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FORMULATION AND INVITRO EVALUATION OF HERBAL SKIN WHITENING CREAM OF GLYCYRRHIZA GLABRA EXTRACT AND SOLANUM TUBEROSUM JUICE

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***Abstract*:** Now a days there is increasing demand for the herbal cosmetics in our country. Present study describes the formulation of cream by using *Glycyrrhiza glabra* root extract and *Solanum tuberosum* juice. The purpose of study is to develop and formulate herbal skin whitening cream with minimum side effects and combine *Glycyrrhiza glabra* root extract was prepared by maceration method and it is evaluated by various phytochemical and pharmacognostic studies. Extract was identified by UV spectroscopic analysis and IR spectroscopy. The *solanum tuberosum* juice was stabilized by using lemon juice, methyl paraben and propyl paraben. By changing the concentration of olive oil and *solanum tuberosum* juice 6 different types of creams are formulated. The concentration of extract used in cream was 1%. In - vitro evaluation of all batches was carried out and other evaluations like colour, appearance, freeze thaw test, sun exposure test, drug content, viscosity, centrifugation testing was carried out. From the above evaluations it is concluded that F4 batch was the optimized batch. F4 batch was found stable for 3 months at room temperature by visual observation.

***Index Terms - Glycyrrhiza glabra* extract, *Solanum tuberosum* juice, cream, drug content, skin whitening.**

1.Introduction

Cosmetics are used to enhance the beauty of human beings. Herbal plants are widely used in cosmetic products. Cream is a o/w or w/o emulsion which contain aqueous and oily phase. Cosmetic preparations are used to treat the various skin disorders like skin pigmentation, skin ageing. There are many people who use skin lightening creams to treat the various skin disorders. skin wrinkles. *Glycyrrhiza glabra* (liquorice) is belonging to family leguminaceae. [1] Glycyrrhizic acid present in glycyrrhiza glabra extract is mainly responsible for the skin whitening effect. Glycyrrhizic acid does not affect the synthesis of DNA. Glycyrrhizic acid decreases the synthesis of tyrosinase enzyme and the also decreases melanin content in the skin. As a result of this it causes skin whitening. [2] Phytochemical screening shows that *Glycyrrhiza glabra* root extract contains saponins, glycosides and carbohydrates. Glabridin, licochalcone, are also present in *Glycyrrhiza glabra* root extract. Ethanolic extract of *Glycyrrhiza glabra* root extract increases viscoelastic and hydration properties of skin. *Glycyrrhiza glabra* root extract and *Solanum tuberosum* juice to get the maximum effect. [3]

Raw *Solanum tuberosum* juice is having invaluable, amazing properties and act as best natural skin lightener with minimum side effects. Raw *Solanum tuberosum* juice is helpful to prevent the wrinkles on face and act as anti-ageing agent. Raw *Solanum tuberosum* juice contains vitamin C, enzymes and starch, zinc, antioxidants, catecholase enzyme, water. [4] Solanum tuberosum juice when mixed with lemon juice then it is used to lighten the darker skin areas. Potato starch act as thickening agent in various cosmetics. Azelaic acid in the juice of solanum tuberosum inhibit tyrosinase enzyme activity and decreases dark spots, pigmentation, acne on skin. [5] *Solanum tuberosum* juice act as exfoliator and used to remove the dead skin cells on the skin. [6] Vitamin C in the juice helps to maintain healthy glow of skin. Lutein, zeaxanthin, are the antioxidants in solanum tuberosum juice that decreases ageing process and decreases the inflammation of skin. [7]

The objective of this study is formulation of herbal skin whitening cream with reduced the side effects than allopathic drugs and combine *Glycyrrhiza glabra* root extract and *Solanum tuberosum* juice to get the maximum effect. To stabilise the *Solanum tuberosum* juice by adding stabilizers is very important. Aim of this study is to give affordable alternative to costly synthetic medicines to poor people with various skin problems.

2.Materials and methods:

2.1 Materials:

Glycyrrhiza glabra root powder was purchased from Manikarnika herbal shop, Chinchwad, Pune, and healthy *Solanum tuberosum* tubers collected from local market. Stearic acid, sodium lauryl sulphate and all other excipients were obtained from Ana lab Fine Chemicals Mumbai. Double distilled water and analytical grade quality solvents were used throughout the research work.

2.2 methods:

2.2.1 Stabilization of *Solanum tuberosum* juice:

Solanum tuberosum juice was not stable at room temperature so we had added different types of preservatives and stabilizers to stabilise *Solanum tuberosum* juice.

Stabilization of potato juice was done by adding different preservatives.

1. *Solanum tuberosum* juice + *Citrus lemon* juice + methyl paraben + propyl paraben. [8]
2. *Solanum tuberosum* juice + Ascorbic acid (0.1%) + methyl paraben+ propyl paraben. [9]
3. *Solanum tuberosum* juice + Ascorbic acid (0.2%) + methyl paraben+ propyl paraben. [10]
4. *Solanum tuberosum* Juice + BHT (0.9%)
5. *Solanum tuberosum* juice + sodium benzoate (0.1%) + benzoic acid (0.1%). [11]
6. *Solanum tuberosum* juice

Figure 1: day 1 of stability test.

Figure 2: day 60 of stability test.

The sample containing *Solanum tuberosum* juice, Citrus lemon juice, methyl paraben, propyl paraben was found to be stable by visual for 60 days.

2.2.2 Preparation of extract of *Glycyrrhiza glabra* roots:

Glycyrrhiza glabra root extract was prepared by maceration method: [12]

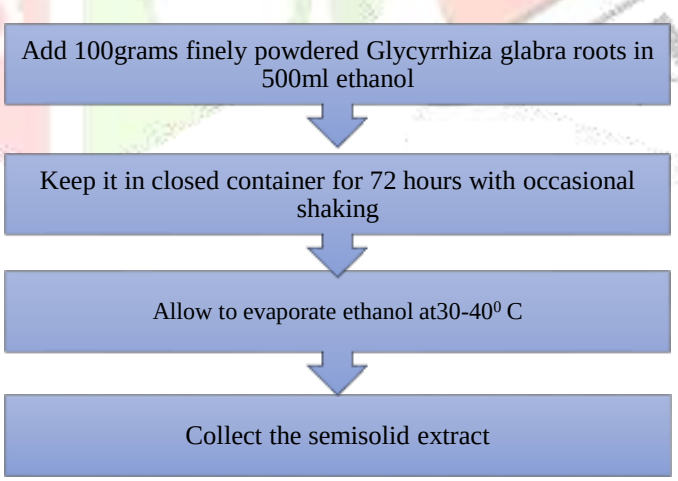

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Figure 3: preparation of extract

Figure 4: *Glycyrrhiza glabra* root extract

2.2.3 Phytochemical and pharmacognostic study of *Glycyrrhiza glabra* extract:

2.2.4 Phytochemical tests of extract:

Preliminary phytochemical screening of the extract of *Glycyrrhiza glabra* root was carried out by established methods. The extract was screened for different chemical as well as physical test**.** [13]

A. Test for alkaloids: The extract was dissolved in water. It was shaken well and filtered. The filtrate was used to perform following tests.
a. Dragendroff's test: few drops of dragendroff"s reagent was added to 2-3 ml filtrate. Presence of alkaloids is indicated by formation of orange brown precipitate.
b. Mayer's test: few drops of Mayer's reagent were added to 2-3 ml filtrate. Presence of alkaloids is indicated by formation of cream coloured precipitate.
c. Hager's test: few drops of Hager's reagent were added to 2-3 ml filtrate. presence of alkaloids is indicated by formation yellow precipitate.
d. Wagner's test: few drops of Wagner's reagent were added to 2-3 ml filtrate. presence of alkaloids is indicated by formation

B. Test for glycoside:

Borntrager's test: to 3 ml extract, add dil. H2SO4.Boil and filter, to cold filtrate add equal volume of benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia, ammonial layer turns pink or red.

C. Test for saponins:

a. Foam test: shake the drug extract or dry powder vigorously with water. Persistent foam observed.
b. Haemolytic test: add drug extract to one drop of blood placed on glass slide. Haemolytic zone appears.

D. Test for carbohydrate:

Molisch's test: 3 ml of the aqueous extract was added to 2ml of Molisch reagent and the resulting mixture shaken properly, then 2ml of concentrated H2SO4 was poured carefully down the side of the test tube. A violet ring at the interphone indicates the presence of carbohydrates.

E. Test for tannins:

About 2ml of aqueous extract was stirred with 2ml of distilled water and few drops of FECL3 solution were added then formation of green precipitate was an indication of presence of tannins.

2.2.5 Pharmacognostic tests of extract:

2.2.6 Determination of ash value

A. Determination of total ash value:

Weigh and ignite flat, thin, porcelain dish. weigh about 1 gm of the powdered drug into the dish. Support the dish on a pipe clay triangle on ring of retort stand. Heat with a burner, using a flame about 2cm high and supporting about 7cm, above the flame, heat till vapours almost cease to be evolved, then lower the dish and heat more strongly until all the carbon burnt off. Cool in desiccator. Weigh the ash and calculate the percentage of total ash with reference to the air-dried sample of crude drug. [14]

B. Acid insoluble ash value:

Using 25ml of dil. HCL wash the ash from the dish used for total ash into a 100ml beaker. Place wire guaze over a Bunsen burner and boil for 5 min. filter through an ash less filter paper, wash the residue twice with hot water. Ignite a porcelain dish in the flame, cool and weigh. Put the filter paper and residue together into the porcelain dish, heat gently until vapours cease to be evolved and then more strongly until all carbon has been removed. Cool in desiccator. Weigh the residue and calculate acid-insoluble ash of the crude drug with reference to the air-dried sample of the crude drug. [14]

C. Sulphated ash value:

This is determined by a similar way to acid insoluble ash, 25ml of dilute sulfuric acid, in place of dil. Hydrochloric acid. [14]

D. Water soluble ash value:

This is determined by a similar way to acid insoluble ash, 25ml of water in place of dil. Hydrochloric acid. [14]

2.2.7 Determination of extractives value

A. Alcohol soluble extractives:

Fill a 100ml graduated flask to the delivery mark with the solvent (90%alcohol) wash out the weighing bottle and pour the washings together with the remain of the solvent into a conical flask. Cork the flask and set weigh about 5gm of the powdered drug in a weighing bottle and transfer it to a dry 250ml conical flask. Aside for the 24 hours, shaking frequently. Filter into a 50ml cylinder. When sufficiently filter has collected, transfer 25ml of filtrate to weighed porcelain dish. Evaporate to dryness on water bath and complete drying in oven. Cool in desiccator, weigh. Calculate the percentage w/w of extractive with reference to air dried drug. [15]

B. Water soluble extractive value

Weigh 5gm of drug in weighing bottle and transfer it to dry 250ml conical flak. Fill 100ml graduated flask to the delivery mark with chloroform water wash out the weighing bottle and pour the washings together with the remain of solvent. Cork the flask and kept aside for 24 hours with shaking. Filter into a 50ml cylinder. Add filtrate to porcelain dish. Evaporate on water bath and then oven. Cool in desiccator. Calculate the percentage w/w of extractive with reference to air dried drug. [15]

2.3 Estimation of extract using UV spectroscopic method:

2.3.1 Preparation of stock culture:

An accurately weighed standard extract (10mg) were transferred to 100ml separate volumetric flask and dissolved in 70ml phosphate buffer 7.4 and 30ml ethanol (70:30). The flask was sonicated for 15 min to give clear solution and volume were made up to mark with to give a solution containing 100ug/ml.

2.3.2 Calibration curve of extract in methanol:

The working standard solution of extract 25 ug/ml was individually scanned in the range 200-400nm to determine maximum wavelength. The maximum wavelength was found to be 255nm of extract. Standard solutions of extract in the range of 5,10,15,20,25,30 ug/ml were obtained by transferring 0.5,1,1.5,2,2.5,3 ml of the stock solution to 10ml of volumetric flask. The volume in volumetric flask was made up with same solvent. The absorbances of these solutions were measured and absorptivity was calculated using Beer's Lambert law.

2.4 Drug excipient compatibility studies:

A. Physical compatibility test: A physical compatibility test was carried out to determine drug-excipient interaction/compatibility. Drug and excipient were mixed uniformly in 1:1 ratio with extracts for individual drugs and excipients. The mixture was placed in glass vials which were kept at room temperature. After 15 days samples were evaluated for colour/appearance for compatibility testing. [16]

B. FT-IR Spectroscopy:

The Fourier Transform Infrared (FTIR) spectroscopy was employed to characterise the possible interaction between the drug and excipient in solid state. It was recorded by using FT-IR spectrophotometer by using KBr technique. The spectra were scanned over a frequency range 400-4000 cm^{-1}.

3.Formulation of herbal cream:

The herbal skin whitening cream was prepared by using the dried ethanolic extract of *Glycyrrhiza glabra* roots and *Solanum tuberosum* juice. The cream was prepared using Stearic acid, cetyl alcohol, olive oil, jojoba oil, Lemon juice, and distilled water in a quantity sufficient to prepare 100gm of herbal cream. Firstly, stearic acid, cetyl alcohol, sodium lauryl sulphate are heated at 70^0C in a china dish on water bath. Then jojoba oil and olive oil are added in it. Extract was dissolved in water and preservatives, lemon juice are added in it in a separate beaker. This mixture was warmed at 70^0C and added in oily phase with continuous stirring. Allow to cool the cream and kept in a closed container. [17]

Table 1: formulation of herbal skin whitening cream.

Sr. No.	Ingredients	F1	F2	F3	F4	F5	F6
1	Extract	1%	1%	1%	1%	1%	1%
2	M.P. (gm)	0.2	0.2	0.2	0.2	0.2	0.2
3	P.P. (gm)	0.02	0.02	0.02	0.02	0.02	0.02
4	Stearic acid (gm)	10	10	10	10	10	10
5	Cetyl alcohol (gm)	5	5	5	5	5	5
6	S.L.S, (gm)	10	10	10	10	10	10
7	Olive oil(ml)	5	6	7	8	9	10
8	Jojoba oil(ml)	5	5	5	5	5	5
9	Potato juice(ml)	10	20	30	40	50	60
10	Lemon juice(ml)	20	20	20	20	20	20
11	Distilled water(ml)	upto 100ml	upto 100ml	upto 100ml	upto 100ml	upto 100ml	upto 100ml

Figure 5: all 6 batches of harbal skin whitening cream

4. Evaluation of herbal cream:

4.1Appearance:

All the 6 batches of herbal cream are tested for apperance by visual observation. [18]

4.2Determination of pH:

The pH of herbal cream was determined by using a pH meter. The measurements was performed at 1, 30, 60, 90 days after preparation to detect any pH changes with time. [18]

4.3 Vicosity:
The viscosity of the prepared herbal skin whitening cream was carried out by Brookfield viscometer (Model RVTDV). The readings were taken at 100 rpm using spindle no.6. [18]

4.4 Drug diffusion:
The drug diffusion of all 6 batches are cacrried out by Franz diffusion cell using cellophane membrane. Franz diffusion cell consist of 2 compartments one is donor and other is receiver. And in between th 2 compartment cellophane mebrane is placed. [18]

4.5 Drug content:
For determination of drug content 1gm of cream was dissolved in 30 ml of methanol and kept for 1hour by continuous stirring. After 1 hour the solution was ultra-sonicated for 15 min to get uniform solution. And then absorbance of that sample was measured at 255nm by using UV spectrophotometer and drug content was calculated. [18]

4.6 Centrifugation testing:
For cetrifugation testing all 6 batches of cream are placed in cetrifugation testing apparatus and the saperation of two phases was observed.

4.7 Freeze thaw test:
In freeze thaw testing herbal creams is placed in freeze at low temperature and then cream was placed at room temperature. This cycle was repeated for 5 times and changes were observed by visual observation. [18]

4.8 Sun exposure evaluation:
In the sun exposure evaluation cream was placed under sun light for 24 hours and the changes are observed by visual observation.

4.9 Homogeneity test:
All creams were tested for physical homogeneity by visual observation. [18]

4.10 Stability study of herbal cream:
The stability study was performed as per ICH guidelines. The formulated harbal creams are filled in well closed containers and stored at different temperatures and humidity conditions, viz. $40^0 \pm 2^0$c and 75 ± 5% RH for period of 3 months. Samples were taken after 3 months and evaluated for appearance, pH, viscosity. [19]

4.11 Antimicrobial study:
We conducted observational study of antibacterial activity of F4 batch which is a optimised batch. First of all the MIC (Minimum Inhibitory Concentration) of the extract was carried out aginst *Escherichia coli*. The harbal skin whitening cream was screened agianst bacterial strains of *Escherichia coli* by using agar disc-diffusion assay. The zone sof inhibition were measured. The prepared herbal skin cream was compared with the marketed formulation of glycyrrhiza glabra root extract craem for antimicrobial activity. [20]

5.Result and discussion:

5.1 Phytochemical tests of extract:

From the phytochemical tests it is concluded that saponins, glycosides, carbohydrates are present in the given extract of *Glycyrrhiza glabra* roots.

Table 2: phytochemical test

Phytoconstituents	Test performed	result
alkaloids	Dragendroff's test Mayer's test Hagger's test Wagner's test	- - - -
Tannins	Gelatin test	-
proteins	Heat coagulation test	-
Saponins	Froth test Haemolytic test	+ +
Glycosides	Bontrager's test	+
Carbohydrates	Molisch's test Benedict's test	+ +

5.2Pharmacognostic tests of extract:

A. Determination of ash value

Ash value was found to be 13%.
Acid insoluble ash value was found to be 4%.
Sulphated ash value was found to be 6%.
Water soluble ash value was 3%.

B. Determination of extractives value.

Alcohol soluble extractive was found to be 8%.
Water soluble extractive value was found to be 22.4%.

5.3 UV analysis of *Glycyrrhiza glabra* extract:

UV Spectrum of *Glycyrrhiza glabra* extract (maximum wavelength was found to be 255nm).

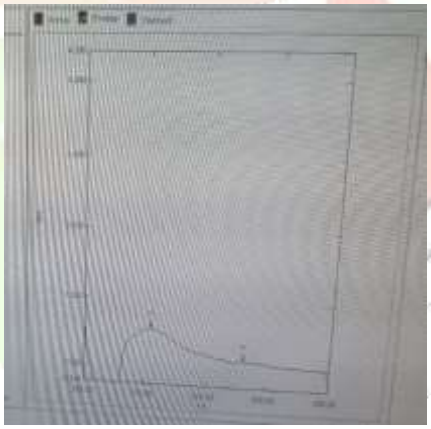

Figure: UV Spectrum of *Glycyrrhiza glabra* extract

Table 3: Absorbance range of extract at different concentration

Conc.(ug/ml)	Absorbance of extract
5	0.1725
10	0.3496
15	0.5088
20	0.6676
25	0.874
30	1.0079
r^2	0.9981
slope	0.0053
intercept	0.0338

5.4 FT-IR Spectroscopy:

During excipient interact is important to study while preparing a formulation from drug extract was mixed with *solanum tuberosum* juice stearic acid, S.L.S. cetyl alcohol and IR grade KBr and was scanned over range of 400 – 4000 cm using FTIR instrument (FTIR – IR affinity -1S Shimadzu Japan). Extract exhibit various peaks due to alcoholic group, carboxyl group, C-O stretch and C=C stretch, C-H bend in plane present in extract. It was observed that main peak of extract does not show changes in peak drug-excipient compatibility studies by FTIR shows that no interaction between extract and excipients used in formulation thus extract and excipient are compatible with each other.

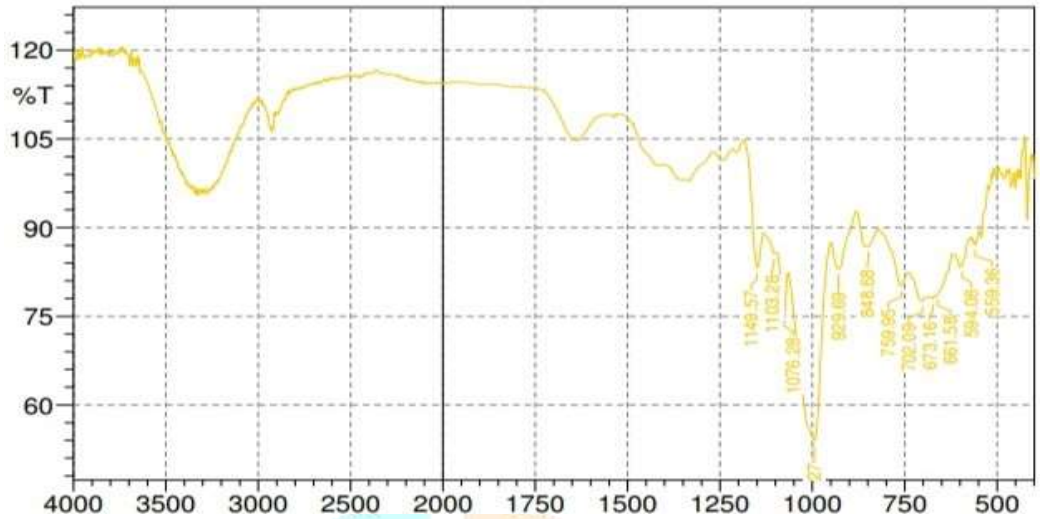

Figure 7: FTIR Spectrum of extract

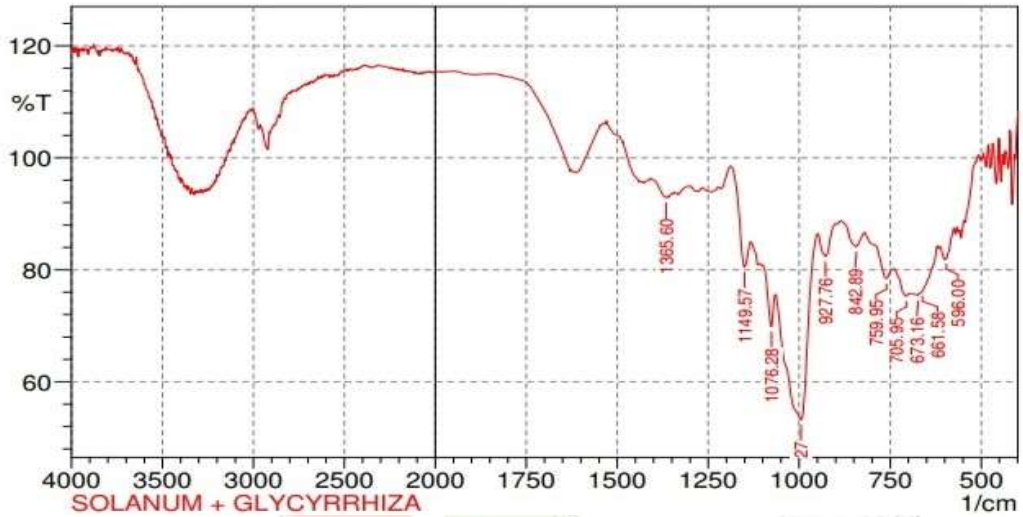

Figure 8: FTIR spectrum of extract and *Solanum tuberosum* juice

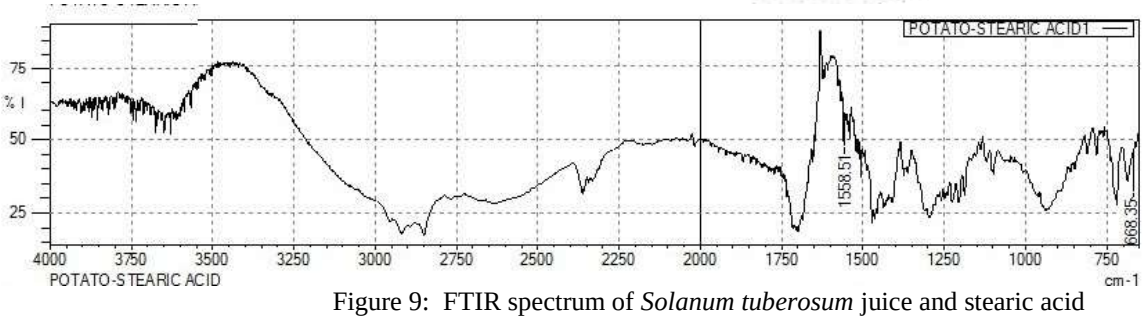

Figure 9: FTIR spectrum of *Solanum tuberosum* juice and stearic acid

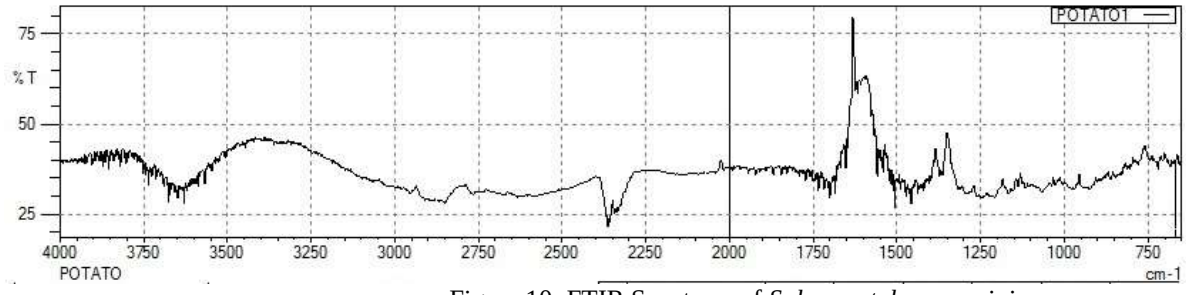

Figure 10: FTIR Spectrum of *Solanum tuberosum* juice

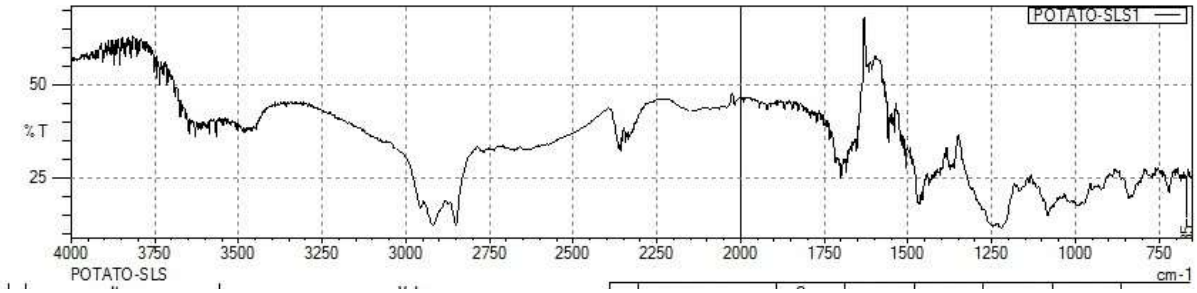

Figure 11: FTIR Spectrum of *Solanum tuberosum* juice and S.L.S.

5.5 Evaluation of herbal skin whitening cream:

Table 4: evaluation parameters of herbal cream

Formulation	Drug diffusion	pH	Viscosity	Drug content	Centrifugation test	Freeze thaw test	Sun exposure test
F1	79.15	6.63	3800	91.58	Pass	Pass	Pass
F2	85.69	6.69	3700	94.35	Pass	Pass	Pass
F3	82.6	6.5	3600	95.45	Pass	Pass	Pass
F4	92.58	6.78	3900	98.65	Pass	Pass	Pass
F5	89.5	6.66	3800	97.46	Pass	Pass	Pass
F6	81.94	6.49	3500	96.99	Pass	Pass	Pass

5.6 Stability study:

Table 5: stability study for first month

Formulation	Appearance	Viscosity(cp)	PH
F1	Yellowish brown	3800	6.97
F2	Yellowish brown	3800	6.98
F3	Brown	3700	6.99
F4	Brown	3900	6.99
F5	Brown	3800	6.73
F6	Brown	3600	6.85

Table 6: stability study of second month

Formulation	Appearance	Viscosity(cp)	PH
F1	Yellowish brown	3900	6.90
F2	Yellowish brown	3800	6.75
F3	Brown	3700	6.89
F4	Brown	4000	6.88
F5	Brown	3900	6.70
F6	Brown	3700	6.81

Table 6: stability study of Third month

Formulation	Appearance	Viscosity(cp)	PH
F1	Brown	3900	6.89
F2	Brown	3900	6.71
F3	Brown	3700	6.83
F4	Brown	4000	6.87
F5	Dark brown	3900	6.69
F6	Dark brown	3800	6.80

All the study shows that all 6 creams are stable for 3 months but all the formulations are showing some deviations in parameters but it was not too much so it was acceptable.

5.7 Antimicrobial study:

Firstly, it is important to calculate the MIC of the extract against the particular microbes that may be bacteria. In this study we had used 1 bacterium (E-Coli). The MIC of extract was found to be 3.5ug/ml. Zone of inhibition for extract was found to be 12mm and for herbal cream zone of inhibition was 14mm. We had also calculated the zone of inhibition of marketed formulation and that was found to be 15mm.

Table 7: zone of inhibition of different sample

Sample	Zone of inhibition(mm)
extract	12
Herbal cream	14
Marketed formulation	15
Standard(tetracycline)	16

Figure 12: the MIC of extract (3.5ug/ml).

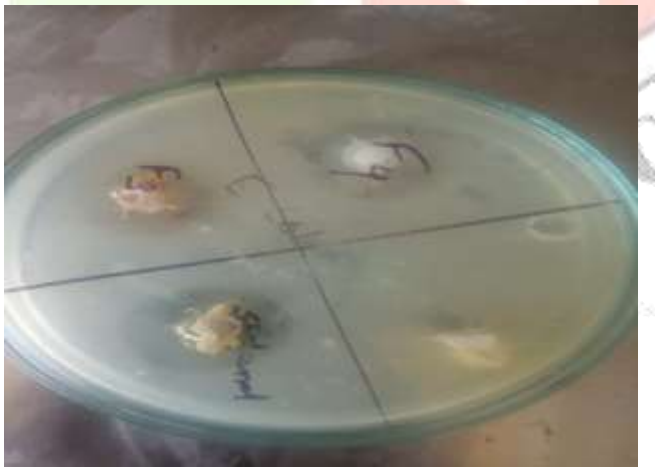

Figure 13: zone of inhibition of cream and extract in E-Coli culture

6. Conclusion:

Herbal skin whitening cream of *Glycyrrhiza glabra* root extract and *Solanum tuberosum* juice by using jojoba oil, olive oil was prepared and evaluated. The cream containing 8ml of olive oil and 40ml of *Solanum tuberosum* juice shows higher values of drug diffusion. So that F4 batch was concluded as optimized batch within these 6 batches. From invitro studies it is concluded that *Solanum tuberosum* juice and olive oil both are used to increase penetration and absorption of cream for 6 hours. Hence the present study demonstrates that *Solanum tuberosum* juice and glycyrrhiza glabra extract were used together with additional skin benefits. And F4 optimized batch was stable for 3 months with higher drug diffusion.

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