Research On - Cosmeceutical Composition and Method for Polyherbal Multi- Active Facewash Gel for Acne Treatment

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Abstract— People today are very concerned about their skin and hygiene. Because facial skin is so delicate, using regular soaps can be harsh on it. Additionally, using a lot of cosmetics can make facial skin even more sensitive, which can lead to moisture loss, oil gland blockage, and acne. 60 to 70 percent of the overall population has acne. Face wash is a safer alternative to other harsh soaps that can dry out the skin on your face and make it seem dull. Anti-acne facewash may contain ingredients that prevent acne from developing. Most individuals steer clear of chemicals while treating acne since they can occasionally lead to a variety of skin issues, such as skin inflammation or skin redness. Many herbal plants have antibacterial, antiaging, and antioxidant properties that are also mentioned in the Vedas. In present study the extracts of Aegle Marmelos, Cinnamomum verum, Miyristica fragrans, Camellia Sinensis, and Aloe barbadensis are used to prepare facewash gel. The manufactured herbal Anti acne facewash gel was submitted to physical characterization such as colour, appearance, pH, viscosity, and spreadability. It was also investigated for stability investigations. The evaluation research demonstrates that formulation batch F2 meets all of the characteristics of Ideal Facewash gel.

Index Terms- Acne , Agle marmelos. Cinnamomum verum, Extract, Antibacterial activity.

I. INTRODUCTION

Skin-

The skin, which participates in hydro-electrolytic balance by limiting water loss & is largest organ of the body , accounting for about 15% of the total adult body weight. It is general protective barrier against external aggression. It performs many vital functions, including protection against external physical, chemical, and biologic assailants , but in this protection it also includes important immunological functions.The skin is continuous ,with the epidermis & mucous membranes lining the body's surface. To keep skin healthy, clear and glossy, a balanced nutrition is required.(1)

Acne-

Common acne or acne vulgaris is the common chronic inflammatory disorder of skin of pilosebaceous units which affects various areas of body such as largest oil glands, which includes face, upper back, & chest.(2) Acne is most common disease in humans which is identified by different scally red areas of skin (seborrhoea), pinheads, black heads, White heads, nodules, & sometimes pimples. Acne may be inflammatory or non-inflammatory which includes skin structure, hair follicles, & sebaceous glands. Propionibacterium acnes and Staphylococcus epidermidis are frequent Pus-forming bacteria that cause various types of acne.(3,4)

Acne classification-

Acne can be characterised as comedonal, popular, pustular, cystic, or nodular. Comedonal acne is non-inflammatory and is classified as whiteheads or blackheads. White heads (closed comedo) seem as fresh or white-colored elevated lumps, but blackheads (open comedo) appear as open pores harbouring dark-colored skin roughage made up of melanin, sebum, and follicular cells. Papules are red, firm, raised lesions that are usually smaller than 5mm in diameter. Pustules are skin elevations with purulent material that are circumscribed. Cysts and nodules are raised, solid lesions that include deeper dermis and subcutaneous tissue. Cysts have a diameter of less than 5 mm, whereas nodules have a diameter more than 5 mm.(4,5)

Acne formation mechanism-

Acne is caused by three pathogenic factors: seborrhoea, sebum retention, and inflammation. Generally acne are related to more sensitivity of sebaceous end-organs to the androgen. Many factors are responsible for acne such as sebum retention & chemical composition of the sebum. Androgen are also responsible for hyper keratinisation.[6]

Causes of acne-

Acne can be caused by a variety of problems in the pilosebaceous gland. There are certain problems. [7,8,9]

1. Hyperkeratinization
2. Hormonal changes
3. Sebum Secretion
4. Genetical factors
5. Dietary factors
6. By plugging oil ducts
7. Hygine
8. Menstrual cycles in females
9. Mental health
10. Climate change
11. Oily makeups
12. Pimple squeezing
13. Greasiness of hairs.

Face wash-

Face wash are products that are used to the face to cleanse it without drying it out. "Cleanser" is another name for face wash. Face washes are generally helpful for all skin types. They assist to eliminate debris and excess oil from the skin while also supplying hydration to dry skin. They maintain the skin clean and germ-free, as well as freshening it, so that it appears healthy and young..[10]

Different face wash has different effect such as, Anti-wrinkle, Anti- acne, Moisturising, & Brightening effect.[10]

Types of face wash[10]

1. For oily/greasy skin
2. For dry skin
3. Normal skin

Forms of facewash [10]

1. Cream based facewash
2. Liquid based
3. Gel base facewash
4. Facewash in form of powder [11]

Feature of face wash [11]

1. Removes the dead cells of skin
2. Rejuvenating the skin cells elevate stress
3. Removes oil, dirt, and impurities from skin.
4. Reduces microbial flora.
5. Leave the skin fresh and breathing of skin.

II. MATERIAL AND METHODS

Materials -

Table No. 1: List of chemicals used

S. No	CHEMICALS	NAME OF THE COMPANY
1	Ethanol	Changshu Hongsheng Fine Chemicals Co. Ltd
2	Carbopol 940	Vishal Chem Pvt Ltd
3	Propylene glycol	Pallav Chemicals Pvt Ltd
4	Sodium LaurylSulphate	Pallav Chemicals
5	Amaranth Solution	Vishal Chem
6	Methyl paraben	Oxford Laboratory Pvt Ltd
7	Propyl paraben	Vishal Chem Pvt Ltd
8	Triethanolamine	Pure Chem Laboratories Pvt Ltd
9	Rose Oil	Pallav Chemicals

Table No. 2: List of Instruments used

S. No	INSTRUMENTS	MAKE
1	Digital Weighing Balance	Shimadzu corporation
2	Heating Mental	Sunbim
3	Digital pH meter	Hanna Instruments

4	Muffle Furnace	Lithey Inc-Laboratory
5	Brookfield viscometer	Brookfield DV-II + Pro
6	UV Visible Double Beam Spectrophotometer	Jasco V- 730
7	Hot air oven	Meta Lab Scientifi Industries
8	FourierTransform Infrared Spectroscopy	Jasco FTIR – 4600

Methods-

Collection and Authentication of the Plant

Crude drugs like Cinnamon, Nutmeg & Green teawere collected from local market in Malegaon and *Aloe barbadensis* leaves were collected from medicinal garden of MalegaonCamp . It was identified as *Aegle Marmelos, Cinnamomum verum, Miyristica fragrans, Camellia sinensis* and *Aloe barbadensis* and a specimen was authenticated by Mahatma Gandhi Vidyamandir's Research Center in Botany MSG Art's Science & commerce College, Malegaon Camp, Nashik.

Experimental Method –Physicochemical Study

The powder drug was used for the evaluation of its physicochemical parameters such as moisture content, ash value.

Moisture content[12]

Water content is the amount of water in a substance, such as soil (also known as soil moisture), rock, pottery, crops, or wood. Water content is utilised in a variety of scientific and technological fields and is stated as a ratio ranging from 0 (totally dry) to the porosity of the material during saturation. It can be provided in either volumetric or mass (gravimetric) form.

Ash values –

Total ash[13-15]

Approximately 2g of the pulverised material was precisely weighed in a previously lit and tarred crucible (often silica). Gradually increase the heat to 500-600°C and spread the material in each layer. The lack of carbon is indicated by the colour white. Desiccators were used to cool the items before they were weighed.

Acid Insoluble Ash:

The remnants from boiling the entire mixture dilate hydrochloric acid and burn the remaining insoluble particles. This determines the quantity of silica present, particularly in sand and siliceous soil.

Water Soluble Ash:

It is the difference between in weight between the total ash and residue after treatment of the total ash with water.

Sulphated Ash:

Heated a silica or platinum crucible to redness for 10 minutes and let it to cool in a desiccator before transferring 1g of the substance being investigated to the crucible and properly weighing the crucible and the end. Ignite the stuff gradually at first, until it is totally scorched. Coil the leftovers and wet it 1ml sulfuric acid, carefully heat until the white sire is no longer evolved, then burn at 800°C until all black particles have gone Ignition was carried out in an area free of air currents. Allow the crucible to cool before adding a few drops of sulphuric acid and heating to ignite as previously.

Extraction of Plant Materials [16]

Maceration Method-

The crude plant material such as bael leaf, cinnamon, nutmeg,& GreenTea. were collected, Leaves of bael are dried well & grinded into fine powder. Similarly cinnamon , nutmeg & green tea were grinded in to powder form. After grinding the required plant material is weighed & macerated with liquid (water or alcohol etc.) for 3 days. After maceration the material is filtered with the help of Watt man filter paper & filtrate is then evaporated to halfof its quantity cooled & used.

III. EVALUATION OF THE EXTRACT

7.2.6.1 Characteristics of extracts [17]

The ethanolic extracts of the *Aegle marmelos, Cinnamomum verum, Miyristica fragrans, Camellia sinensis* were evaluated for its physical state, colour, odour, and taste.

Phytochemical investigation of the extract [18-22]

The active components found in the alcoholic extracts of *Aegle marmelos, Cinnamomum verum, Miyristica fragrans,* and *Camellia sinensis* were identified using preliminary qualitative phytochemical analysis. To test for the presence of different ingredients in *Aegle marmelos, Cinnamomum verum, Miyristica fragrans,* and *Camellia sinensis,* the following techniques were used.

Test for Tannins & Phlobatannins

Make a 5% ferric chloride solution in distilled water. In 100 l of sample, 0.5ml of this solution was added. Tannins were indicated by dull green or dark blue coloration.

Test for Alkaloid (Wagner's test)

In 1 mL of extract, add 1 mL of Wagner's reagent (iodine in potassium iodine). The presence of alkaloids is indicated by the precipitate's reddish brown coloration.

Test for Saponin

(500 µl) of sample was combined with 7 ml of distilled water. The presence of saponin is indicated by the production of foam.

Test of Glycosides

A brown ring was produced at the interface after dissolving 2ml of test solution in 4ml of glacial acetic acid containing one drop of 5% ferric chloride solution and underlaying with 1 ml of concentrated H_2SO_4.

Test of Terpenoids (Chloroform test)

2 mL of chloroform was combined with 2 mL of each of the test solutions. 2 mL of concentrated H_2SO_4 was added to this mixture and cooked in a water bath (65°C). Terpenoids were present because a reddish brown tint appeared at the contact.

Test of Flavonoids

Flavonoids were evaluated by adding ten percent lead acetate solution to one millilitre of each extract. The presence of flavonoids was shown by the formation of a yellow precipitate.

Test of Anthocyanin's

A combination of HCl (2M, 1mL) and ammonia (4M, 1mL) in the amount of 2 mL was added to 1mL of test solution. The presence of anthocyanins is shown by the colour change from pink-red to blue-violet.

Thin Layer Chromatography [23-25]

The separation takes place on a neutral platform like transparent plastic, glass, or metallic foil which was previously treated with a tiny amount of a material that adsorbs water, usually a silicon dioxide, aluminium oxide (alumina), or cellulosic gel. The stationary phase symbolises this adsorbent layer. Capillary action is the process that pushes a solvent or solvent combination (also referred to the phase of mobility) up the plate after the sample is applied to the plate. Separation occurs when analytes climb at different rates on the TLC plate. This may be performed on a laboratory scale to monitor the course of a reaction or on a preparative scale to purify trace quantities of an intermediate. TLC is a common analytical technique due to its ease of application, inexpensive price, superior sensitivity, and quick separation. TLC, such as all chromatography, operates on a similar concept: the binding capacity of a chemical for both stationary and mobile stages impacts the speed at which it moves. TLC's objective is to produce well-defined, well-separated commercials.

UV Callibration of Extracts [26]

Extracts of *Aegle marmelos, Cinnamomum verum, Miyristica fragrans,& Camellia sinensis* were calibrated by using UV visible Spectrophotometer . 0.1 mg of sample were diluted with ethanol & 100mlof stock solution were prepared & 5 dilutions were prepared as 5ppm, 10ppm, 15ppm,20ppm, 25ppm & 30ppm. Abrosbance were measured at specific nm.

Preparation of standard curve of *Aegle Marmelos, Cinnamomum verum, Miyristica fragrans,&Camellia Sinensis* –

Preparation of standard solution A stock standard solution of *Aegle marmelos, Cinnamomum verum, Miyristica fragrans,& Camellia sinensis* (1mg/ml) was prepared by dissolving *Aegle Marmelos, Cinnamomum verum, Miyristica fragrans,& Camellia Sinensis* in 50% ethanol separately.. Various dilutions ranging from 5 – 30 ppm were prepared from the stock solution and standard curve obtained at λmax 285 nm, 273nm,273nm,& 232nm respectively.

Sample Preparation for UV spectrophotometry
The herbal extract (1mg/ml) was prepared by dissolving in 50 % ethanol. It was then further diluted to obtain absorbance within the standard curve rang.
Selection of the detection wavelength

The absorption spectra of the standard and sample solution were scanned between 200-800 nm. All hada maximum absorption λmax at 415 nm which was then chosen as the wavelength for detection.

Fourier transform infrared spectroscopy [27]
The spectroscopic technique has become an analytical and most powerful tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. The Fourier Transform Infrared Spectroscopy (FTIR) was used to identify functional groups of the chemical constituents present in plant extracts based on the peak values in the IR region.

Antimicrobial activity of the extract [28-31]
The following Standard cultures of Microbial Type Culture Collection (MTCC) strains wereused in the study

1. *Staphylococcus aureus*
2. *S Epidermidis* (MTCC- 2639)
3. *Propioni bacterium* (MTCC- 1951)

Antimicrobial activity by Agar Well Diffusion method[32-33]
The bactericidal activity of various solvent extracts were tested using the agar well diffusion technique. In the centre of a sterile Petri plate, one millilitre of new bacterial or fungal culture was pipetted. Molten cooled Muller Hinton agar (MHA) for bacteria strains or Potato dextrose agar (PDA) for fungus was then poured and thoroughly mixed into the Petri plate holding the inoculum. Following solidification, wells were drilled into agar plates containing inoculums with a sterile cork borer (6 mm in diameter). Then, in each well, 100 l of each extract (20% w/v) was added. The extract concentration (20% w/v) was chosen based on our preliminary studies and existing literature. The plates were chilled for 30 minutes to allow the extracts to fully diffuse into the agar. The dishes were then left to incubate for 18 hours at 37°C. The zone of inhibition (including the diameter of the wells) that developed after the incubation time was used to detect antimicrobial activity. A 10% concentration of DMSO was used as a negative control.

Formulation and Optimization Of Gelling Agent
Carbopol is a polymer that dissolves in water that works as a strong gelling thickening in the production of transparent gels. Different concentrations of Carbopol 940, such as 1%, 1.5%, and 2%, were attempted and optimised to produce the necessary gel consistency and spreadability.

Table No. 3: Formulation of Carbopol gel

INGREDIENTS	G1	G2	G3
Carbopol 940	1%	1.5%	2%
Propylene glycol	5ml	5ml	5ml
Methyl paraben	0.15gm	0.15gm	0.15gm
Propyl Paraben	0.30gm	0.30gm	0.30gm
Triethanolamine	5ml	5ml	5ml
Water	q. s	q. s	q. s

Formulation of herbal Anti acne Facewash gel [34,35]

Table No. 4: Formulation of herbal Anti acne Facewash gel

Ingredients	F1	F2	F3	F4
Bael Extract	1 %	1.5 %	2%	2.5%
Cinnamon Extract	1 %	1.5 %	2 %	2.5 %
Nutmeg Extract	1 %	1.5 %	1.5 %	1.5 %
Green Tea Extract	1 %	1.5 %	1 %	0.5 %
Aloe barbadensis gel	5ml	5ml	5ml	5ml
Carbopol	2%	2%	2%	2%
Propylene glycol	5ml	5ml	5ml	5ml
Methyl Paraben	0.15gm	0.15gm	0.15gm	0.15gm
Propyl Paraben	0.05gm	0.05gm	0.05gm	0.05gm
Triethanolamine	0.30gm	0.30gm	0.30gm	0.30gm
Sodium Lauryl Sulphate	1gm	1gm	1gm	1gm

Rose Oil	25µl	25µl	25µl	25µl
Amaranth Dye	1 ml	1 ml	1 ml	ml

Evaluation of Herbal Anti Acne Facewash Gel

The prepared herbal Anti acne facewash gel were subjected to physical characterization such as color, appearance, pH, viscosity, spreadability. It was also evaluated for its stability property, antimicrobial activity and skin irritation study.

Physical appearance [36]

The prepared herbal gel was optically evaluated for shade, odour, uniformity and texture. After setting the gels in the proper vessel, all produced gels were visually inspected for uniformity. They were examined for their texture and presence of aggregates.

Measurement of pH [37]

Using a digital pH meter, the pH of several formulations was determined. For two hours, one gramme of gel was dissolved in 100ml of distilled water. The pH of each mixture was measured in triplicate.

Determination of Viscosity [37]

The viscosity of produced gels was measured using a Brookfield viscometer (Brookfield viscometer) with spindle No. 62.

Spreadability [38-39]

The ability to spread refers to how far the gel spreads after being applied to the skin or afflicted region. We took a pair of standard-sized slides made of glass.. One of the slides was covered with the gel formulation. The other slides were put on top of the gel, with the gel sandwiched between the two slides at a spacing of 6.0 cm along the slide.A 100gm weight was put on the upper slides, pressing the gel between the two slides to produce a thin layer. The weight was removed, and the excess gel on the slides was removed by scraping away. The two slides in place were attached to a stand without the slightest movement and in such a way that only the top slide could easily fall off because of the weight attached to it. A 20gm weight was securely connected to the upper slide. The time required for the upper slide to travel 6.0 cm and separate from the lower slide under the influence of the weight was recorded. The experiment was done three times, and the mean time was calculated each time.

Consistency:

The consistency was verified by applying to the skin.

Washability:

Formulations were applied to the skin, and then the ease and extent of washing with water werepersonally assessed.

Homogeneity

All developed gels were tested for homogeneity by visual inspection after allowing them tocongeal in a container. They were examined for the appearance of aggregates and the existence of any aggregates.

Stability Analysis [38-39]

The gel formulation's stability was investigated under various storage conditions (80°C and 400°C). At 7, 15, and 30 days, samples were extracted and examined for physical properties such as appearance, homogeneity, pH, viscosity, and spreadability.

Skin irritancy test:

After gaining agreement from all subjects, this test was done on ten human volunteers in good health of either sex. 0.5 gms of facewash gel was dabbed to a 6 cm^2 area of skin on the hand, which followed by covering with a gauze patch. A semi-occlusive dressing was used to keep the patch in contact with the skin for an hour. After 1 hour of exposure, the gauze was removed and the leftover test material was washed with tap water without affecting the current reaction or epidermal integrity. After the gauze was removed, the skin was examined at 1 hour, 3 hours, 6 hours & 12 hours for any obvious reaction on the skin.[78]

IV. RESULTS AND DISCUSSION

Ash Value –

Table No. 5- Ash value and Moisture content

Ash value	Bael leaf	Cinnamon	Nutmeg	Green tea
Total ash value	17% w/w	5.5% w/w	2.5% w/w	8.5% w/w

Acid insoluble ash	4.5% w/w	1% w/w	0.5% w/w	2.1% w/w
Water soluble ash	12.5 % w/w	4.5% w/w	2% w/w	3.2% w/w
Sulphated ash	14.5 % w/w	6% w/w	3% w/w	7.6% w/w
Moisture content	10%	6%	4%	8%

Evaluation of *Aegle marmelos, Cinnamomum verum, Miyristica fragrans* and *Camelliasinensis* extracts .
Characteristics of extracts

The physical state, color, odor, taste of the ethanolic extract of *Aegle marmelos, Cinnamomum verum, Miyristica fragrans, Camellia sinensis.* Were mentioned in Table No:6

Table No. 6: Characteristics of the extract

Characteristics	Observation			
	Bael leaf extract	Cinnamon extract	Nutmeg extract	Green tea extract
Physical state	Liquid	Liquid	Liquid	Liquid
Color	Green	Brown	Yellowish Brown	Green
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Characteristic	Characteristic	Characteristic	Characteristic

Phytochemical investigation of the extract
Phytochemical screening –

Table No .7: Phytochemical screening of Extracts

Test	Bael Leaf	Cinnamon	Nutmeg	Green Tea
Tannins	+	+	+	+
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Cardiac Glycosides	+	+	-	+
Steroids	+	-	-	+
Terpenoids	+	+	+	+
Flavonoids	+	+	+	+
Phlobatannins	+	-	-	-
Carbohydrates	-	-	-	-
Coumarins	-	+	-	+

(+) Indicates presence of chemical constituent (-) Indicates absence of chemical constituents

Thin Layer Chromatography -

Table No. 8 – TLC of Extracts

Sr. no	Solvent system	Detection	Rf Value
1	Bael Chloroform: methanol (7:3)	UV Light	0.71
2	Cinnamon Toluene: ethyl acetate (93: 7)	UV Light	0.58
3	Nutmeg Ethyl acetate: Methanol (4: 6)	UV Light	0.83
4	Green tea Chloroform: Methanol: water (65: 35: 1)	UV Light	0.88

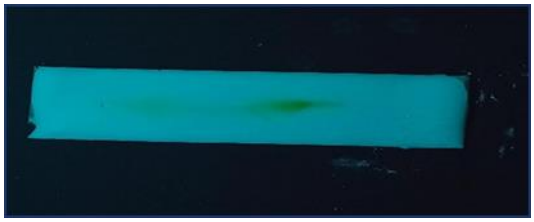


Figure No.-1 - TLC of Bael leaf

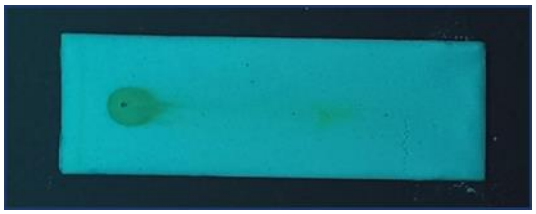


Figure No. – 2 – TLC of Cinnamon

Figure No. – 3 – TLC of Nutmeg

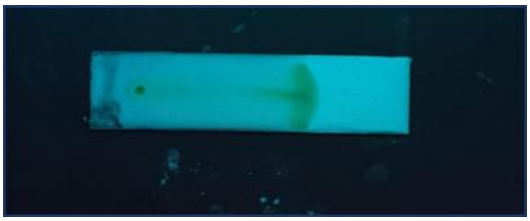


Figure No. – 4- TLC of Green tea

UV Calibration of Extracts of *Aegle marmelos, Cinnamomum verum, Miyristica fragrans, & Camellia sinensis* -

Table No. 9 – Calibration Curve of *Aegle marmelos, Cinnamomum verum, Miyristica fragrans, & Camellia sinensis* in Ethanol

Concentration (in PPM)	Absorbance(nm)			
	Aegle Marmelos	*Cinnamomum verum*	*Miyristica fragrans*	*Camellia Sinensis*
0	0	0	0	0
5	0.2114	0.2133	0.1344	0.2362
10	0.3722	0.3304	0.2341	0.4119
15	0.5249	0.4409	0.3356	0.5672
20	0.6661	0.5825	0.42203	0.7162
25	0.8017	0.7112	0.5032	0.8669

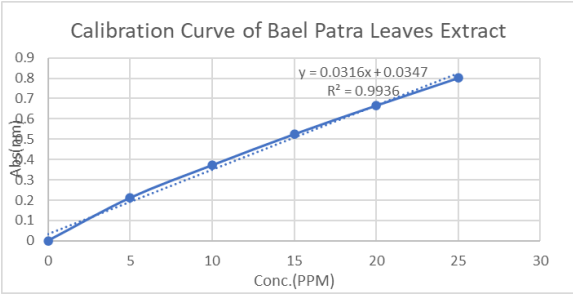


Figure no.5 - Calibration Curve of Bael Patra Leaves Extract

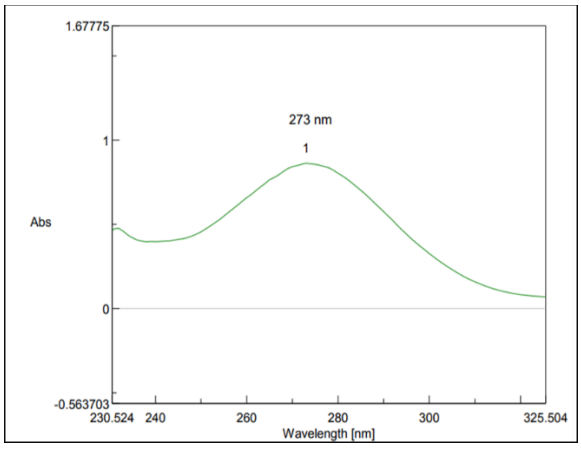


Figure No.6 - UV Absorption Spectra of Bael Leaves Extract

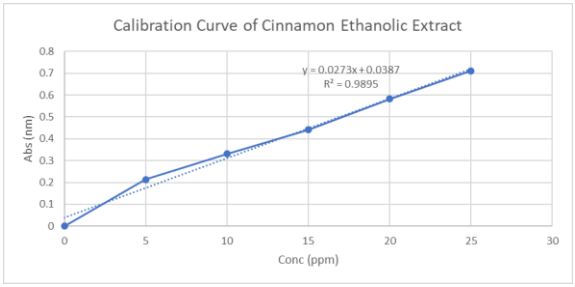


Figure no.7 - Calibration Curve of Cinnamon Extract

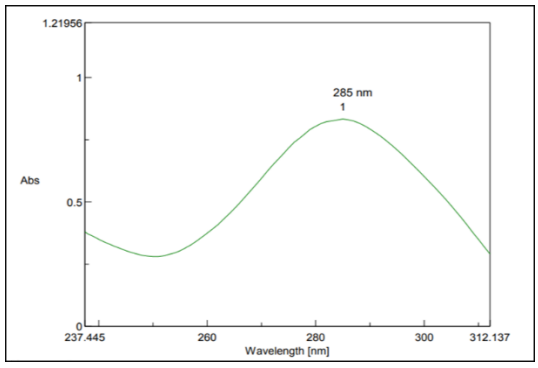


Figure No.8 - UV Absorption Spectra of Cinnamon Extract

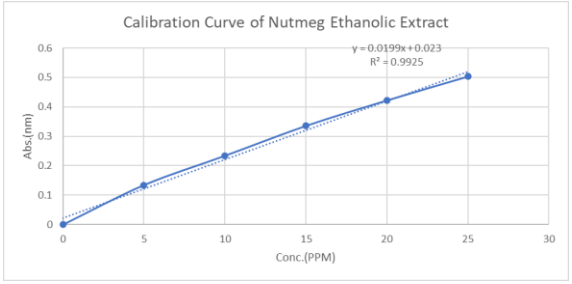


Figure no. 9- Calibration Curve of Nutmeg Extract

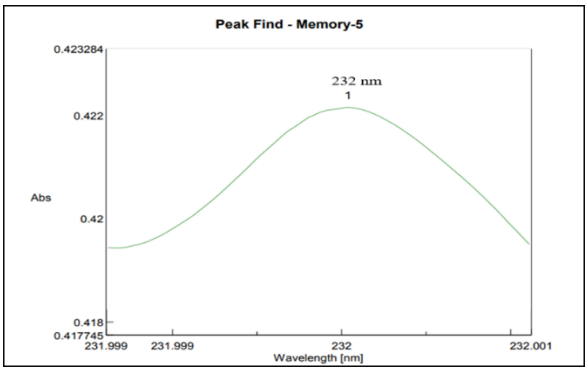


Figure No.10 - UV Absorption Spectra of Nutmeg Extract

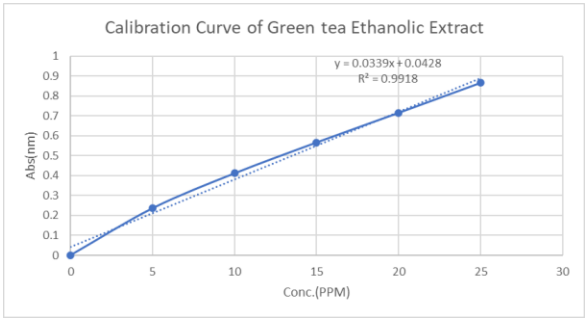


Figure no. 11- Calibration Curve of Green Tea Extract

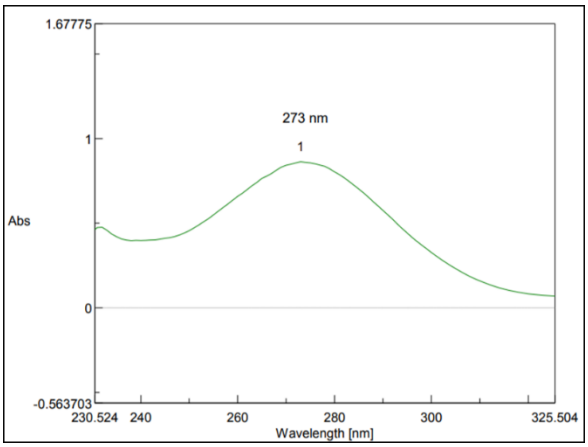


Figure No.12 - UV Absorption Spectra of Green tea Extract

Fourier Transform Infrared Spectroscopic Analysis –

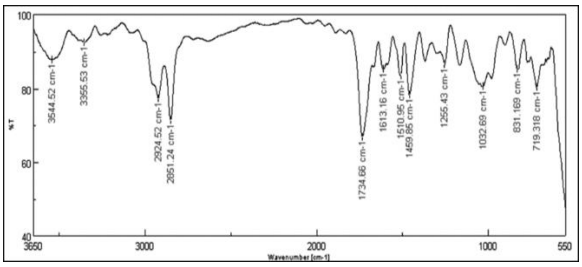


Figure No. 13 – FTIR spectra of Ethanolic Extract of *Agle marmalose*

Table No. 10 – FTIR Study *of Agle mamalose L*

Sr. No	Functional Group	Observed Value Wave numbers (cm-1)	Standard Value Wave numbers (cm-1)
1	Alkanes		
	C-H(stretch)	2924.5	3000-2850
	-CH_2 (bend)	1459.8	1375-1465
2	Alkenes		
	C-H(stretch)	2851.2	3100-3000
	C=C(stretch)	1613.1	1000-650
	C=C (bend)	719.3	1000-650
3	Aromatic rings		
	C=C (bend)	1510.9	1600 & 1475
4	Aldehydes	1734.6	1740 - 1720
5	Esters C-O (stretch)	1255.4	1300 - 1000
6	Primary Amines		
	N-H (stretch)	3355.5	3500 - 3600
	C-N (stretch)	1032.6	1350 - 1000
	N-H (stretch) N-H (bend)	3544.5 1631.1	3500 – 3600 1550 - 1690
7	Ethers C-O (stretch)	1032.6 1255.4	1300 - 1000

8	Alcohols O-H (stretch)	3355.5 3544.5	3650 - 3600
9	Ketone C=C (stretch)	1613.1	1680 - 1600
10	Nitro compounds N=O (stretch)	1510.9	1550 - 1350

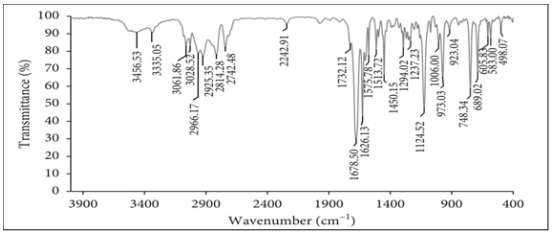


Figure No. 14 – FTIR spectra of Ethanolic Extract of *Cinnamomum verum.*

Table no.11- FTIR Study of *Cinnamomum verum.*

Sr. No	Functional Group	Observed Value	Standard Value
1	Carbonyl Group C=O	1678 & 1626	1720 – 1740
2	Alkenes C-H	973	650 – 1000
3	Esters C-O	1124	1000 – 1300
4	Alkanes -CH_2 (bend)	1294	1375 - 1465
5	Alcohols C-OH	1450	1000 – 1300
6	Aromatic rings C=C	1575	1500 - 1600
7	Carbonyl group C-H	2814	2700 - 2800

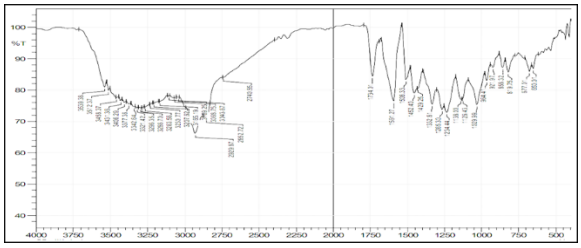


Figure No. 15 - FTIR spectra of Ethanolic Extract of *Myristica fragrans.*

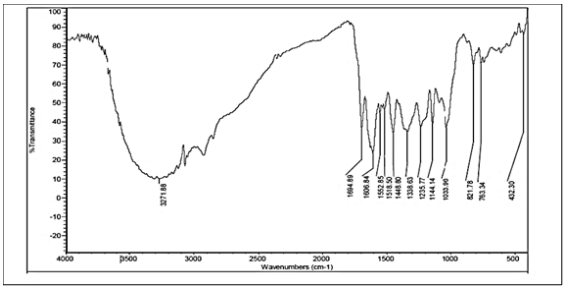


Figure No.16 - FTIR spectra of Ethanolic Extract of *Camellia sinensis*

Table No.12 - FTIR study of Ethanolic Extract of *Myristica fragrans*

Sr. No.	Functional Group	Observed Value	Standard Value
1	Alkanes (stretch)	2740.8	3000 - 2850
	-CH2 (bend)	1452.4	1465
2	Alkenes (stretch)	3068.7 3043.6	3100 - 3000
3	Aromatic Ring(stretch)	3165.1	3150 - 3050
		921.9 964.4	900 - 690
4	Esters	1734.0	1750 - 1730
5	Aldehyde	1734.0	1740 - 1720

Table No.13 - FTIR study of Ethanolic Extract of *Camellia sinensis*

Sr. no.	Functional group	Observed value	Standard value
1	Alkanes (Stretch)	3271.88	2850 - 3000
2	Imines & Oximes C=N	1694.8	1690 – 21640
3	Amines & Amides	1552.8	1550 - 1640
	N-H		
4	Alkanes $-CH_2-$	1448.8	1465
5	Aromatics	821.7 763.3	690 - 900

Anti- microbial activity of the extract

TableNo.14: Zone of inhibition of the *Aegle marmelos* extract

Aelge marmelos extract	Name of the organism											
	Staphylococcus aureus			Mean (in mm)	*Propionibacterium acne*			Mean (in mm)	*Staphylococcus Epidermidis*			Mean (in mm)
	1	2	3		1	2	3		1	2	3	
20µl/ml	13.5	13.4	13.4	13.4± 0.03	13.4	13.3	13.3	13.3± 0.03	12.7	12.6	12.6	12.6± 0.03
30µl/ml	13.5	13.6	13.4	13.5± 0.1	13.5	13.5	13.4	13.5± 0.03	12.7	12.7	12.6	12.7± 0.03
Control (Ethanol)	-	-	-	-	-	-	-	-	-	-	-	-

Table No. 15: Zone of inhibition of the *Cinnamomum verum* extract

Cinnamomum verum *extract*	Name of the organisms											
	Staphylococcus aureus			Mean (in mm)	*Propionibacterium acne*			Mean (in mm)	*Staphylococcus Epidermidis*			Mean (in mm)
	1	2	3		1	2	3		1	2	3	
20µl/ml	12.7	12.7	12.6	12.7± 0.03	13.5	13.5	13.4	13.4± 0.1	12.7	12.6	12.6	12.6± 0.03
30µl/ml	13.4	13.3	13.3	13.3± 0.03	13.5	13.5	13.4	13.5± 0.03	12.4	13.2	12.4	12.6± 0.07
Control (Ethanol)	-	-	-	-	-	-	-	-	-	-	-	-

Table No. 16: Zone of inhibition of the *Miyristica fragrans* extract

Miyristica fragrans Extract	Name of the organism											
	Staphylococcus aureus			Mean(in mm)	*Propionibacterium acne*			Mean(in mm)	*Staphylococcus Epidermidis*			Mean(in mm)
	1	2	3		1	2	3		1	2	3	
20µl/ml	13.5	13.4	13.4	13.4± 0.03	13.4	13.3	13.3	13.3± 0.03	12.7	12.6	12.6	12.6± 0.03
30µl/ml	13.5	13.6	13.4	13.5± 0.1	13.5	13.5	13.4	13.5± 0.03	12.7	12.7	12.6	12.7± 0.03
Control (Ethanol)	-	-	-	-	-	-	-	-	-	-	-	-

Table No. 17: Zone of inhibition of the *Camellia sinensis* extract

Camellia Sinensis *extract*	Name of the organisms											
	Staphylococcus aureus			Mean(in mm)	*Propionibacterium acne*			Mean(in mm)	*Staphylococcus Epidermidis*			Mean(in mm)
	1	2	3		1	2	3		1	2	3	
20µl/ml	8.2	9.3	9.4	8.9± 0.6	9.2	9.2	9.3	9.2± 0.6	9.2	9.2	10.2	9.5± 0.3
30µl/ml	9.4	10.3	10.4	10.0± 0.03	10.5	10.5	10.4	10.4± 0.6	9.4	10.3	10.4	10.0± 0.03
Control (Ethanol)	-	-	-	-	-	-	-	-	-	-	-	-

Zone of inhibition of the *Aegle Marmelos, Cinnamomum verum, Miyristica fragrans,& Camellia Sinensis* extract

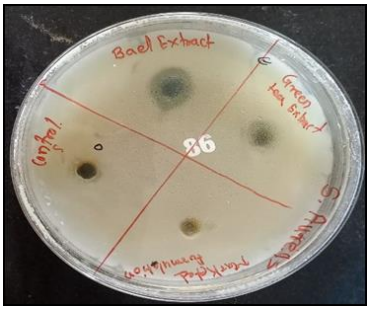


Figure No. 17 : Zone of inhibition of *Aegle Marmelos & Camellia Sinensis* extract towards *Staphylococcus aureus.*

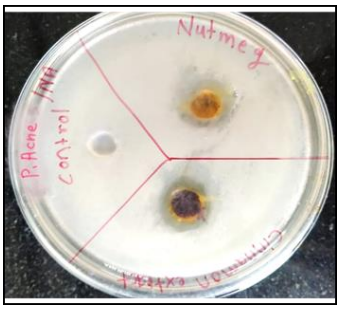


Figure No. 18 : Zone of inhibition of *Cinnamomum verum & Miyristica fragrans* extract towards *Staphylococcus aureus.*

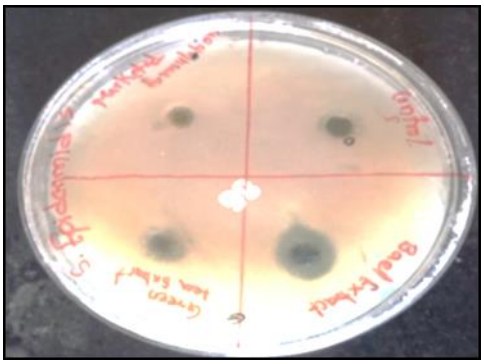


Figure No. 19: Zone of inhibition of *Aegle Marmelos & Camellia Sinensis* extract towards *Staphylococcus epidermidis*

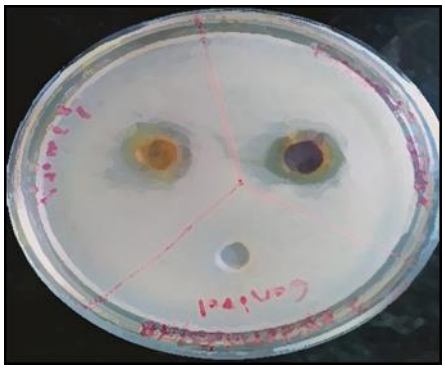


Figure No. 20: Zone of inhibition of *Cinnamomum verum & Miyristica fragrans* extract towards *Staphylococcus epidermidis.*

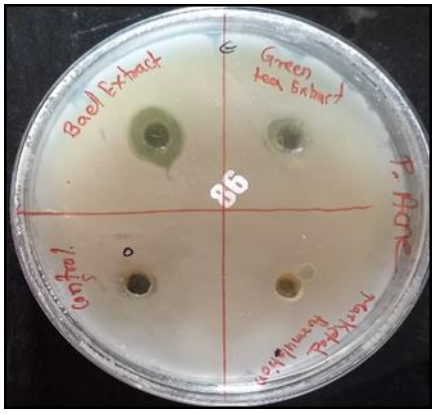


Figure No. 21: Zone of inhibition of *Aegle Marmelos & Camellia Sinensis* extract towards *P. Acne*

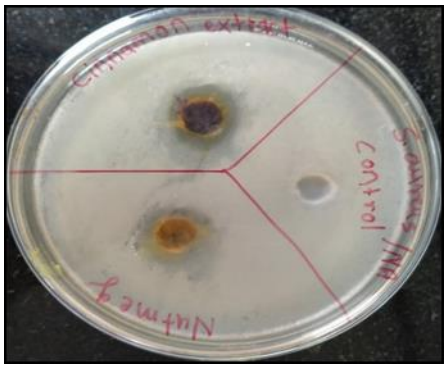


Figure No. 22 : Zone of inhibition of *Cinnamomum verum & Miyristica fragrans* extract towards *P. Acne*

OPTIMIZATION OF GELLING AGENT

Various carbopol-940 concentrations, such as 1,1.5 and 2%, were optimised to produce gel with the appropriate physical properties. Carbopol gel 2% has good physicochemical qualities for integrating ethanolic extracts of *Aegle marmelos, Cinnamomum verum, Miyristica fragrans, Camellia sinensis,* and *Aloe barbadensis.*

Formulation of herbal Anti acne Facewash gel containing *Aegle marmelos, Cinnamomum verum, Miyristica fragrans, Camellia sinensis* and *Aloe barbadensis* –

Aegle marmelos, Cinnamomum verum, Miyristica fragrans, Camellia sinensis, and Aloe barbadensis were mixed into an optimised 2% Carbopol gel basis. Carbopol gel base was infused with ethanolic extracts of Aegle Marmelos, Cinnamomum verum, Miyristica fragrans, and Camellia Sinensis at various concentrations of 1,1.5, 2%, and 2.5%. The concentration of Aloe barbadensis in all Carbopol gel bases was kept constant [5 ml]. Figures 39 and 40 depict the formed Hearbal facewash gel.

Figure No.23 : Image of Formulated of Herbal Anti acne Facewashgel containing *Aegle Marmelos, Cinnamomum verum, Miyristica fragrans , Camellia Sinensis* and *Aloe barbadensis*

Evaluation of herbal anti acne facewash gel –
Physical appearance

Table No. 18: Physical appearance of formulated gel.

	F1 [1% ethanolic Extract of herbal drugs]	F2 [1.5% ethanolic Extract of Herbal drugs]	F3 [2% ethanolic Extract of Herbal drugs]	F4 [2.5% ethanolic Extract of Herbal drugs]
Physical appearance	Transparent Reddish-Brown Gel	Transparent Reddish Brown Gel	Transparent Reddish Brown Gel	Transparent Reddish Brown Gel
Color	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown
Homogeneity	Slight aggregates	Absence of aggregates	Slight aggregates	Absence of aggregates
Washability	Easily washable	Easily washable	Easily washable	Easily washable
Greasiness	Non-Greasy	Non-Greasy	Non-Greasy	Non-Greasy

Table No. 19: Results of Evaluation tests

Formulation code	pH	Viscosity [cps]	Spreadability (gm.cm/sec)
F1	6.9	1428±0.1	19.37
F2	7.7	1425±0.75	21.35
F3	7.9	1358±0.25	22.13
F4	7.5	1422±0.25	22.22

Skin Irritancy test –
After 1 hour of exposure, the gauze was removed and the facewash is washed with tap water without affecting the current reaction or epidermal integrity. After the gauze was removed, the skin was examined at 1 hour, 3 hours, 6 hours & 12 it Shows no reaction on skin surface or no any redness on skin.

Stability studies
Stability study of different formulations were carried out at storage condition of 8^0C and 40^0C for a period of one month. Samples were withdrawn at the time interval of 7,15 and 30 days. During the study period, all the formulations [kept at 8^0C & 40^0C] were found to be homogenous and free from microbial growth which may attributed to the presence of preservatives. The colour of F1 formulation has changed somewhat, and F3 formulation has a terrible odour when stored at 40 °C on the 30 th day, and the pH of the gel has also altered in both F1, F3, and F4 formulation.

Selection of optimized formulation
Gel should have appropriate properties and be stable over lengthy periods of time in order to provide positive outcomes on the skin. Based on the findings of the stability tests and physical criteria like spreadability, pH, viscosity, and spreadability, formulation F2 was determined to be excellent and was chosen for further characterisation such antimicrobial activity testing..

FTIR Spectroscopy of Formulated face wash –

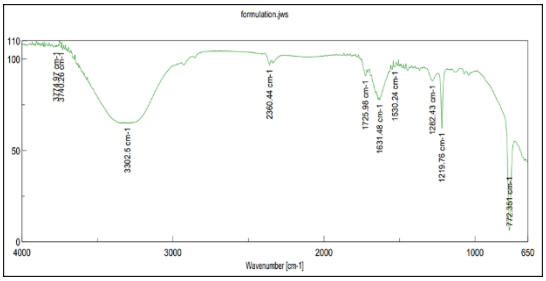


FIGURE NO. 24 – FTIR spectra of Formulation

TABLE NO. 20 - FTIR study of Formulation

Sr. No.	Functional Group	Observed Value	Standard Value
1	Alkyne	2360.4	2100 -2250
2	Aldehydes	1725.9	1740 -1720

3	Amide N-H (bend)	1631.4	1550 - 1640
4	Nirto compounds N=O	1530.2	1350 -1550
5	Ethers C-O	1282.4 1219.7	1000 -3000
6	Aromatic	772.3	690 - 900

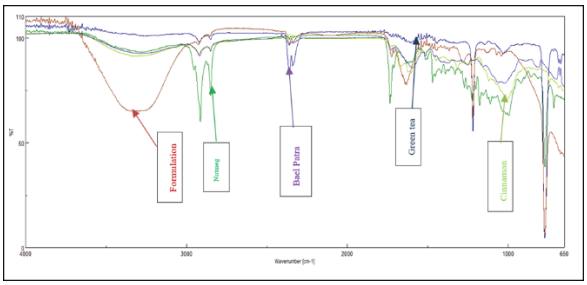
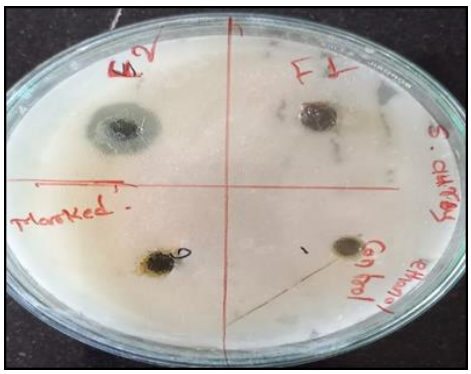


Figure No. 25 – Overlay of FTIR spectra of formulation and extracts.

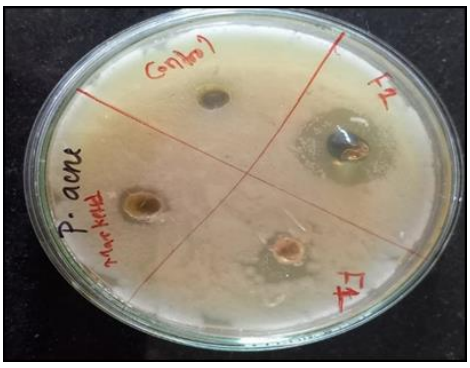
Antimicrobial activity testing by Agar well diffusion method.

Table No. 21: Zone of inhibition of the formulated Anti acne herbal gel

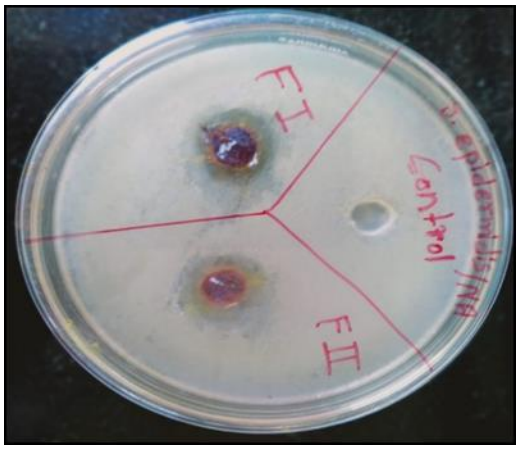
	Organism										
	Staphylococcus aureus			Mean (in mm)	*Propionibacterium Acne*			Mean (in mm)	*Staphylococcus Epidermidis*			Mean (in mm)
	1	2	3		1	2	3		1	2	3	
Herbal Antiacne facewash gel (F2)	12.2	12.4	12.2	12.3± 0.1	13.2	13.3	13.2	13.2± 0.06	12.5	12.3	12.5	12.4± 0.1
Maketed Fcewash (f1)	–	–	–	–	11.2	11.3	11.3	11.2± 0.06	12.2	12.3	12.3	12.2± 0.6
Maketed Facewash (f3)	–	–	–	–	10.2	10.2	10.3	10.2± 0.3	–	–	–	–
Control (ethanol)	–	–	–	–	–	–	–	–	–	–	–	–



FigureNo.26: Zone of inhibition ofFormulation F2 towards *Staphylococcus aureus*



FigureNo.27: Zone of inhibition ofFormulation F2 towards *Propionibacterium Acne*



FigureNo.28: Zone of inhibition ofFormulation F2 towards *Staphylococcus epidermidis*

V. SUMMARY AND CONCLUSION

Summary

About 650 million people are affected by acne worldwide. It is Eighth most prevalent disease worldwide. Because of acne problem most of people may suffer psychologically mostly teenagers suffer from lack of confidence in them, which may leads to depression & anxiety in person. Hence, to reduce this acne problem herbal anti acne Face wash gel is formulated by using aqueous extracts of bael leaves, cinnamon bark, nutmeg fruits & green tea leaves , which will reduce the acne and excess oil secretion without losing natural moisture of skin & also get rid from the use of synthetic drugs which may cause harmful effects on skin, Also the formulation is checked by using various parameters like microbial test, ph, viscosity , spredability etc.

Conclusion

All of these researches have shown the final reasons, leading to the following conclusions:

1. This study targets the chronic skin condition acne with the aim of formulating an effective and safe herbal Anti acne Facewash gel by using Aegle marmelos, Cinnamomum verum, Miyristica fragrans, Camellia sinensis and Aloe barbadensis.
2. The ethanolic extract of Aegle marmelos, Cinnamomum verum, Miyristica fragrans, Camellia sinensis and collected Aloe barbadensis gel were incorporated in to optimized Carbopol gel base.
3. The combination of these herbal constituents may produce an effect to minimize the Acne problem.
4. An antimicrobial investigation revealed that there was no microbial contamination and that the zone of inhibition was good.
5. Overall, this study reports concluded that the formulation of herbal Anti acne facewash gel may offer an effective and safe dosage form which leads to patient adherence and compliance to the therapy.

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