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FORMULATION AND EVALUATION OF HERBAL TREATMENT OF VITILIGO

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Keywords:

Vitiligo, Antioxidant, Anti-inflammatory, Polyherbal lotion

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ABSTRACT: Vitiligois depigmentation disorder, and it causes loss of melanocytes. Denaturation of melanocytes caused by oxidative stress, genetics, auto immune response and then synthesis of inflammatory mediators. Vitiligo is classified into two types segmental and non-segmental. There is various treatment to cure vitiligo and some treatment causes side effects. So, through herbal plants we can cure the diseases the herbal plants are *Ginkgo biloba*, *Nigella sativa*, *Psoralea corylifolia* there are plants which has antioxidant properties, anti-inflammatory, these antioxidant helps to neutralizes free radicals and reduce oxidative damage in the skin. *Psoralea corylifolia* it has ability to stimulate melanin production in the skin through this repigmentaion occurs in the skin. In the pursuit of developing effective treatments for vitiligo, understanding the chemical composition and molecular structure of a topical formulation is essential. GC-MS analysis is used to identify the compound present in polyherbal lotion, FT-IR spectroscopy is used to analzye the functional group present in the lotion, HPLC quantitative analysis is used to determine exact amount of active ingredients present in the polyherbal lotion. The lotion formulation has enriched activity against occident stress and inflammation. Skin irritation test has done for the formulation check whether formulation produce's edema and redness in the skin. The topical formulation of lotion was prepared and evaluated, and the lotion was prepared for 25 grams. The two *in-vitro* studies were done H_2O_2 and albumin denaturation assay through this oxidant stress and inflammation will be reduced.

INTRODUCTION: Vitiligo affects around 0.5% to 2% of population all around the world it's both equivalent in both male and female and it causes in both adults and children of both genders together. Non segmental vitiligo occurs at every age but normally occurs in young age people between 10 years and 30 years. One epidemiology study showed that almost 50% of people affected by vitiligo at the age of 40 years and above.

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In spite of this fact that vitiligo occurs at every age whether it is youthful or moderate ages [1]. The range of segmental vitiligo (SV) is 5% to 30% and SV accounts for 5% to16% of overall vitiligo cases [2]. Vitiligo is classified into two types 1. Segmental vitiligo 2. Non- segmental vitiligo and second consists of variations central, generalized, universal and acrofacial vitiligo. segmental vitiligo occurs less frequently compared to non-segmental vitiligo. The person who affected by vitiligo has loss of pigmentation from skin, overlying hair and mucosa persists.

1. **Localized Vitiligo:** Discoloration occurs in the particular part of the body and categorized into two subclasses.

2. **Focal Vitiligo:** Small injury occurs in the part of the body.

3. **Segmental Vitiligo:** White patches occur in skin on the one side of the body, Fast expansion of the patches in a specific part of the body it is caught and unchanged in a considerable length of time.

4. **Generalized Vitiligo:** Discoloration occurs in different parts of the body. Typically described by the continuous expansion of the skin injuries to a vast locale. Generalized vitiligo often begins from the face and extremities.

5. **Acrofacial Vitiligo:** White patches apparent on the face, hand& feet. Frequently begins in the fingertips and facial opening.

6. **Vitiligo Vulgaris:** Recognized by means of its dissipated dispersion all the through the body.

7. **Vitiligo Universalis:** universal vitiligo means wide distribution of white patches throughout the body. This leads complete discoloration of the skin, mucosae and hair. Mucosal vitiligo influences the mucous layer in the body. Mucosal vitiligo influences either the oral or the genital section. Occupational vitiligo is sort vitiligo which is exanimated it occurs due to direct contact with synthetic that annihilate the melanocytes

The most significant symptom is skin patch depigmentation. Initially, patches will be tiny, but over time, they will become larger. On the wrists, hands, and face, there are visible skin lesions. Depression will also be experienced by the person who has these illnesses [3].

MATERIALS AND METHODS:

Pharmacognostical Studies:

Collection and Authentication of Plant Material: *Ginkgo biloba* (Ginkgoaceae) leaf was purchased from the Indian Jadi Booti online website East Gorakh Park, shahdara which was collected in the month of December 2023. Leaf was recognized and verified by siddha central research institute in Chennai (code: G31012413B). *Nigella sativa* (Ranunculaceae) and *Psoralea corylifolia* (Fabaceae) was collected from the local marked which is located in Thirukkalukundram, Chengalpattu, Tamil Nadu in the month of December 2023. Seed was recognized and verified by siddha central research institute in Chennai (code: N31012412S), (code: C31012411C).

Phytochemical Studies:

Physico-chemical Analysis:

Ash Value: Ash value useful in evaluating the quality and purity of the drug these values are important in qualitative standards. Ash is usually representing the phosphate, carbonates, calcium, magnesium, etc. occur in the drug these're added into the drug for the proses of adulteration. The crude powdered drug was heated in a muffle furnace at 70-degree to 80- degree ash will be obtained.

Acid- insoluble Ash: Take the total ash and dilute with 25 ml of HCL acid and boil for 5 min and filter through filter paper and then take that residue heated by using muffle furnace and cool and keep it in a desiccator.

Water Soluble Ash: Take the total ash and dilute with 25 ml of water and boil for 5 min and filter through filter paper and then take that residue heated by using muffle furnace and cool and keep it in a desiccator.

Alcohol Soluble Extractive Value: 5 gram of crude powder drug is macerated 100ml of alcohol in a stopper flask placed in a orbital shaker 8 hours and allow to stand for 16 hours. Through filter paper filtrate the drug and collect the filtrate & evaporate 25 ml filtrate and allow to dry at 150 degree and weight keep it in a desiccator.

Water Soluble Extractive Value: 5 gram of crude powder drug is macerated 100ml of distilled water in a stopper flask placed in a orbital shaker 8 hours and allow to stand for 16 hours. Through filter paper filtrate the drug and collect the filtrate & evaporate 25 ml filtrate and allow to dry at 150 degree and weight keep it in a desiccator.

Loss on Drying: Tale a porcelain dish weigh weight of it then adds 5 gm of crude powdered drug and kept in an oven at the temperature of 100-105 °C for some time then take the porcelain dish keep it in a desiccator until it cool. Now calculated moisture content of the through this formula [4, 5].

pH Test: First rinse the electrode before using. Then weigh the sample in to a beaker then dissolve it in water for making % solution required and start to calibrate the pH meter by using buffer solution of 7 and then the electrode should be moved to the test solution and start to record the pH when the reading is constant [6].

Phytochemical Studies:

Preliminary Test for Phytochemical Screening: Phytochemical screening is done for all the three extracts with a hydro alcoholic solution with a standard procedure.

Detection of Flavonoids:

Alkaline Reagent Test: The extract was mixed with (NaOH) to form a yellow colour that conforms the presence of Flavonoids and when dilute acid was added to the solution it becomes colourless.

Lead Acetate Test: Upon addition of lead acetate solution to the extract, a precipitate will form, characterized by a yellow coloration indicating the presence of flavonoids.

Detection of Carbohydrates:

Molisch Test: The appearance of a violet colour ring at the junction of the two layers, following the addition of concentrated sulfuric acid to the mixture of aqueous extract and alpha-naphthol solution, suggests the presence of carbohydrates, particularly pentoses, in the sample. Further characterization and quantification of the specific carbohydrate constituents may be necessary for a comprehensive analysis.

Fehling's Test: The observation of a reddish color upon mixing 1ml each of Fehling's A and Fehling's B solutions, boiling the mixture for 1 minute, followed by the addition of an equal volume of the test solution, and subsequent heating on a water bath for 5-10 minutes, confirms the presence of reducing sugars in the test solution.

Benedict's Test: Upon mixing the Benedict's reagent with the test solution in a test tube and subjecting it to a 5-minute boiling water bath, observe for the formation of any yellow or red coloration, indicating the presence of reducing sugars in the test solution.

Barford Test: Mix the Barford's reagent with the test solution and boil the mixture for 2 minutes in a boiling water bath. Allow the mixture to cool, then observe for the formation of any red precipitate, indicating the presence of pentoses in the test solution.

Seliwanoff's Test: 3 ml of seliwanoff's reagent and 1ml test solution is added kept it in a water bath for 1-2 mins. Observed if any red colour is formed.

Detection of Alkaloids: The extract was dissolved in 1N hydrochloric acid, filtered, and the filtrate was utilized to determine the presence of alkaloids.

Mayer's Test: The addition of potassium mercuric iodide to the filtrate resulted in the formation of a yellow-colored precipitate, confirming the presence of alkaloids.

Wager's Test: To the filtrate iodine-potassium iodide was added to form brown/ reddish colour precipitate shows alkaloids is present.

Dragendorff's Test: Potassium bismuth iodide was added to the filtrate to form a red precipitate that shows the existence of alkaloids.

Hager's Test: The addition of saturated picric acid solution to the filtrate resulted in the formation of a yellow-colored precipitate, indicating the presence of alkaloids.

Detection of Proteins and Amino Acids:

Ninhydrin Test: When 1ml of the extract is combined with 0.25%w/v ninhydrin reagent and heated for a few minutes, the emergence of a blue color confirms the presence of amino acids.

Xanthoproteic Test: The addition of concentrated nitric acid to the filtrate results in the formation of a yellow color, indicating the detection of proteins.

Detection of Glycosides: To the extract add 2N HCL, boiled and filtered and it's used for detection of alkaloids.

Modified Bontrager's Test: Filtrate add few drops FeCl3 and keep it in a water bath for boiling then cool it further treated with an equal amount of benzene and then organic layer is separated from the aqua's layer & treated with ammonia. In the ammonia layer pink colour is formed it shows the presence of anthranol glycosides.

Legal's Test: The addition of sodium nitroprusside, pyridine, and sodium hydroxide to the filtrate results in the formation of a pink to dark red color, indicating the detection of cardiac glycosides.

Detection of Phenols:
Ferric Chlorides Test: To the extract add ferric chloride produced bluish- black colour conforms the presence of phenolic compounds.

Detection of Saponins:
Forth Test: Extract add few drops of water in a test tube & shake it 15 min and foam will be formed for 1 cm this confirms the presence of saponins.

Foam Test: Extract add few quantity of H_2O and foam will be appear and persists for 10 min that shows the saponins is present.

Detection of Tannins:
Gelatin Test: Filtrate add 5ml of distilled water and then add 1% of gelatin solution and then add 10%of sodium chloride white precipitate is formed [7].

Quantitative Estimation:
Determination of Total Flavonoid Content (TFC): The determination of flavonoid content was carried out using the AlCl3 colorimetric assay method, as described by Jia and Tang. Total flavonoid content was estimated based on the colorimetric reaction, where the colour intensity is directly proportional to the number of flavonoids present. The absorbance was measured at a wavelength of 510 nm. To establish the calibration curve, various concentrations of quercetin (0.025, 0.050, 0.075, 0.10, and 0.15 mg/ml) were prepared in methanol, each with a volume of 0.5 ml, and then diluted to 2.5 ml with distilled water. To each of these solutions, 0.15 ml of NaNO2 was added and allowed to stand for 5 minutes. Subsequently, 0.3 ml of aluminium chloride (AlCl3) was added and again allowed to stand for 5 minutes. Following this, 1 ml of sodium hydroxide (NaOH) and 0.55 ml of distilled water were added. The absorbance of each solution was then measured at 510 nm. By plotting the absorbance against the concentration of quercetin, a calibration curve was established. The regression equation derived from thiscurve provides a relationship between quercetin concentration and absorbance, facilitating the determination of flavonoid content in samples based on their absorbance values [8].

Total Phenolic Content (TPC): The Folin-Ciocalteu test was employed to determine the total phenolic content (TPC) of the extract. Specifically, 10 mg of the extract was dissolved in 10 ml of the solvent to achieve a concentration of 1 mg/ml. Then, 100 µl of the extract was mixed with 0.75 ml of the Folin-Ciocalteu reagent in a test tube and allowed to stand for 5 minutes. Following this, 0.75 ml of sodium carbonate (Na2CO3) was added to the mixture in another test tube, and the solution was gently shaken. After 90 minutes, the absorbance of the mixture was measured using a UV-Vis spectrophotometer at 725 nm [9].

Total Alkaloid Content (TAC): The extract, prepared at a concentration of 1 mg/ml, was treated with 2N HCl (hydro alcoholic acid) and kept in a separating funnel. Subsequently, 1 mL of phosphate buffer with a pH of 4.7 was added to facilitate complex formation, and this mixture was allowed to react for 10 minutes. Following the formation of the complex, the mixture was partitioned with 3 ml of CHCl3 (chloroform), and the CHCl3 layer was separated. This CHCl3 layer was then diluted to 10 ml with chloroform. The absorbance of the resulting mixture was measured at 415 nm using a UV spectrophotometer [10].

Preparation of Polyherbal Hydroalcoholic Extract: Weigh 25 gram of ginkgo biloba leaf, 25 gram of nigella sativa and psoralea coryliforia and then transfer into a beaker from that beaker weigh 50 grams of polyherbal compound transfer into 1000ml container of maceration bottle and then add hydroalcoholic solution. Keep it for maceration up to 7days after that by using filtration filter the extract.

Preparation of Polyherbal Hydroalcoholic Extract: Weigh equal proportion (20 grams) of crude herbal powder and mix them thoroughly and then take 50 grams of polyherbal mixture in a maceration bottle and then add hydro alcoholic solution (370 ml of methanol: 130 ml of water) and cover the bottle through lid/ cap kept for 7 days. After 7 days of maceration of a polyherbal extract hydro alcoholic extract is filtered through filter

paper and then filtrate is kept in a water bath for evaporation. Then final dry extract is stored for future formulation.

Take a three beaker and weigh the equal amount of herbal extract (10 grams) and then add hydro alcoholic solution in ratio of (70 ml of methanol: 30 ml water) into the 3 beaker and kept aside for 7 days and through filter paper, filter the extract and kept in a water bath and wait for the evaporation. Finally, extract is stored and exanimated further for formulation [11].

Preparation and Formulation of Lotion: Take a beaker add all the water phase material into the beaker and keep it in a water bath for 70° C. Take separate beaker add all the oil phase material into the beaker and keep it in a water for 40° C. By using thermometer check both phases obtain specific degree now transfer oil phase into a mortar and pestle then add water phase little by little and started to triturate while triturating add 2ml of Triethanolamine and rose oil for Frances and emulsion is formed.

TABLE 1: EXCIPIENT AND ITS USES

S. no.	Ingredients	Category	Uses
1	Glycerine	Humactant	Improve skin barrier function
2	Triethanolamine	Neutralizer	Stabilizer or emulsifier
3	Liquid paraffin	Emollients	Moisturizer, decrease itching and flaking
4	Stearic acid	Emulsifier	Retains the skin natural moisture
5	Cetyl alcohol	Co-emulsion	Conditioning, softing
6	Methyl paraben	Preservative	Prevents fungus growth
7	Rose oil	Francene	Hydrating
8	Distilled water	Diluent	Enhances the absorption

Evaluation of Lotion: Evaluation research is characterized as a method of methodical, disciplined investigation used to determine the value or merit of a product, initiative, procedure, activity, or system with the aim of offering data that will assist in making decisions.

Organoleptic Characteristics: Through visualisation method we can determine organoleptic characteristics of ploy herbal lotion. Colour, Odour, Texture was examined and determined.

Homogeneity Test: Homogeneity test was done by visualization and touch.

pH Test: Potential of hydrogen of the polyherbal lotion is tested by pH di*gi*tal meter.

Spread Ability Test: Position two slides horizontally, apply lotion to the center of one slide, then place the other slide over it. Apply a specific load to the upper slide to evenly press the lotion between the two layers, forming a thin layer. After removing the load, scrape off the material adhered to the slides.

Absorption Test: Absorption test was done by applying lotion on the left side of your skin and rubbed gently until it gets completely absorbed.

Washability Test: The polyherbal lotion was applied on your left-hand side of the skin by using tap water check whether the lotion is removed completely.

Irritancy Test: The polyherbal lotion was applied on your left-hand dorsal side surface of 2 sq.cm and observed any irritancy, sensitivity and edemaappears on the skin.

Phase Separation: Lotion is placed in a container at the temperature of 25-100 ° C keep away from the light. Check whether any separation occurs within 24 hours.

Greasiness: Lotion was applied on the skin and check whether any oily or grease is present [12, 13].

Drug Excipient Compatibility Studies using Fourier- transforms Infrared Spectroscopy [FT-IR] Analysis: Infra-red ATR (attenuated total reflectance) is used to determine FT-IR and then software used is software opus 6.5 or 7.0 or higher. Before using ensure that the plat form is empty and free from materials then clean with 90 % of ethanol and then pour blank sample into the platform and then run the software after that through ethanol clean the platform then pour small quantity of liquid sample into the platform that sample should

be surrounded by platform then run the sample peak will be determined [14].

Chromatographic Technique for Fingerprint Analysis and Quantification of Biomarkers in Polyherbal Formulation Using:

Gas Chromatography- Mass Spectroscopy [GC-MS] Analysis:

GC-MS Fingerprint Profiling: The hydro alcoholic extract of the polyherbal formulation was analysed for the determination of volatile compounds using GC-MS analysis [15].

Sample Preparation: The conc. of 1 mg/mL of sample is prepared using methanol as a diluent.

Instrument: A silica column is fused with Elite-5 mass spectroscopy and carrier gas is a helium and operate at a constant flow rate 1ml/min is part of the Clarus 680 gas chromatography system.

Chromatographic – Spectrometry Condition:

Ion source temperature	:	220°C
Column temperature	:	110-280°C
Ionization mode	:	Electron- impact ionization
Flow rate	:	1.0 mL/min
Interface temperature	:	250°C
Split ratio	:	1:20
Ionization energy	:	70 eV
Injector temperature	:	250°C
Ion source temperature	:	220°C
Scan range	:	40-700m/z
Scan rate	:	0.50 s/scan
Run time	:	50 min
Carrier gas	:	Helium (99.999%)
Column	:	Phenomenex ZB-5MS Elite-1 fused silica capillary column (30 mm X 0.25 mm i.d., 0.25 µm thickness)

HPLC Analysis:

Quantification of Quercetin:

Instrumentation and Chromatographic Condition: The sample was analyzed for phytomarkers using the RP-HPLC-PDA method employing a Shimadzu LC-20AD HPLC system equipped with a CT0-20A controller, CBM-20A communication bus module, and a column oven. A Rheodyne 7725 injection valve with a 20 µl loop volume was utilized for sample injection. Detection was performed using an SPD-M20A photo-diode array detector. Lab Solution version 7.1 software was employed for data interpretation and acquisition. The phytomarkers were assessed based on their specific wavelengths and maximum absorbance.

Preparation of Standard and Sample Solution for HPLC Analysis: The stock solution of 1mg/mL concentration were prepared using methanol as diluent from which calibration standards were prepared in methanol by diluting the stock solution of standards to attain desired concentration range of 12.5-200 µg/ml. The polyherbal formulation was prepared by diluting 100 mg of lotion in 1 mL of methanol which was further sonicated in water bath for 15 min and filtered [16].

***In-vitro* Antioxidant Activity:**

Procedure: The methodology, as described by Ruch *et al.*, was slightly modified to evaluate the potential of plant extracts in scavenging hydrogen peroxide. A hydrogen peroxide solution with a concentration of 43 mM was prepared using phosphate buffer (1 M, pH 7.4). Various concentrations of the polyherbal formulation (PHF) ranging from 10 to 500 µg/ml were mixed with 0.6 ml of the hydrogen peroxide solution. Following a 10-minute incubation period, the absorbance of hydrogen peroxide at 230 nm was measured, with a blank solution containing only phosphate buffer (without hydrogen peroxide) used as reference. Ascorbic acid was employed as the standard. The percentage inhibition of free radical scavenging activity was calculated using a specified formula [17].

$$\% \text{ inhibition} = [(\text{Control- Test})/\text{control}] \times 100$$

Pharmacological studies:

Anti- Inflammatory Test:

Anti-Inflammatory Activity - Inhibition of Albumin Denaturation:

Procedure: With slight adaptations, the method by Mizushima, Kobayashi, and Sakat *et al.* was employed to assess the inhibition of protein denaturation, a key contributor to inflammation. Test samples (R) were prepared at concentrations ranging from 100 to 500 µg/mL. Each test sample was mixed with 500 µL of 1% bovine serum albumin. Following a 10-minute incubation period at room temperature, the mixture was heated at 51°C for 20 minutes to induce protein denaturation. Upon cooling to room temperature, the absorbance of the mixture was measured at 660 nm.

Acetyl salicylic acid was included as a positive control. The percentage inhibition of protein denaturation was determined using the specified formula during the triplicate experiment [18, 19].

$$\% \text{ Inhibition} = 100 - (A1-A2) / A0) \times 100)$$

Skin Irritation Test: The formulation was applied on the skin and leave it for 24 hours and then check whether any inflammation or irritation and redness occurs on the skin [20, 21].

RESULTS AND DISCUSSION:

Physico-Chemical Analysis: Physico chemical analysis of the three herbal plants were discussed here in **Table 2** includes a extractive values, ash value pH and loss on drying.

TABLE 2: PHYSICO CHEMICAL ANALYSIS OF A MEDICINAL PLANTS

Test	*Ginkgo biloba*	*Nigella sativa*	*Psoralea corylifolia*
Ash value	3.0%w/w	4.9 %w/w	5.3%w/w
Water soluble extract	1.3%w/w	1.70%w/w	1.45%w/w
Acid insoluble extract	0.15%w/w	0.59%w/w	0.5%w/w
Alcohol soluble extractive value	10%w/w	17.5%w/w	9%w/w
Water soluble extractive value	15%w/w	10.6%w/w	6.3%w/w
Loss on drying	10	4.65	3.5
pH	7.7	6.5	6.9

Physico-Chemical Analysis: Physico chemical analysis of the three plants were subjected here with ash value, extractive value, pH, loss on drying value is mentioned in the table**.**

TABLE 3: PHYSICO CHEMICAL ANALYSIS OF A MEDICINAL PLANTS

S. no.	Test	*Ginkgo biloba*	*Nigella sativa*	*Psoralea corylifolia*
1	Ash value	3.0 %w/w	4.9%w/w	5.3
	Water soluble extract	1.3	1.70	1.45
	Acid insoluble extract	0.15	0.59	0.5
2	Extractive values			
	Alcohol soluble extractive value	10	17.5	9
	Water soluble extractive value	15	10.6	6.3
3	Loss on drying	10	4.65	3.5
4	pH	7.7	6.5	6.9

Phytochemical Studies:

Preliminary Test for Phytochemical Screening: The qualitative analysis of a three plants extract was identified for the phytochemical constituents such as Glucosides, saponins, steroids, Tannins, Flavonoids, protein, Alkaloids, carbohydrates, phenols this test is involved to detect the presence or absence of these three compounds.

TABLE 4: PRELIMINARY PHYTOCHEMICAL SCREENING OF THREE PLANTS

S. no.	Test	*Ginkgo biloba*	*Nigella sativa*	*Psoralea corylifolia*
1	Glucosides	+	+	+
2	saponins	+	+	+
3	steroids	+	+	-
4	Tannins	+	-	+
5	Flavonoids	+	+	+
6	protein	+	-	-
7	Alkaloids	+	+	+
8	carbohydrates	-	+	+
9	phenols	-	+	-

+ = present, - = absent

Quantitative Estimation: The quantitative estimation of polyherbal formulation were described in the **Table 6.** The calibration graph and serial dilution of Alkaloid content (TAC), Flavonoid content (TFC) and phenolic content (TPC) was represented in **Fig. 1-6.**

TABLE 5: QUANTITATIVE ESTIMATION OF POLYHERBAL FORMULATION

S. no.	Total content estimation	Polyherbal formulation
1	Alkaloid	0.6 mg/g
2	Flavonoid	1 mg/g
3	Phenol	1.1 mg/g

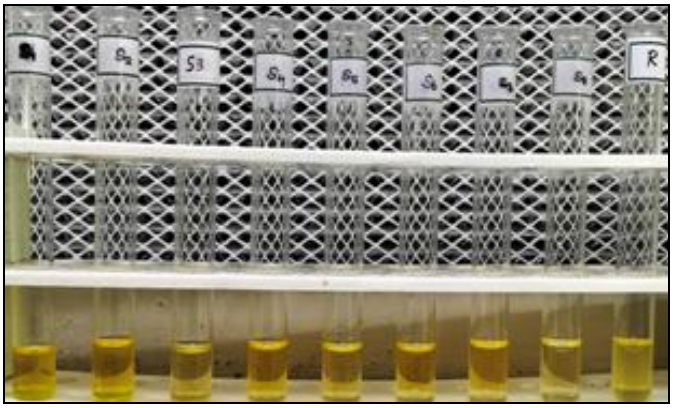


FIG. 1: DILUTION FOR TOTAL ALKALOID CONTENT TAC) OF POLYHERBAL FORMULATION

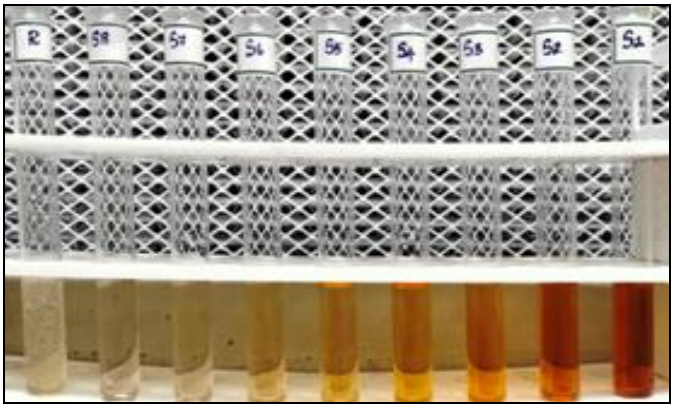


FIG. 2: DILUTION FOR TOTAL FLAVONOID CONTENT (TFC) OF POLYHERBAL FORMULATION

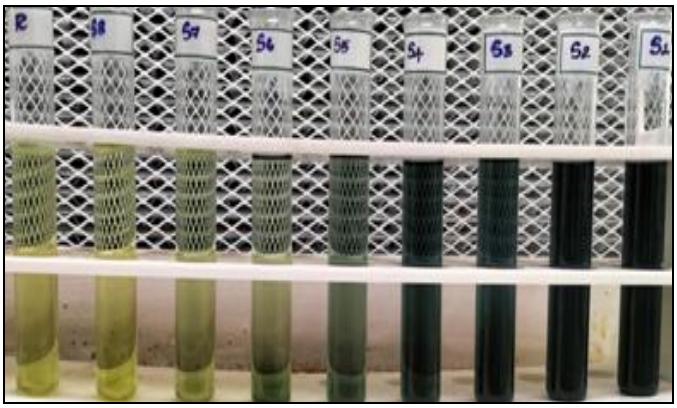


FIG. 3: DILUTION FOR TOTAL PHENOLIC CONTENT (TPC) OF POLYHERBAL FORMULATION

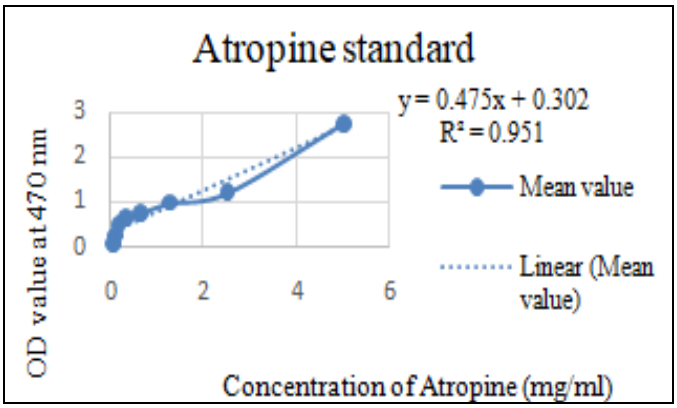


FIG. 4: CALIBRATION GRAPH FOR TOTAL ALKALOID CONTENT (TAC)

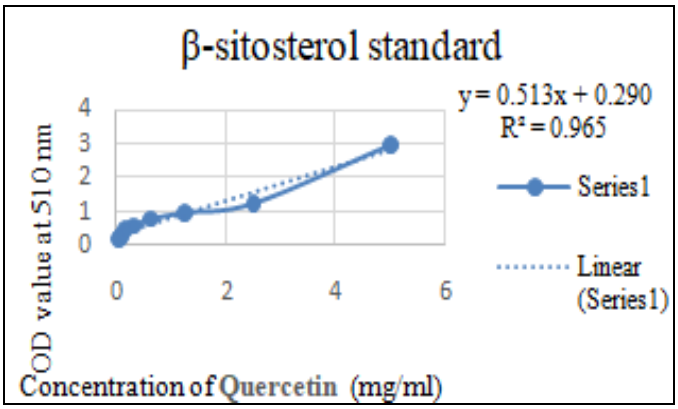


FIG. 5: CALIBRATION GRAPH FOR TOTAL FLAVONOID CONTENT (TFC)

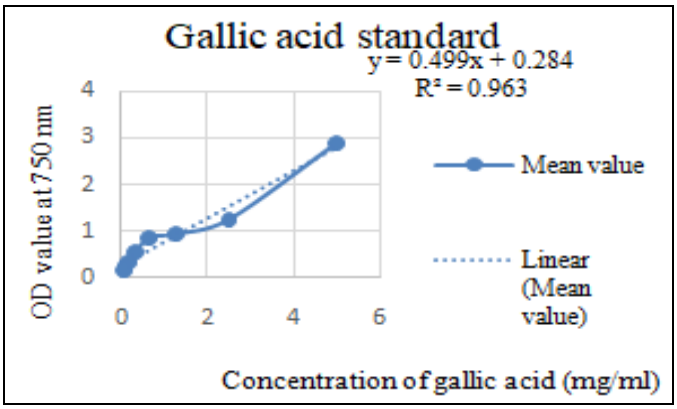


FIG. 6: CALIBRATION GRAPH FOR TOTAL PHENOLIC CONTENT (TPC)

Formulation of Lotion: Take one beaker add all the water phase compounds such as glycerin, water, methyl paraben kept in the water for 70°C. Take separate beaker add all the oil phase compounds such as stearic acid, liquid paraffin, cetyl alcohol kept in a water bath for 40°C. Now using thermometer check both phases are at correct degree after that take mortar and pastel pour oil phase into it then add little by little of water phase started to triturate while triturating add triethanolamine and rose oil until it forms lotion. Comparing the three formulation of lotion which we have done for trial and error, trial 4 has achieved most effective and has successful outcome and excipients present in formulation has good properties and uses which is beneficial for the skin.

TABLE 6: FORMULATION OF 25 GRAM OF LOTION

S. no.	Ingredients	Quantity
1	Extract	5 gm
2	Glycerine	5 ml
3	Triethanolamine	2 ml
4	Liquid paraffin	5 ml
5	Stearic acid	3 gm
6	Cetyl alcohol	5 gm
7	Methyl paraben	0.1 gm
8	Rose oil	0.1 ml
9	Distilled water	q.s

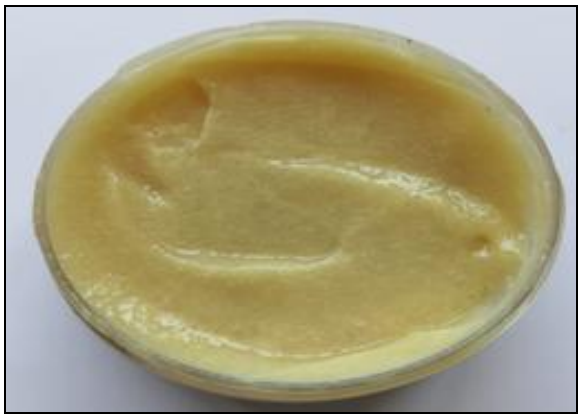


FIG. 7: FORMULATION OF 25 GRAM OF LOTION

Evaluation of Lotion: The lotion was evaluated with a Organoleptic characteristics, Homogeneity, pH test, spread ability, Absorption test, wash ability, Irritancy test, Phase separation, greasiness of lion were false under the limits of Indian pharmacopeia which were mentioned in **Table 6.**

TABLE 7: EVALUATION OF LOTION

Test	F1	F2	F3
Colour	Creamy white	Light brown	Dark brownish
Texture	smooth	smooth	Smooth
State	Semi solid	Semi solid	Semi solid
Odor	Pleasant	Pleasant	Pleasant
Homogeneity	Good	Good	Good
pH test	5.5	7	7.6
Spread ability	Easily spreadable	Easily spreadable	Easily spreadable
Absorption test	Absorbed	Very well absorbed	Very well absorbed
Wash ability	Easily washable	Easily washable	Easily washable
Irritancy	Irritancy was not formed	Irritancy was not formed	Irritancy was not formed
Phase separation	phase separation does not occur	phase separation does not occur	phase separation does not occur
Greasiness	No - oily and greasiness	No oily and greasiness	No oily and greasiness

Drug Excipient Compatibility Studies using FT-IR Analysis: Fourier transform infrared (FT-IR) spectroscopy was employed to investigate the physicochemical compatibility between the polyherbal formulation and the excipients utilized in its formulation. Examination of both the drug and the physical mixture of the polyherbal formulation with the excipients showed no noticeable differences, indicating compatibility between the polyherbal formulation and the excipients. The FT-IR spectrum of herbal plants, excipients, PHF are shown in the figure 11-15. This spectrum results of PHF without excipients exhibited the characteristics absorption peaks at

2934.28 cm^{-1} (C-H stretching (alkane)). Spectrum result of PHF with excipients exhibited the characteristics absorption peaks at 1111.51 cm^{-1} (C=S stretching). Spectrum result of *G B* exhibited the characteristics absorption peaks at 1256.94 cm^{-1} (C-O stretching (alcohols)), 1513.80 cm^{-1} (N-H bending). Spectrum result of *N S* exhibited the characteristics absorption peaks at 1262.05 cm^{-1} (C-O stretching (alcohols)). Spectrum result of *P S* exhibited the characteristics absorption peak at 1711.48 cm^{-1} (C=O stretching (acid).

Fingerprint Analysis of *Ginkgo biloba*:

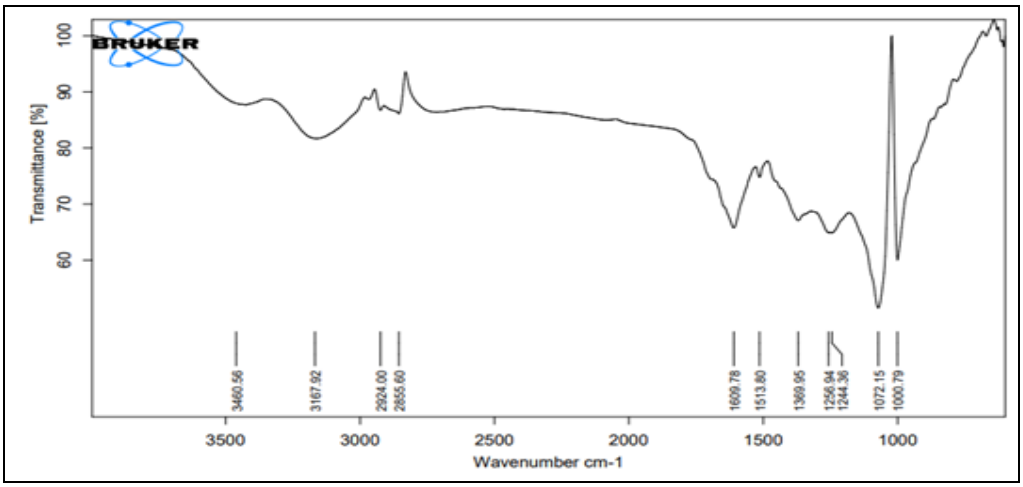


FIG. 8: FT-IR SPECTRUM OF *GINKGO BILOBA*

TABLE 8: FT-IR SPECTRUM OF *GINKGO BILOBA*

Wavenumber	Range
1000.79	C-N vibrations
1072.15	C-N vibrations
1244.36	C-O stretching (alcohols)
1256.94	C-O stretching (alcohols)
1369.95	C-O stretching (phenols)
1513.80	N-H bending
1609.78	N-H bending
2855.60	C-H stretching (alkane)
2924.00	C-H stretching (alkane)
3167.92	N-H stretching
3460.56	N-H stretching

Fingerprint Analysis of *Nigella sativa:*

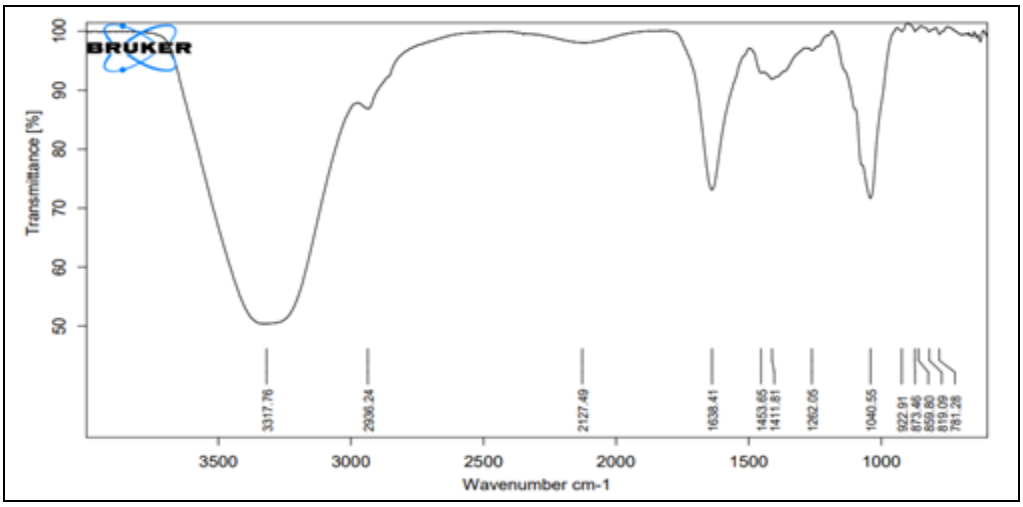


FIG. 9: FT-IR SPECTRUM OF *NIGELLA SATIVA*

TABLE 9: FT-IR SPECTRUM OF *NIGELLA SATIVA*

Wavenumber	Range
922.91	C-N vibrations
1040.55	C-N vibrations
1262.05	C-O stretching (alcohols)
1411.81	C-O stretching (phenols)
1453.65	C=C stretching (aromatic)
1638.41	C=C stretching
2127.49	C=C stretching (alkyne)
2936.24	C-H stretching (alkane)
3317.76	N-H stretching

Fingerprint Analysis of *Psorela corlifoilia:*

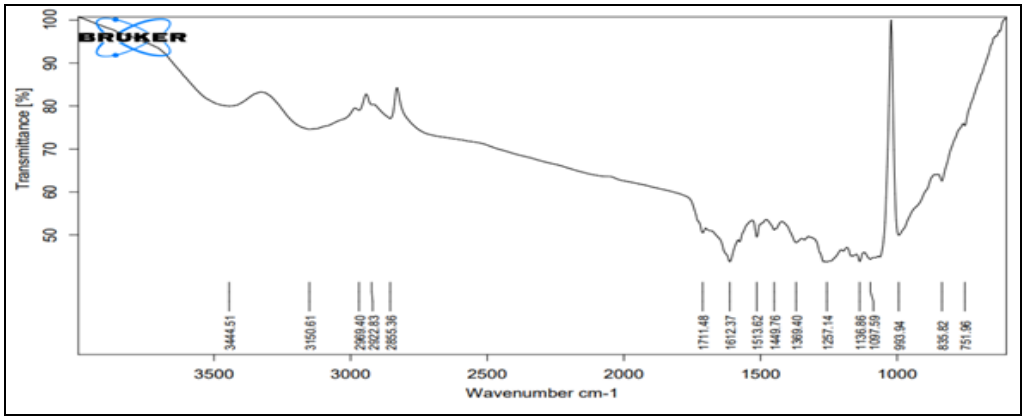


FIG. 10: FT-IR SPECTRUM OF *PSORELA CORLIFOILIA*

TABLE 10: FT-IR SPECTRUM OF *PSORELA CORLIFOILIA*

Wavenumber	Range
835.82	C-H bending (aromatic)
993.94	C-N vibrations
1097.59	C-N vibrations
1136.86	C-N vibrations
1257.14	C-N vibrations
1369.40	C-N vibrations
1449.76	N-H bending
1513.62	N-H bending
1612.37	N-H bending
1711.48	C=O stretching (acid)
2969.40	C-H stretching (alkane)
3150.61	N-H stretching
3444.51	N-H stretching

Fingerprint Analysis of Polyherbal Formulation:

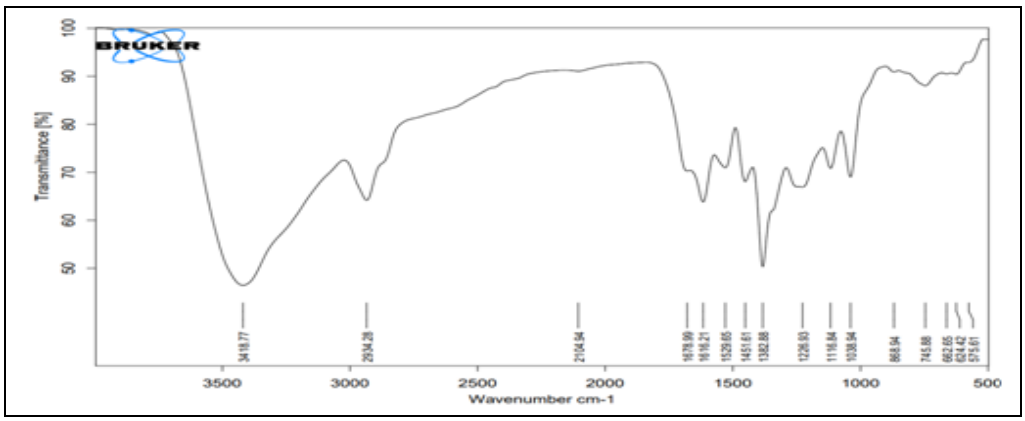


FIG. 11: FT-IR SPECTRUM OF FORMULATION OF LOTION WITHOUT EXCIPIENTS

TABLE 11: FT-IR SPECTRUM OF FORMULATION OF LOTION WITHOUT EXCIPIENTS

Wavenumber	Range
868.94	C-H bending (aromatic)
1038.94	C-N vibrations
1116.84	O-H bending (alcohols)
1226.93	S=O stretching
1382.88	C-O stretching (phenols)
1451.61	N-H bending
1529.65	N-H bending
1616.21	N-H bending
1678.99	C=N stretching
2104.94	C=C stretching (alkyne)
2934.28	C-H stretching (alkane)
3418.77	N-H stretching

Fingerprint Analysis of Lotion:

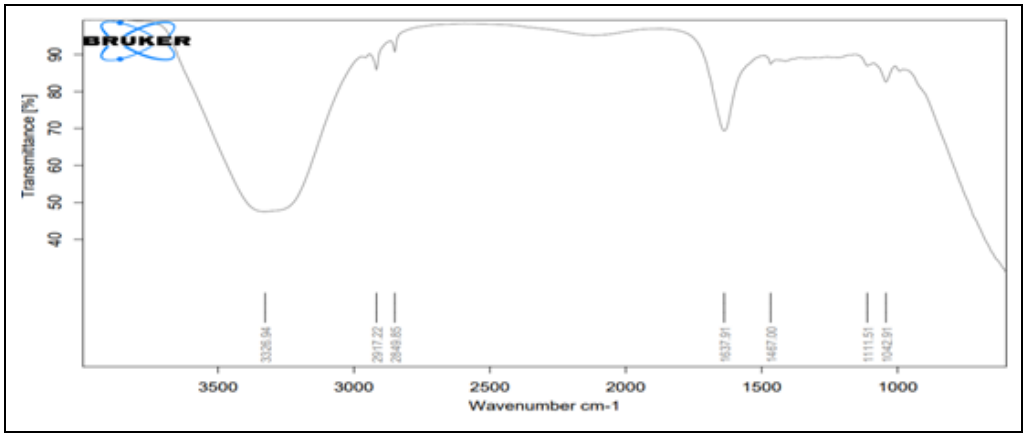


FIG. 12: FT-IR SPECTRUM OF FORMULATION OF LOTION WITH EXCIPIENTS

TABLE 12: FT-IR SPECTRUM OF FORMULATION OF LOTION WITH EXCIPIENTS

Wavenumber	Range
1042.91	C-N vibrations
1111.51	C=S stretching
1467.00	C=C stretching (aromatic)
1637.91	C=N stretching
2849.85	C-H stretching (alkane)
3326.94	N-H stretching

GC-MS Analysis:

GC-MS Identification of Compounds from a Polyherbal Formulation:

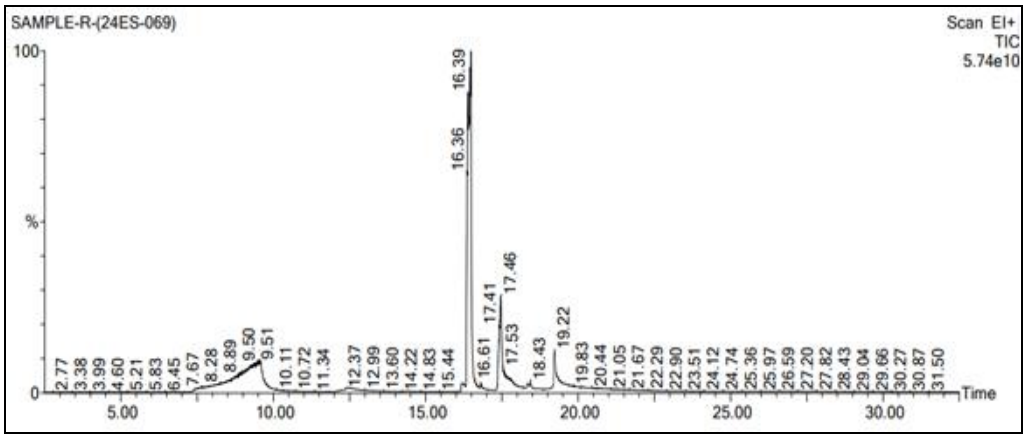
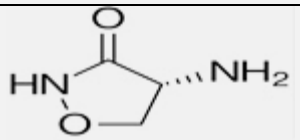
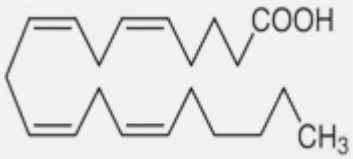
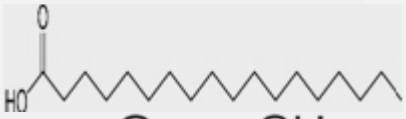
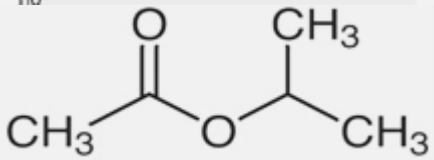
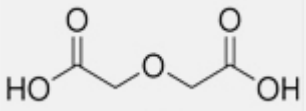
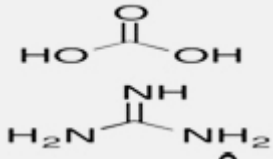
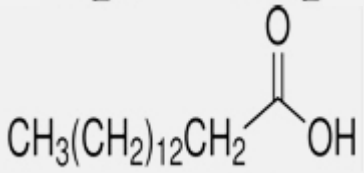
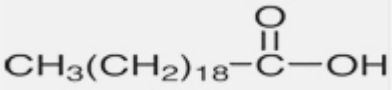
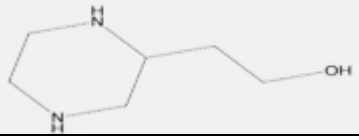


FIG. 13: GC-MS CHROMATOGRAM OF HYDRO ALCOHOLIC EXTRACT OF POLYHERBAL FORMULATION

TABLE 13: COMPOUNDS IDENTIFIED BY GC-MS IN HYDROALCOHOLIC EXTRACT OF PHF

S. no.	Compound name	Molecular weight	Molecular formula	Biological Activity	Structure
1	Cycloserine	102	$C_3H_6O_2N_2$	Tuberculosis	
2	Eicosanoic acid	312	$C_{20}H_{40}O_2$	Modulating the intensity and duration of immune and inflammatory response.	
3	1-Hexadecanol	242	$C_{16}H_{34}O$	Skin softer and help skin to keep its moisture.	
4	Octadecanoic acid	284	$C_{18}H_{36}O_2$	Anti-inflammatory	
5	Acetic acid,1-methylethyl ester	102	$C_5H_{10}O_2$	antiseptic	
6	Diglycolic acid	134	$C_4H_6O_5$	Vasodilation	
7	Guanidine carbonate	180	$C_3H_{12}O_3N_6$	Anti-tumour, Cardiovascular, Diuretic	
8	Tridecanoic acid	214	$C_{13}H_{26}O_2$	Anti-inflammatory, anti-microbial activity	
9	Eicosanoic acid	312	$C_{20}H_{40}O_2$	Anti-inflammatory	
10	1-Docosene	308	$C_{22}H_{44}$	Anti-bacterial	

HPLC Analysis:

Quantification of Quercetin:

Optimized Chromatographic Condition: After optimization, high-performance liquid chromatography (HPLC) analysis of the polyherbal formulation was carried out under the following conditions: using a C18 reverse-phase column with a mobile phase consisting of methanol (phase A) and 0.1% formic acid (phase B), at a flow rate of 1 mL/min employing an isocratic elution method. The compound exhibited responses at wavelengths of 208 nm and 365 nm, and the column temperature was maintained at ambient levels. The retention time (Rt) for quercetin and the standard was determined to be 2.364. By utilizing the regression equation, the specified biomarker in the polyherbal formulation was quantified.

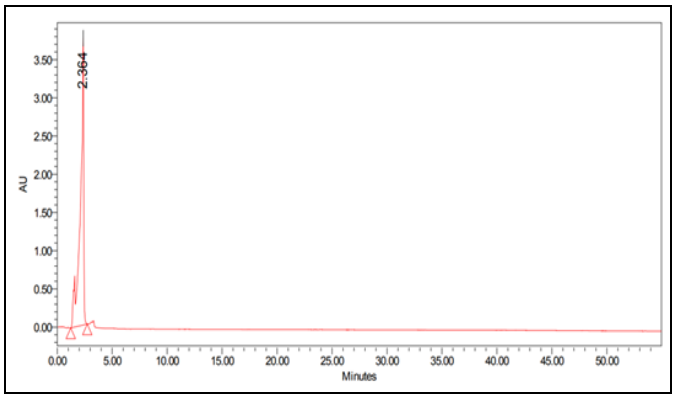


FIG. 14: HPLC CHROMATOGRAM OF STANDARD QUERCETIN

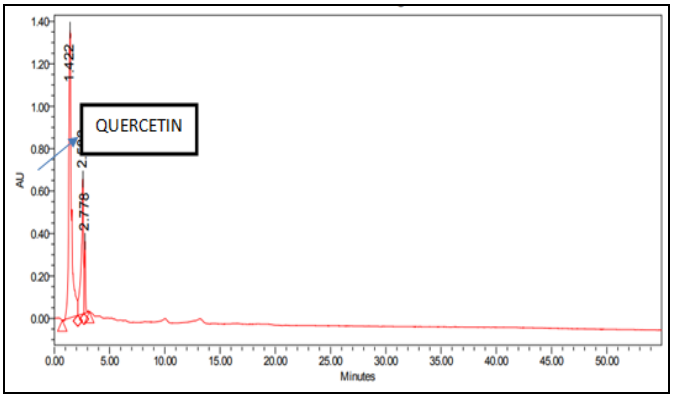


FIG. 15: HPLC CHROMATOGRAM OF PHF IN LOTION

In-vitro Antioxidant Activity

Hydrogen Peroxide Scavenging Assay: The polyherbal formulation was evaluated using the method described by Ruch and Cheng. A phosphate buffer H_2O_2 solution was prepared and maintained at a pH of 7.4. Each individual extract and the polyherbal formulation were tested at different concentrations with 0.6 ml of solution added to each. Ascorbic acid served as the standard. The absorbance of H_2O_2 at 230 nm was measured to determine the level of hydrogen peroxide aggregation, indicative of antioxidant activity. The results of the H_2O_2 assay, along with the radical scavenging activity of the hydroalcoholic extract of the polyherbal formulation, demonstrated significant inhibitory effects on radical scavenging activity. These findings were compared to those of ascorbic acid, as detailed in **Table 13** and **14**, and represented graphically in **Fig. 10** and **11.**

OD Value at 230 nm:

TABLE 14: CONTROL FOR PHF

S. no.	PHF (µg/ml)	Mean Value
1.	Control	0.281 ±0.039
2.	500	0.088±0.002
3.	250	0.092±0.001
4.	100	0.098±0.001
5.	50	0.113±0.001
6.	10	0.152±0.023
7.	Ascorbic acid	0.012±0.001

Value was in ± mean standard deviation, n=3

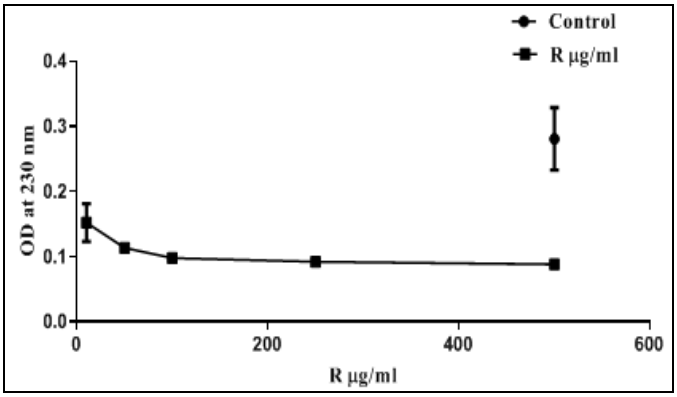


FIG. 16: COMPARATIVE GRAPH OF PHF AND CONTROL

Percentage of Inhibition:

TABLE 15: PERCENTAGE OF INHIBITION OF PHF

S. no.	PHF (µg/ml)	% of inhibition	IC_{50} value
1.	Ascorbic acid	95.6±0.443	
2.	500	68.6±1.006	42.48
3.	250	67.2±0.503	
4.	100	65.1±1.331	
5.	50	59.6±0.443	
6.	10	45.7±8.453	

Value was in ± mean standard deviation, n=3

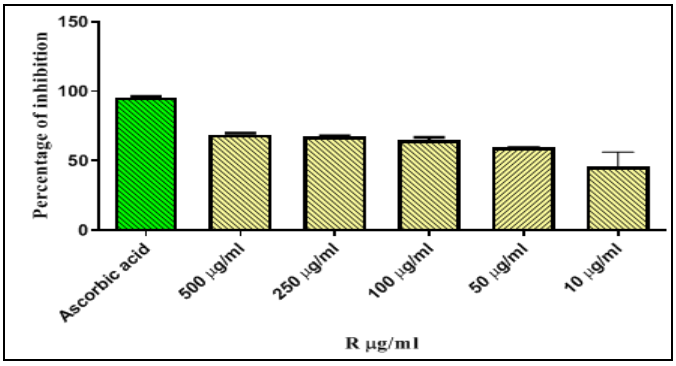


FIG. 17: COMPARATIVE GRAPH OF PHF

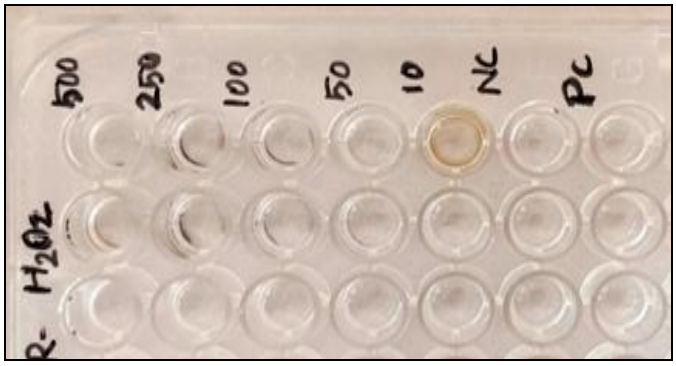


FIG. 18: PHF IN DIFFERENT CONCENTRATION

Anti-inflammatory Activity:

Inhibition of Albumin Denaturation: Polyherbal formulation is estimated and in this denaturation of proteins is estimated by 500 µL of bovine serum albumin is added to the PHF and then resulting was observed. Acetyl salicylic acid is a positive control.

OD Value at 660 nm:

TABLE 16: CONTROL FOR PHF

S. no.	PHF (µg/ml)	Mean Value
1.	Control	1.99±0.003
2.	500	1.59±0.006
3.	250	1.66±0.017
4.	100	1.72±0.023
5.	50	1.82±0.027
6.	10	1.92±0.044

Value was in ± mean standard deviation, n=3

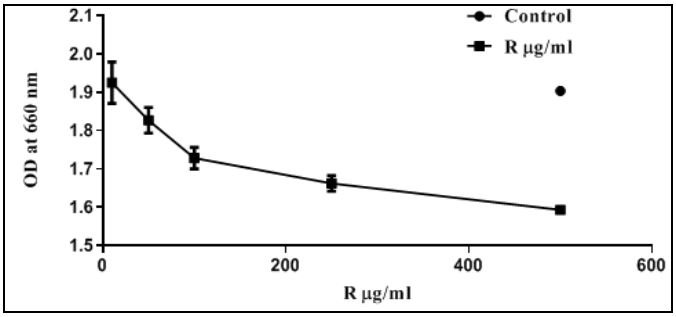


FIG. 19: COMPARATIVE GRAPH OF CONTROL AND PHF

Inhibition Percentage of Albumin Denaturation (%):

TABLE 17: INHIBITION% OF ALBUMIN DENATURATION

S. no.	PHF (µg/ml)	Inhibition % albumin denaturation (%)	IC50 value
1.	Control	100±0	
2.	500	20.05±0.343	84.58
3.	250	16.60±0.858	
4.	100	13.27±1.154	
5.	50	8.34±1.377	
6.	10	3.411±2.213	

Value was in ± mean standard deviation, n=3

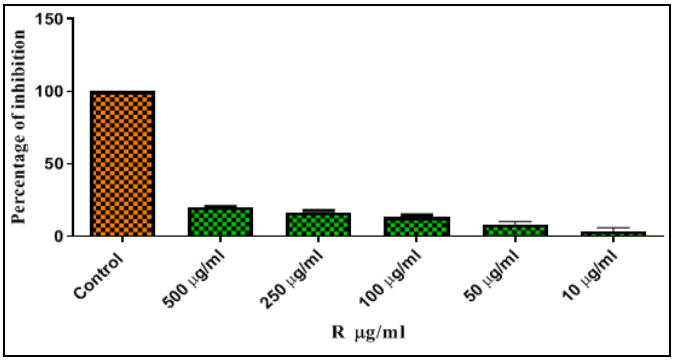


FIG. 20: GRAPH OF PHF

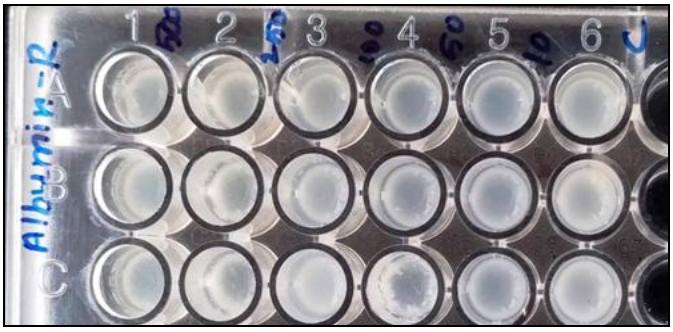


FIG. 21: PHF IN DIFFERENT CONCENTRATION

Skin Irritation Test: Skin irritation test has done for the polyherbal formulation, and it has shown that formulation does not produce any edema, inflammation, redness, and irritation. During this test the formulations are found to be very safe, and it can apply in the skin.

TABLE 18: SKIN IRRITATION TEST FOR PHF

S. no.	Name of the test	Result of test PHF
1.	Irritancy test	No redness and irritation

Summary:

Pharmacognostical Studies: Standardization for a crude drug is very critical to ensure the safety, efficacy, and consistency in its therapeutic effects. Organoleptic characteristics of the drug involves physical character such as odour, taste, colour and appearance of the drug.

Through quality and purity, we can determine standardization of the herbal drug through which we can reduce adulteration and substitution of other plant species or synthetic compounds. Indian pharmacopeia (IP) provides a guideline for the herbal drug through which we can determine specifications, identification, purity, quality of the herbal drug. In this *Ginkgo biloba, Nigella sativa, Psoralea coryliforila* are the herbal drug which is used to identify phytochemical constituents which is responsible for its therapeutic effects, identification, and authentication. This can develop the standardized herbal formulation and dosage forms as well as integration with traditional herbal medicine with modern healthcare systems.

Physical parameters are evaluated to determine quality of the crude powdered drug which includes moisture content this indicates presence of water in the drug and check the stability and shelf life. Ash value is one of the important parameters to check the quality and purity of herbal drug. Ash value is used to determine amount of total inorganic matter/ compound present in the drug after incineration. The total ash value of the *Ginkgo biloba, Nigella sativa, Psoralea coryliforila* was found to be 3.0 % w/w, 4.9%w/w, 5.3 %w/w.

Phytochemical Studies: Extractive value is used to measure the quantity of matter present in the substances. The percentage value of extractive value of a alcohol soluble extractive of a herbal drug *Ginkgo biloba* 10%w/w, *Nigella sativa* 17.5% w/w, *Psoralea coryliforila* 9%w/w. water soluble extractive value *Ginkgo biloba* 15%w/w, *Nigella sativa* 10.6 %w/w, *Psoralea coryliforila*6.3%w/w. The preliminary phytochemical was evaluated and performed to identify the presence of chemical constituents in herbal drug. It shows the presence of alkaloids, saponin, flavonoids, tannins, carbohydrates, steroids. Quantification estimation was done for the polyherbal compounds such as determination of total phenolic compound, alkaloid compound, total compound was found to be 0.6 mg/g, 1 mg/g, 1.1 mg/g. Trial and error method for the formulation of lotion in these 4 trials has done out of this one method was selected for formulation of lotion and evaluation test was done for the lotion formulation. Drug excipient compatibility studies using FT-IR Analysis in this spectrum of the herbal drug, excipients, Polyherbal formulation. formulation without excipients the absorption peaks at 1678.99 cm^{-1} (C=N stretching), polyherbal formulation with excipients the absorption 1111.51 cm^{-1} (C=S stretching), Absorption peak at 1256.94 cm^{-1} (C-O stretching (alcohols)) for *G B.* Absorption peak at 1262.05 cm^{-1} (C-O stretching (alcohols)) for *N S.* Absorption peak at 1612.37 cm^{-1}(N-H bending), is estimated. GC-MS is estimated and to determine major compound form the polyherbal formulation such as Cycloserine, Eicosanoic acid, 1-Hexadecanol, Octadecanoic acid, Acetic acid1-methylethyl ester, Diglycolic acid, Guanidine carbonate, Tridecanoic acid, Eicosanoic acid, 1-Docosene. high performance liquid chromatography (HPLC) has done comparative study with polyherbal formulation and standard, in this quertin is found in the polyherbal lotion 2.582.

Pharmacological Studies: *In-vitro* activity studies have been done in Hydrogen peroxide (H_2O_2) in this ascorbic acid is used as standard and then PHF is a test solution in this free radical scavenging activity is determine and then IC_{50} value is 42.48. Anti- inflammatory activity- inhibition of albumin denaturation in this acetyl salicylic acid is taken as positive control and then PHF is taken has sample. The absorbance of sample was determined and then IC_{50} value is 84.58. In skin irritancy test we can determine any redness; irritancy is formed in the skin when PHF is applied and there no redness and irritancy is formed.

CONCLUSION: Vitiligo is a depigmentation disorder is caused by loss or reduction of melanocytes from the skin. Vitiligo is classified into two types Segmental and Non-segmental vitiligo symptoms depigmentation in the skin, hair, and mucosal part the time increases patch will also increases. There are various method to treat vitiligo topical methos, laser method but causes side effects that is reason for choosing herbal drug to treat vitiligo, herbal drug doesn't produce any side effects. The Pharmacognostical studies for this research can be utilized to determine the standardization of the crude drug that is selected for the study. The preliminary phytochemical analysis was performed and identified presence of maximum number of phytoconstituents present in methanol. Through FT-IR analysis peak value is determined for herbal drug, polyherbal formulation

with excipients and without excipients. The GC-MS analysis of the polyherbal formulation has determined some compound which as biological activity and that produces response. HPLC is used to determine biomarker present in polyherbal formulation. The *in-vitro* antioxidant, anti-inflammatory activity, and skin irritation test has been done. Through Hydrogen peroxide scavenging assay confirmed that polyherbal formulation has a activity of antioxidant. Anti-inflammatory activity has done through Inhibition of albumin denaturation Assay has confirmed that anti-inflammatory cavity in polyherbal formulation. Skin irradiation test is done and there no redness and irradiation are found in the skin.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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