferric Chloride Test:

About 50mg of extract was dissolved in 2ml of distilled water added two drops of neutral 5% ferric chloride solution and observed for blue, green of violet color, which indicates the presence of phenloic compounds. Test solution treated with few drops of ferric chloride solution gives dark colour.

Lead Acetate Test:

About 50 mg of extract was dissolved in 2ml of distilled water and to this 3ml of 10% lead acetate solution was added. Formulation of bulky white precipitate indicates the presence of Phenolic compounds.

Bromine Water Test:

About 50mg of extract was dissolved in 2ml of distilled water and 1ml of bromine water was added an observed discoloration of bromine water. Discoloration of bromine water indicates the presence of Phenolic compounds.

Iodine Test:

About 50mg of extract was dissolved in 2ml of distilled water.1ml of iodine solution was added and observed for formation of transient red color indicates the presence of phenolic compounds.

Detection of Fixed Oils & Fats:

Spot Test:

A small quantity of extract was taken and rubs off against whatman filter paper. If permanent oil stains formed, it indicates presences of fixed oils.

Solubility Test:

About 50mg of the extract was taken and dissolved in polar and non solvents. If the extract is solubilized in non polar solvents and insoluble in polar solvents, it indicates the presence of fats.

Detection of Glycosides:

Legal Test:

About 50mg of extract is dissolved in few drops of pyridine and 1 drop of 2% sodium nitroprusside and a drop of 20% sodium hydroxide solution is added. Formation of deep red color indicates the presence of cardiac glycosides.

Keller-Killiani Test:

To about, 50mg of extract 10ml of hydrogen peroxide and 0.5ml of strong solution of lead acetate. Shake well and separate the filtrate. The clear filtrate is treated with equal volume of chloroform and evaporated to yield the extractive. The extractive is dissolved in glacial acetic acid and after cooling, 2 drops ferric chloride solution are added it. These contents are transferred to a test

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tube containing 2ml concentrated sulphuric acid. A reddish brown layer acquiring bluish-green colour after standing is observed due to the presence of glycosides.

Test for Athraquinone Glycosides:
Borntrager's Test:

About 50mg of extract was hydrolyzed with 2ml of concentrated hydrochloric acid for 2 hrs on a water bath and filtered. To 2ml of filterate, 3ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added. Formation pink color indicatees the presence of anthraquinone glycosides.

Test for Saponin Glycosides:
Foam Test:

About 50mg of extract was dissolved in 2ml of alcohol, diluted with 20ml of distilled water and shaken for 15 mins in a graduated cylinder. A layer of stable foam indicates the presence of saponin glycosides.

Alkaline Reagent Test:

50mg of the extract was dissolved in 2ml of alcohol and to the extract, increasing amount of sodium hydroxide was added. It shows yellow coloration, which decolorizes after addition of an acid if flavonoids of an acid if flavonoids are present.

Test for Flavonoids:
Shinoda Test or Magnesium-Hydrochloric acid Reduction Test:

About 50mg of the extract was dissolved in 1ml of alcohol and two fragments of magnesium turnings were added. To this conc. hydrochloric acid was added drop wise and observed for colouration. If any pink or crimson red colour develops, presence of flavanol glycosides is inferred.

Formulation of Anti-Acne Cream:

1. The composition of anti-acne cream was shown in Table.2. The oil phase consists of Octadecanoic acid and other oil soluble component like Cetyl alcohol and liquid paraffin were dissolved within the oil phase.
2. The oil phase was placed inside the beaker within the water bath.
3. The temperature of water bath was set to 70°C during the heating time.
4. The water soluble components and preservatives (glycerine, Methyl Paraben and Borax) were dissolved within the aqueous phase and heated within the same water bath at temperature 70°C.
5. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until the cooling of emulsifier occurred.
6. Different proportion of Lemmon Grass extract (5% & 10%) was mixed with the bottom together with fragrance.

Table.2 Composition of Anti-acne Cream Formula.

S. No.	Composition	F 1	F 2	F 3	F4	F5	F6
1	Lemmon Grass Extract	20ml	20ml	20ml	20ml	20ml	20ml
2	Methyl Parbean	1mg	2mg	-	-	-	3mg
3	Borax	0.4mg	0.5mg	0.10mg	0.11mg	0.12mg	0.13mg
4	Stearic Acid	0.8g	1g	1.2g	1.5g	1.7g	-
	Glycerin	0.5ml	-	-	1ml	2ml	-
	Liquid Paraffin	2ml	4ml	-	6ml	-	-
5	Cetyl Alcohol	9ml	8ml	10ml	12ml	14ml	16ml
6	Distilled H2O	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

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Fig.4 Anti-acne Cream

Evaluation of Anti-Acne Cream:

The physical appearance of the detailing was checked outwardly which included Colour. The shade of the definitions was looked at against white foundation. The consistency was checked by applying on skin. The oiliness was evaluated by the application onto the skin. The scent of the gels was checked by blending the cream in water and taking the smell.

Colour: The color of the cream was observed by visual examination.

Odor: The odor of cream was found to be characteristics.

Consistency: The formulation was examined by rubbing cream on hand manually. The cream having smooth consistency. Cream did not leave greasy substances on skin surface after application.

State: The state of cream was examined visually. The cream having a semisolid state.

pH: The pH meter was calibrated using standard solution. About 0.5g of the cream was weighed and dissolved in 50ml of water and its pH was measured.

Viscosity: Viscosity of cream was done by using Brooke field viscometer at the temp of 25°C using spindle no. 63 at 5rpm.

Spreadability:

Two sets of a glass slide with standard dimension were taken. Polyherbal formulation cream was placed in between two slides & sandwiched about the length of 60mm. Removed the adhered excess gel on surface of the glass slides & fixed to a stand without any disturbance. In the upper slide, 20g weight was tied and noted the time taken for movement of the upper slide to the distance of 60mm under the influence of weight. Meantime was calculated by repeating the experiment three times & spreadability was calculated using the following equation 1 [101].

Spreadability = m×L/t

Where,

m=Weight tied to upper slide.

L=Length of the glass slide (6 cm).

t=Time in seconds.

Antibacterial Activity Studies:

Transferred 20ml of liquefied agar medium previously inoculated with 0.1ml bacteria into the sterile petri dish having an internal diameter of 8.5cm & allowed to form the uniform thickness of the medium in the petri dish. After complete solidification of the liquefied inoculated medium, the wells were made aseptically with cork borer having 6mm diameter. 100mg concentrations of polyherbal gel were weighed and diluted with 2ml of sterile water in sterile test tubes. The drug solution was carefully transferred into the cup & incubated at 37°C for 24h & zones of inhibition were measured [12].

2.8 Ex-Vivo Skin Irritation Studies:

The albino Wistar rats were divided into two groups with 3 rats in each group. On the previous day of the experiment, the hairs on the backside area of rats were removed. The animals of group I was served as the control, without any treatment. Another group of animals (Group II) was applied with prepared polyherbal Cream. The Cream was applied once a day for 7days and the site was covered with a cotton bandage and observed for any sensitivity. The score of erythema is read and recorded as Score 0 for no erythema; Score 1 for Mild erythema (barely perceptible-light pink); Score 2 for Moderate erythema (dark pink); Score 3 for Severe erythema (Extreme redness) [13].

2.9 Drug Content:

The medication substance of the plans was controlled by dissolving a precisely gauged amount of Cream 1g in 100ml of dissolvable (phosphate cradle pH 6.8 + ethanol in proportion 40:60). The

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arrangements were saved for shaking for 4hrs and afterward saved for 6hrs for complete disintegration of the definitions. At that point the arrangements were sifted through 0.45mm film channels and legitimate weakenings were made, and the arrangement was exposed to the Spectrophotometric investigation. The medication content was determined from the direct relapse condition acquired from the adjustment information.

2.10 In-vitro Diffusion Studies:

The in-vitro dispersion reads for all definitions (F1-F6) were completed utilizing the Franz-dissemination cell. The dispersion cell device was created as an open finished round and hollow cylinder. A gauged amount of definition identical to 1gm of the medication was set onto the dialysis film 70 (Hi-Media) and was drenched somewhat in 100ml of receptor medium (phosphate support pH 6.8+ ethanol in proportion 40:60) which was persistently blended and the temperature was kept up at 37±1°C. Aliquots of 1ml were pulled back from every one of the framework at time periods, 10, 15, 30, 60, 120, 240, and 360 minutes were broke down for drug content utilizing bright spectrophotometer [15].

2.11 Stability Examines:

Stability testing of the formulation is a part of drug discovery and ends with the commercial product, to assess the drug and formulation stability, stability studies were done. The stability study was carried out for the most satisfactory formulation. The optimized gel formulation was sealed in a glass vial and kept at 40 ±2°C at 75 ± 5 % RH for 1months and analyzed. At the one month ending the samples were analyzed for the physicochemical analysis, pH, viscosity, Phytochemical constituents [15].

III. RESULTS AND DISCUSSION

Preformulation Study:

LOD:

Loss on drying Loss on drying represents the amount of moisture present in plant. Air dried part is used for extraction process as moisture interfers in analysis and extraction. So moisture had to be removed before extraction procedure. The percentage loss on drying of the plants were Lemon Grass leaves Powder 0.4 % respectively.

Table.3 LOD of Lemmon Grass leaves Powder

Drug	**LOD**
Lemmon Grass leaves Powder	**2-3%**

MC:

Table.4 % Moisture Content

Lemongrass Powder	**Moisture content (%)**
Control (Lemmon Grass leaves Powder)	5.54 ± 3.08a
Oven dried	4.0± 1.03b

Calibration Curve of Lemongrass Extract:

A calibration curve was prepared in phosphate buffer pH 6.8 in the range of 5-30µg/ml by UV-Visible spectrophotometer. The abs. of these solutions was measured at 446nm. This procedure was performed in triplicate to validate the calibration curve. The data is given in Table.5.

Table.5 Data of concentrations and absorbance in phosphate buffer pH 6.8

S.No.	**Conc. (µg/ml)**	**Abs. at 446nm* (Mean ±SD)**
1	0	0
2	5	0.156
3	10	0.298
4	15	0.357
5	20	0.601
6	25	0.741
7	30	0.825

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A calibration curve was constructed by plotting absorbance vs. conc. in µg/ml as shown in Figu.5 & a regression equation was found to be Y = 0.168 with regression coefficient 0.968.

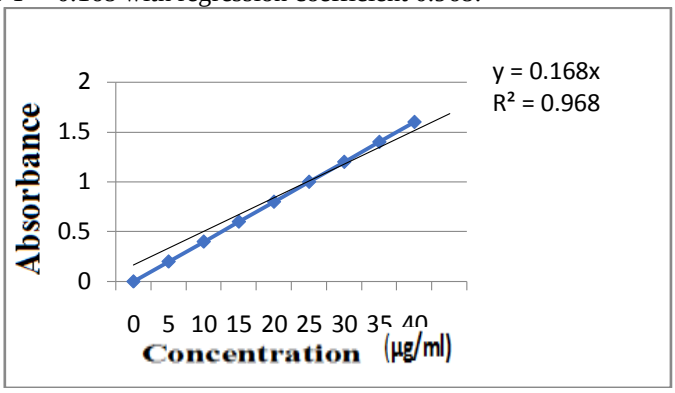

Fig.5 Calibration curve of Lemongrass Extract in phosphate buffer pH 6.8

FTIR Spectroscopy Study:

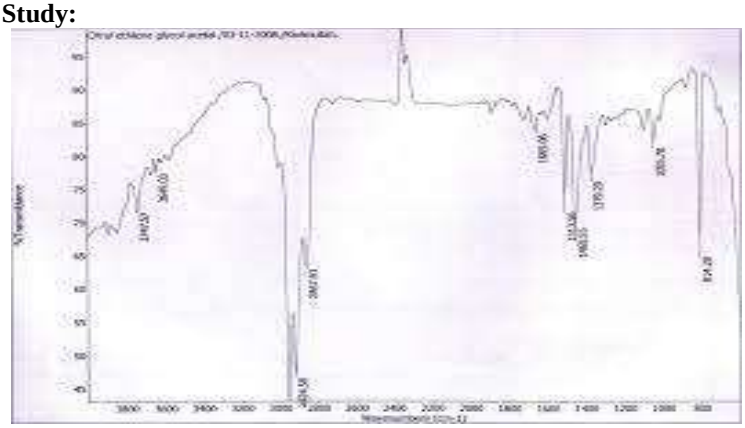

Fig.6 FTIR of Pure Lemongrass Extract

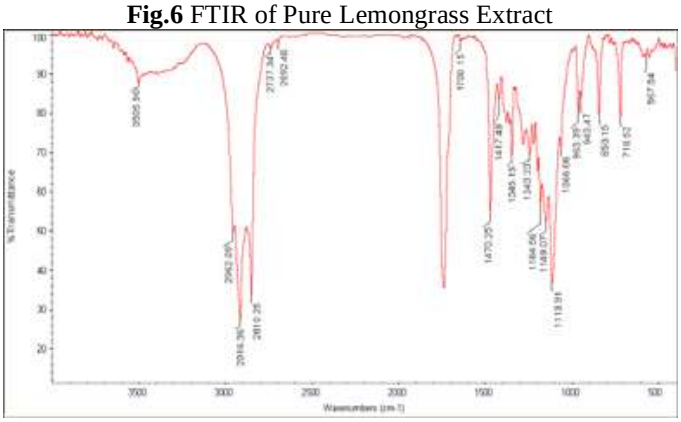

Fig.7 FTIR of Stearic Acid

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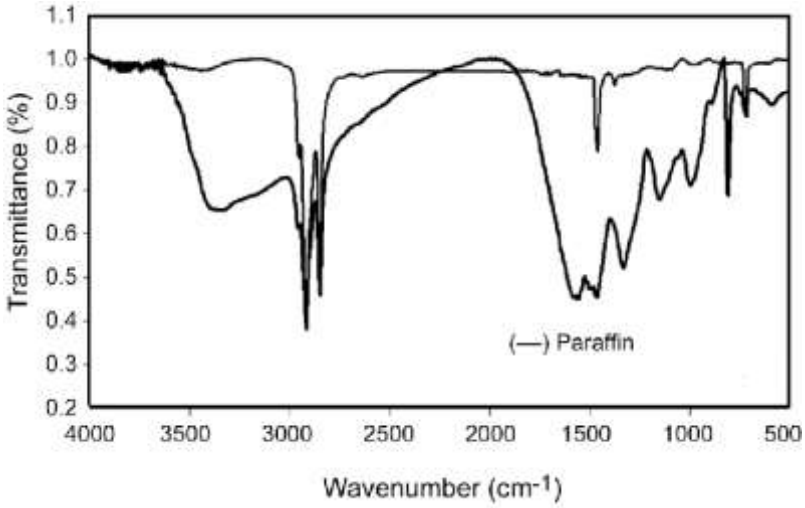

Fig.8 FTIR spectra of Liquid Paraffin

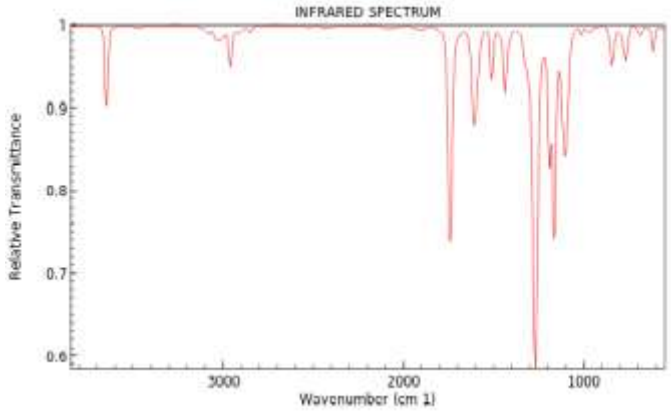

Fig.8 FTIR spectra of Methyl Paraben

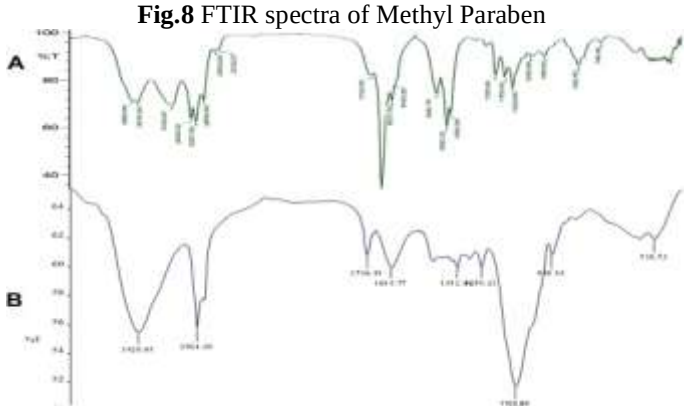

Fig.9 FTIR Spectra of Lemongrass Extract **and** Stearic Acid

Table.6 Characteristic bands between Drug and physical mixture

Functional Groups	**Reported frequency cm^{-1}**	**Observed Frequencies cm^{-1}**
N=N stretching	1725.58	1725.58
-C=N-N stretching	1644.52	1644.52
Aromatic –C-H stretching	2856.98	2856.98

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Aliphatic –C-H stretching	3124.35	3124.35
-C-N stretching	928.45	928.45
NH2 stretching	3270-3043	3113.34
C-H stretching	3032-2980	3267.56
R-O-CH3 stretching	3645-3590	3589.23
O-H stretching	3400-2400	2400

Phytochemical Screening:
The Phytochemical analysis extracts results are shown in Table.7.

Table.7 Photochemical Screening

S.NO	**TESTS**	**Results**
I	**Tests for alkaloids**	
1	Mayers test: potassium mercuric iodide solution	+
2	Hagers test: picric acid	+
3	Dragendroffs test: potassium bismuth iodide solution	+
4	Wagners test: potassium iodide solution	+
II	**Test for proteins**	
1	Millons test:	-
2	Biuret test: 4% NaOH + 1%$CuSO_4$	+
II	**Test for amino acids**	
1	Ninhydrin test:	-
IV	**Test for steroids**	-
1	Salkowski test: 2ml of chloroform +2ml of conc. H_2SO_4	+
V	**Test for terpenoids**	
1	Liebermann-buchard test: 2ml of acetic anhydride+2ml of Chloroform +1ml conc.H_2SO_4.	-
VI	**Test for Phenolic compounds:**	
1	Ferric chloride test :2ml distilled water+5%$FeCl_2$	+
2	Lead acetate test:2ml distilled water+3ml of 10% lead Acetate	-
3	Bromine water test: 2ml of distilled water+1ml of bromine water	-

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4	Iodine test: 1ml of iodine Solution	-
VII	**Test for oils:**	
1	Spot test:	+
VIII	**Test for fats:**	+

Note:- (+) Presence and (-) Absence

MIF Determination:

The MIC estimation of the product extract is expected to decide the measurements in the recipe. This in vitro portion was then duplicated 2-4 times to acquire an in vivo portion or the test portion utilized in the equation. MIC results can be seen in beneath. In light of the information in the table, it was realized that the MIC estimation of Cymbopogon Citratus extracts against S. epidermidis and Staphylococcus Aureus was lower than against P. acnes. This indicated that the extract was more viable work as anti-acne against S. epidermidis and Staphylococcus Aureus. The MIC estimation of S. epidermidis was 5-10 % w/v, though against P. acnes was 20-40 % w.

Table.8 MIC study

S. No.	**Bacterial Growth**			
	Conc. % w/v	**P. acnes**	**S. epidermidis**	**Staphylococcus Aureus**
1.	40	-	-	+
2.	20	-	-	-
3.	10	+	-	-
4.	5	+	+	-
5.	2.5	+	+	+

Evaluation of Anti-acne Cream:

Table.9 Evaluation of Anti-acne Cream

Days	**Formulation Code**	**Ph**	**Viscosity** (cps)	**Spreadability (g/second)**	**Colour**	**Odor**	**Consistency**
1	F1	4.8±0.04	39010	7.8	White	Pleasant	Semi-Solid
7	F2	5.0±0.01	38099	8.0	-	-	-
14	F3	6.2±0.02	38100	8.4	-	-	-
21	F4	6.6±0.04	39125	8.6	-	-	-
28	F5	6.9±0.02	38095	9.0	-	-	-
35	F6	7.0±0.05	37076	9.5	-	-	-

Drug Content and In-vitro Release Studies:

We need to discover the % drug content and % total arrival of all detailing F1 to F6, the medication content information found between 94.4 to 98.5% aggregate delivery b/w 94% to 99%.

Table.10 Drug content and In-Vitro release

S. No.	**Formulation Code**	**% Drug Content (M/M)**	**% Cumulative release**
1	F1	94.4	94
2	F2	95.6	94
3	F3	96.3	95
4	F4	97.7	96
5	F5	98.5	99

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6	F6	97.1	98

Antibacterial Activity of all Formulations:

The antibacterial activity studies were performed by well diffusion method by measuring the zone of inhibition (in mm). The study results of the poly-herbal gel showed antibacterial activity in a dose-dependent manner against the bacteria causing acne. The antibacterial activity study of the formulation is shown in Fig. From the antibacterial activity of all formulation, the formulated poly-herbal Cream F5 shows better antibacterial activity with the zone of inhibition 36mm for S. Epidermidis, Staphylococcus aureus, P. Acnes and 44mm for Staphylococcus epidermis. In addition to this, the poly-herbal cream showed a synergistic effect as compared to individual extract which can be useful for the treatment of local inflammation. Thus this topical formulation was suitable for the treatment of local anti-acne application and selecting for further tests.

Table.11 Antibacterial Activity of Formulations

Formulation	**Zone of inhibition (mm) mean ± SD**		
Inhibitory diameter (mm)	**S. Epidermidis**	**P. Acnes**	**Staphylococcus aureus**
F1	17±1.8	20.7±1.2	38.9±0.5
F2	17.4±0.4	20.4±1.01	39.8±0.7
F3	18.2±0.3	18.7±1.09	40.01±0.6
F4	18.6±1.9	21.4±1.3	42.6±0.10
F5	19.8±1.12	23.1±0.9	42.9±0.3
F6	21.8±1.14	24.6±0.7	43.0±0.6

Stability Studies:

Table.12 Stability Study for best formulation F5

S.No.	**Parameter**	**F5**				
		Initial	**15day**	**30days**	**60days**	**90days**
1	**pH**	6.9	6.7	6.7	6.6	6.5
2	**Viscosity**	38095	38090	38087	38075	37093
3	**Spreadability**	9.0	9.0	8.8	8.5	7.0
4	**Colour**	White	-	-	-	-
5	**% Cumulative release**	99	99	98.5	98	97

The duration of Stability studies of the formulation 5, there is no change in colour, but Variation of a pH, Viscosity, Spreadability and % Cumulative drug Release.

IV. CONCLUSION:

It is a very good attempt to formulate and evaluate the Polyherbal anti-acne cream along with the stability studies. Based on this study, poly-herbal anti-acne cream prepared from the extract of Lemon Grass Extract, Stearic Acid, Borax, Liquid Paraffin, Cetyl Alcohol, Glycerin & Methyl Paraben. Formulation F5 Prepared from various ratio of the extract of Lemon Grass Extract (20ml), Stearic Acid (1.7gm), Borax (0.12gm), Liquid Paraffin (6ml), Cetyl Alcohol (14ml), Glycerin (2ml) and Methyl Paraben (2mg) showed significant good antibacterial activity on P. Acnes, Staphylococcus aureus and staphylococcus epidermis with no irritation. The polyherbal cream showed a synergistic effect as compared to individual extract with good stability. Thus the study result concludes that the formulated poly-herbal gel with extract of Lemon Grass Extract, Stearic Acid, Borax, Liquid Paraffin, Cetyl

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Alcohol, Glycerin and Methyl Paraben respectively can be used for the treatment of acne. the formulated poly-herbal Cream F5 shows better antibacterial activity with the zone of inhibition 36 mm for S. Epidermidis, Staphylococcus aureus, P. Acnes and 44 mm for Staphylococcus epidermis. In addition to this, the poly-herbal cream showed a synergistic effect as compared to individual extract which can be useful for the treatment of local inflammation. Thus this topical formulation was suitable for the treatment of local anti-acne application and selecting for further tests. The duration of Stability studies of the formulation 5, there is no change in colour, but Variation of a pH, Viscosity, Spreadability and % Cumulative drug Release.

REFERENCES

[1]. Adityan, Balaji, Rashmi Kumari, and Devinder Mohan Thappa. "Scoring systems in acne vulgaris." Indian Journal of Dermatology, Venereology, and Leprology 75, no. 3 (2009): 323.

[2]. Benner, Nicholas, and Dawn Sammons. "Overview of the treatment of acne vulgaris." Osteopathic Family Physician 5, no. 5 (2013): 185-190.

[3]. James, Kirk A., Craig N. Burkhart, and Dean S. Morrell. "Emerging drugs for acne." Expert opinion on emerging drugs 14, no. 4 (2009): 649-659.

[4]. James, William D. "Acne." New England Journal of Medicine 352, no. 14 (2005): 1463-1472.

[5]. Arndt, Kenneth A., and Jeffrey TS Hsu. Manual of dermatologic therapeutics. Lippincott Williams & Wilkins, 2007.

[6]. Laurence, Anderson. "Looking Good, the Australian Guide to Skin Care." (2006).

[7]. Thiboutot, Diane M., and John S. Strauss. "Diseases of the sebaceous glands." Burns, Tony; Breathnach, Stephen; Cox, Neil; Griffiths, Christopher. Fitzpatrick's dermatology in general medicine (6th ed.). New York: McGraw-Hill (2003): 67287.

[8]. Goodman, Greg. "Acne and acne scarring: the case for active and early intervention." Australian family physician 35, no. 7 (2006): 503.

[9]. Purvis, Diana, Elizabeth Robinson, Sally Merry, and Peter Watson. "Acne, anxiety, depression and suicide in teenagers: A cross-sectional survey of New Zealand secondary school students." Journal of paediatrics and child health 42, no. 12 (2006): 793-796.

[10]. Picardi, Angelo, Eva Mazzotti, and Paolo Pasquini. "Prevalence and correlates of suicidal ideation among patients with skin disease." Journal of the American Academy of Dermatology 54, no. 3 (2006): 420-426.

[11]. Nambudripad, Devi S. Freedom from Eczema. Delta Publishing, 2008.

[12]. Simos, Dimitrios S., Alexandros Kokkinos, Nikolaos Tentolouris, Charilaos Dimosthenopoulos, Emily Mantzou, Artemios Artemiadis, Flora Bacopoulou et al. "Pythagorean self-awareness intervention: A novel cognitive stress management technique for body weight control." European journal of clinical investigation 49, no. 10 (2019): e13164.

[13]. Tilles, Gérard. "Acne pathogenesis: history of concepts." Dermatology 229, no. 1 (2014): 1-46.

[14]. Dasgupta, Trisha, and Joya Sahu. "Origins of the KOH technique." Clinics in dermatology 30, no. 2 (2012): 238-242.

[15]. Brumberg, Joan Jacobs. The body project: An intimate history of American girls. Vintage, 1998.