FORMULATION, EVALUATION AND METHOD DEVELOPMENT OF ANTIFUNGAL CREAM BY PLANT EXTRACTION OFAGARICUS BISPORUS

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Abstract- **The scientific name of mushroom is Agaricus Bisporus. Mushrooms have played a significant role in traditional medicine across numerous Cultures. The use of medicinal mushrooms has a long history in India, where their therapeutic benefits were highly regarded.People have been using mushrooms for food and medicine and lowers the risk of various diseases by controlling and modulating several bodily functions.**

The mushroom has the medical effects of mushrooms due to their antifungal, antibacterial, antioxidant, and antiviral qualities in addition to their usage as functional foods are supported by contemporary pharmacological research.The antifungal cream has fewer adverse effects and is highly beneficial. Every natural ingredient is readily available on the market. The antifungal cream is used to treat fungal infections, which typically damage our skin and hair. Jockey itch, ringworm, and athletes' foot are examples of fungal skin infections that are treated with antifungal creams. This herbal antifungal cream helps to reduce fungal infections and is safe and natural to use.

Index Terms- **Mushroom, Antifungal, Agaricus Bisporus, Ringworm,Natural**

I. INTRODUCTION

The use of medicinal mushrooms has a long history in India, where their therapeutic benefits were highly regarded. People have been using mushrooms for food and medicine. More and more people are realizing that eating a healthy diet helps to maintain excellent health, which lowers the risk of various diseases by controlling and modulating several bodily functions. Large portions of conventional knowledge about the medical effects of mushrooms due to their antifungal, antibacterial, antioxidant, and antiviral qualities in addition to their usage as functional foods are supported by contemporary pharmacological research.

The Various Extraction methods used for mushroom extraction

1. Drying
    i. Microwave Drying
    ii. Oven Drying
2. Extraction with Microwave Assisted
3. Soxhlet apparatus
4. Traditional Method of Extraction
    i. Maceration
    ii. Infusion
    iii. Percolation
    iv. Decoction
5. Reflux Extraction
6. Ultrasound assisted Extraction or sonication extraction.

Principle of UV Spectroscopy:

The UV visible principle the distinctive spectra that are created when chemicals absorb visible or ultraviolet light are the basis of spectroscopy. The foundation of spectroscopy is the relationship between matter and light. When matter absorbs light and experiences excitation and de-excitation phases, a spectrum is produced. When an electromagnetic wave strikes a substance, it can cause transmission, absorption, reflection, and scattering, among other effects. The observed spectrum demonstrates the interactions between various wavelengths and discrete-dimensional objects, including molecules, macromolecules, and atoms. Absorption occurs when the frequency of the incoming light matches the difference in energy between the excited and ground states of a molecule. This is the fundamental mechanism of molecular spectroscopy

UV-Visible spectroscopy is based on the principle that molecules absorb light in the ultraviolet (UV) and visible regions of the electromagnetic spectrum. When light passes through a sample, certain wavelengths are absorbed by the molecules, while others are transmitted.

Fig. No. 1 UV Spectroscopy

Principle Infrared (IR) spectroscopy:

FTIR spectroscopy is based on the principle that molecules absorb IR radiation at specific frequencies, resulting in changes in their vibrational and rotational energy levels. The IR radiation is absorbed by the sample, causing the molecules to vibrate and rotate. The resulting spectrum is a plot of the absorbed IR radiation against the wavenumber (cm-1).

Infrared (IR) spectroscopy is based on the principle that molecules absorb specific wavelengths of infrared radiation, causing vibrations in their bonds.

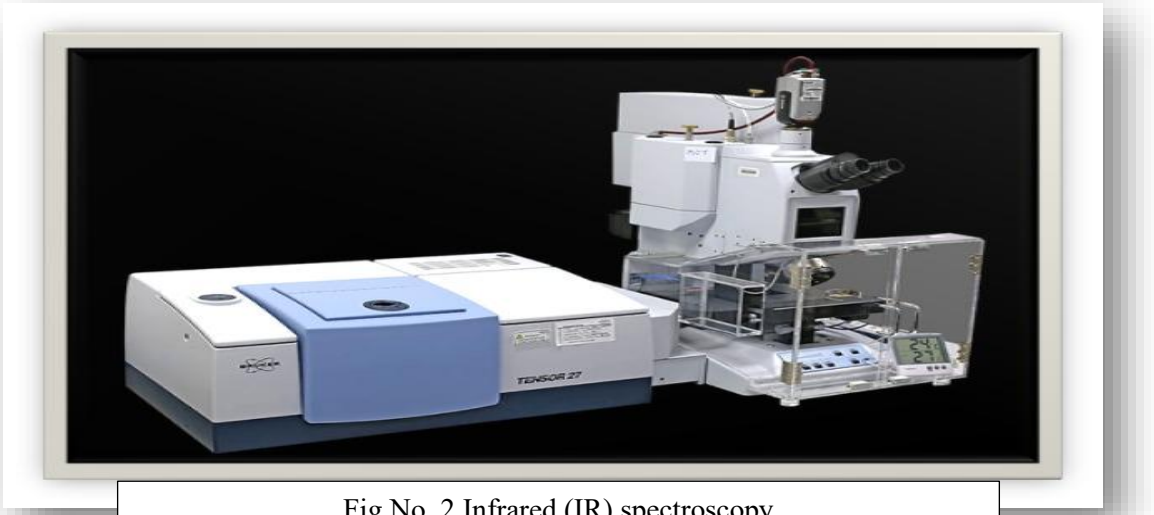


Fig No. 2 Infrared (IR) spectroscopy

Evaluation Parameter

1. Solubility
2. Irritancy
3. Sprediablity
4. Viscosity
5. PH
6. Homogenecity
7. Microbial Growth Test

Method Development

1. Wavelength
2. Linearity
3. Precision
   i. Interday Precision
   ii. Intraday Precision
4. Robustness
5. Ruggedness
6. LOD & LOQ

Novelty of Work:

1.The mushroom extract is used as an antifungal agent.

2.Also, we prepared a new formulation of cream.

3.We prepared our self-cream formulation method as well as evaluation of cream, and new method development for analytical technique.

II. REVIEW OF LITERATURE

1. Miss. Tembhe Kajal[1] Miss. Harad Aratis [2], Miss. Asawe Tejaswini, Miss. Vikander Siddhi[4], Miss. Mharse Tanuja. To study the

growing herbal plants for natural skin care product, this study Introduces a novel approach to the fungal or bacterial infection by formulating and evaluating and antifungal or Antibacterial cream. That utilizes bottom Mushroom (Agaricus bisporus mushroom). Powder as the primary active ingredient. To gain a comprehensive understanding of the active ingredient, the study includes and analysis of its chemical test, cultivation, and microbial assay. Additionally, the phytochemical testing is conducted to formulated and evaluate the Antifungal activity of the Agaricus bisporus mushroom powder extract, using Soxhlation method.

2. Aarti Kotnala1, Priya Chaudhary2, Kiran Bisht3, Neetu Neg4 Many of the mushrooms and fruiting bodies of fungi are edible in nature and act as a great source of protein, whereas some other mushrooms reported to have narcotic effect and utilized as a medicine. They are enriched in nutritional components such as trace elements, fibers, minerals, vitamins, and proteins, and have low content of cholesterol. They are utilized by the individual as an additional vegetable due to its great quality and have beneficial influence on the human fitness and health. Mushrooms reported to have healing properties and utilized for the treatment of various diseases. They are used as anticancer, antiviral, and antibacterial agents due to the presence of active bio-constituents.
3. Maryam Eskandari-Nojedehi, Hoda Jafarizadeh-Malmiri* and Javad Rahbar-Shahrouzi Edible mushroom (Agaricus bisporus) extract was used to synthesize gold nanoparticles (AuNPs) through hydrothermal process (at a pressure of 15 psi and a temperature of 121°C for 15 min). Response surface methodology was applied to monitor the influence of the synthesis parameters, namely: the mushroom extract concentration (1–9 gr DP/100 ml distilled water) and the amount of HAuCL4 · 3H2O solution (8–12 ml) on the particle size and concentration of fabricated AuNPs.Abhishek Bisai2, Vinita Singh1, Nitin Kumar2, Deborah Yukti Tandi1, Harish Sharma1 and Gyanesh Kumar Sahu1*Fungal diseases become a major medical problem. Fungal disease is difficult to manage because they tend to be chronic, hard to.
4. The fungal infection is a common condition caused by fungi. The herbal antifungal cream was formulated by using various herbs such as neem and aloe vera. Herbal medicine is one of the oldest and most universal system of health care system. The herbal antifungal cream is very helpful and it is fewer side effects. All herbal ingredients are easily available in market. The herbal antifungal cream is used to treat fungal infection which most commonly affect our skin, hair and nails. Herbal antifungal cream is used to treat fungal skin infection such as athletes foot, ringworm and jock itch. This herbal antifungal cream represents a natural and safe to use, and this herbal antifungal cream is beneficial in reduction of fungal infection.
5. M. Krishnaveni and M. Manikanda Extract prepared from mushrooms were tested for their antimicrobial activity against E. coli, Klebsiella sp. Three different solvent system like acetone, ethanol, methanol was used for the extraction process. The mushrooms such as Ganoderma lucidum, Pleurotus florida, Calocy be indica were selected for the present study. The antimicrobial study was done. The zone of inhibition observed was compared with standard antibiotics. The zone of inhibition observed was slightly smaller when compared to standard antibiotics. Among the three species selected Ganoderma lucidum showed better zone of inhibition compared to other two. The observed difference in the zone of inhibition might be due to the solvent system used in the extraction process.

III. MATERIAL & METHOD

Sr. No	Sample	Grade
1.	Standard sample	Lab Scale
2.	Bees Wax	Lab Scale
3.	Peppermint Oil	Lab Scale
4.	Borax	Lab Scale
5.	Silica Gel	Lab Scale
6.	Bentonite	Lab Scale
7.	Methyl paraben	Lab Scale
8.	Steric Acid	Lab Scale
9.	Propylene Glycol	Lab Scale

Table no. 1: Material & Method

HYPOTHESIS: EXTRACTION PROCEDURE

After being regularly cleaned with double-distilled water to get rid of any organic contaminants on their surface, the mushrooms were broken up into tiny pieces and allowed to dry in the shade for three days. A home miller was used to powder the dried mushroom bits. 5gm of powdered mushroom was combined with one 100ml of double-distilled water in 250 milliliter Erlenmeyer flasks to create the reduction broth. The mixture was then heated for ten minutes and filtered using Whatman No. 1 filter paper. Until it was needed again, the supplied mushroom extract was kept in the refrigerator at 4°C.

Extraction Procedure for Mushroom

I. They are wash with distilled Water.
II. They are taken for drying for Few min.
III. Cut into small pieces.
IV. They are given in beaker & Heat.
V. They are standing until the moisture is removed.
VI. To Obtain the pure extract of mushroom.

Method of Preparation of Cream:

In Beaker A

Take the mushroom extract and boil it on a water bath at 70°C while stirring constantly.

In Beaker B

i. Take Glycerine and boil.
ii. Add bees wax with continuous stirring.
iii. Add borax and silica gel with continuous stirring.
iv. Then add methyl paraben.
v. Mix beaker A&B together with continuous stirring.
vi. Add bentonite with continuous stirring.
vii. Then the formulation cools in room temperature for 5 min.
viii. Then add the Fragrance.

EVALUATION PARAMETER:

1. Solubility
2. Irritancy
3. Sprediablity
4. Viscosity
5. PH
6. Homogenecity
7. Microbial Growth Test
8. Thermal Stability

1. Solubility:

Take a Distilled water, Methanol, Aceto nitrile. Then the take a 0.1 gm cream dissolved in all solvent. To determine the cream is dissolved in Distilled water.

2. Irritancy:

Mark a 1sq.cm.area on the dorsal surface of left hand. Time was recorded as the cream was administered to the designed area. Edema, Erythema And irritation ware monitored for up to 24hours at regular interval and reported.

3. Sprediablity:

The spreadability test evaluate an herbal antifungal creams Capacity to cover and disperse a certain surface area uniformly after application. this test usually entails applying a predetermine amount of cream on standardized surface like substrate mimetic skin and then measuring the spread's diameter after a substrate amount of time.

The creams spredability is affected by variables such as viscosity, texture, and formulation Ingredient.

4. Viscosity:

The viscosity of Formulated creams was measured by Brook Field Viscometer LVD using Spindle SPL4 At 6 rpm Speed And shear Rates. The Measurements were done over the Range of speed 60 rpm between two successive speeds as equilibrium with the Shear rate. Viscosity determination was Performed at 32℃ Temperature.

5. PH:

The PH of Semi solid (cream) Formulation were Determined by using Digital PH meter. Weight 0.1gm of cream and dispersed in 10 ml distilled water and stored the 1hr. Then measurement of PH by using digital PH meter.

6. Homogeneity:

The herbal antifungal cream to be consistently effective and applied it must be homogeneous. It is crucial to assure that the active component is distributed uniformly throughout the product to ensure that every application provide the desired effects. Achieving homogeneity requires careful ingredient mixing quality control procedure, and formulation and manufacturing procedure, Particle size reduction, mixing and emulsification are some methods used to attain the required uniformly. Furthermore, Analytical Technique like Spectroscopy, Microscopy and visual inspection are used homogeneity.

7. Microbial Growth test:

To test for microbial growth in designed creams, the creams were made as a control and inoculated using the cup plate method on Agar media plates. The plates were put in the incubator and left there for 72 hours at 37℃. Plates were removed after the incubation period and the microbial growth was examined by contracting it with the control.

Method Development Parameter:

- Wavelength:

The extent to which a sample absorbs light depends upon the wavelength of light. The wavelength at which a substance shows maximum absorbance is called absorption maximum or Amax.

Preparation of Stock solution:

1. Cream containing 0.1ml was weighed using an electronic balance.
2. This cream and distilled water were placed in a 10-ml volumetric flask. to the mark.
3. The cream was dissolved by giving the flask a good shake. This was the standard solution.

Procedure:

1) Adjust the spectrophotometer's wavelength to 200 nm and establish the reference level of the device using a cuvette filled with distilled water.
2) The cuvette with the prepared dilution should be placed in the sample container. Note down the absorption.
3) At 200 nm to 400 nm wavelength increments, repeat step 2 and note absorbance at each setting.
4) Plot the absorbance vs wavelength findings.

- Linearity:

This parameter deals with the potency to obtain test findings that have a concentration-based correlation with the analyte For linearity study, Linearity was assessed by visually inspecting the plot area as a function of concentration. four solutions at different concentrations 2, 4, 6, 8 were prepared using four different portion and the obtained data were used for the linearity study of

mushroom.

- Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

I. Interday Precision:

The and reliability of results obtained from analysing the same sample on different days.

The interday precision of the method was checked by considering the effect of analyst variation and by comparing the average responses which were obtained in three different days. Results were reported as RSD.

Calculation:

Standard Deviation (SD) = 0.0416

% RSD =SD/mean × 100

=0.0416/0.092 × 100

= 0.452 × 100

% RSD = 45.22 %

II. Intraday Precision:

The consistency of results when a measurement is repeated multiple times on the same day under identical conditions.

1. A stock solution of the synthetic combination was made with 0.1 mg of cream.
2. To get 10ml as a workable solution, this solution was diluted 6 times.
3. In the specified linearity range, spot areas were acquired three times in a single day, and the relative standard deviation of the peak responses at each level was calculated.

Calculation:

Standard deviation (SD) = 0.0367

%RSD = SD/Mean ×100

= 0.0367/0.213×100

=0.1723×100

%RSD =17.23%

- Accuray:

Accuracy is a measure of how close a measurement is to the correct or accepted value of the quantity being measured. Precision is a measure of how close a series of measurements are to one another. Precise measurements are highly reproducible, even if the measurements are not near the correct value.

Formula:

1. Amount Added = Weight of API × Purity of API/100
2. Amount found = Test Response/Standard Response × Standard Weight/Standard Dilution × Test Dilution/1 × Standard potency/100
3. %Recovery = Amount found /Amount Added×100

Calculation:

1. Standard Deviation (SD)= 0.0022

% RSD = SD/Mean × 100

= 0.0022/0.599 × 100

= 0.0037 × 100

%RSD = 0.37%

2. Standard Deviation (SD) = 0.0029

% RSD = SD/Mean × 100

= 0.0029/0.804 × 100

= 0.0036 × 100

% RSD = 0.36%

3. Standard Deviation (SD) = 0.0134

%RSD = SD/Mean × 100

= 0.0134/0.999 × 100

%RSD = 1.34%

- Robustness:

Robustness is correlated with the testing method's capacity to endure slight changes. Robustness for assay estimates was assessed in this study by allowing for slight variations in the mobile phase flow rate and detection wavelength.

The test result, including the percentage RSD of the individual sample, was then assessed after the flow rate and detection wavelength were adjusted by ± 0.2 mL/min and ± 2.0 nm, respectively.

Calculation:

Standard Deviation (SD) = 0.00252

%RSD = SD/Mean × 100

= 0.00252/0.227 × 100

= 0.0111 × 100

%RSD = 1.11%

- Ruggedness:

The ability of an analytical technique to continue operating even when there are minor, sporadic variations in experimental parameters is known as ruggedness. These variables might be various analysts, tools, chemicals, or even the time of day.

It assists in identifying conditions that are essential to the method's operation and in expressing the method in a clear and concise manner, which facilitates the technique's transfer to other labs**.**

Calculation:

Standard Deviation (SD) = 0.003728

%RSD = SD/Mean×100

= 0.003728/0329×100

= 0.01133×100

%RSD = 1.13%

- LOD & LOQ:

The lowest amount of analyte in a sample that is readily detectable but cannot be quantified to the specified value is known as the LOD of an analytical procedure.

The lowest quantity of analyte in a sample that can be readily quantified with the right level of accuracy and precision is known as the limit of quantification (LOQ) in analytical methods.

IV. RESULT & DISCUSSION

1. Solubility:

Solubility	Soluble in water

2. Irritancy:

The Formulation shows no redness, edam, inflammation and irritation during studies. These Formulation are safe to use for skin.

3. Sprediabilty:

Cream is uniformly distributed on skin.

4. Viscosity:

Viscosity cream was determined using Book field rotational viscometer at 60 rpm.

Viscosity	1427.1 mpass

Fig no. 3 Viscosity

5. PH:

Measurement of PH values of prepared formulation is 6.51 which are Considered Acceptable to avoid the risk the irritation upon the application to the skin because adult skin PH is 3.

PH	6.15

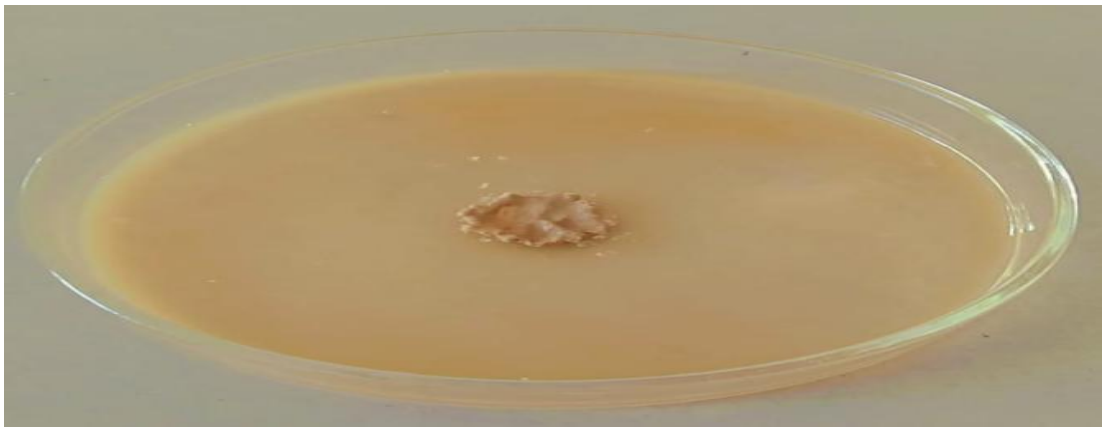
Fig no.4 Digital PH Meter

6. Homogeneity:

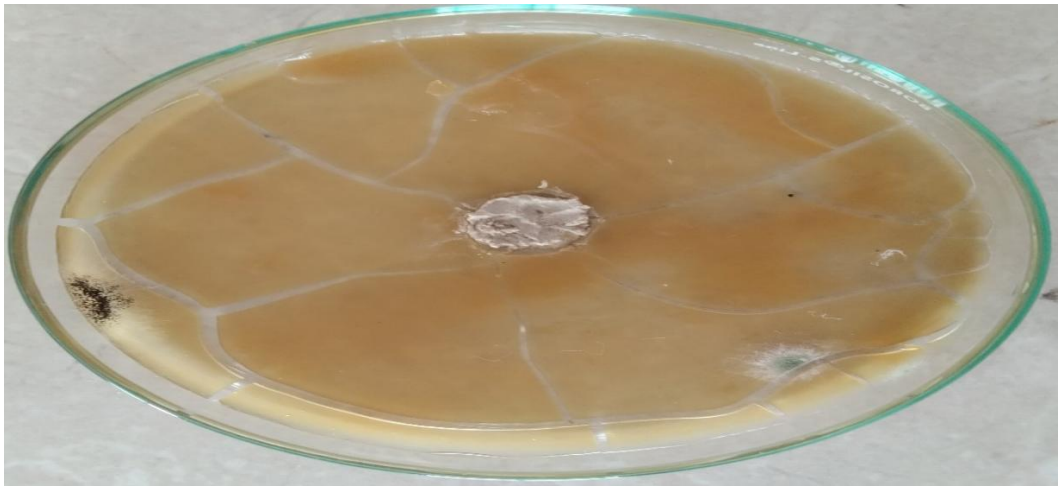
The developed cream was tested for homogeneity testing through visual inspection. Cream is uniformly distributed on skin.

7. Microbial Growth test:

When apply the cream, it inhibits the growth of fungi and kill them.



Before Test



After test

Method Development:

- Uv Spectroscopy:

Fig no.5 UV

Maximum Wavelength of Extract

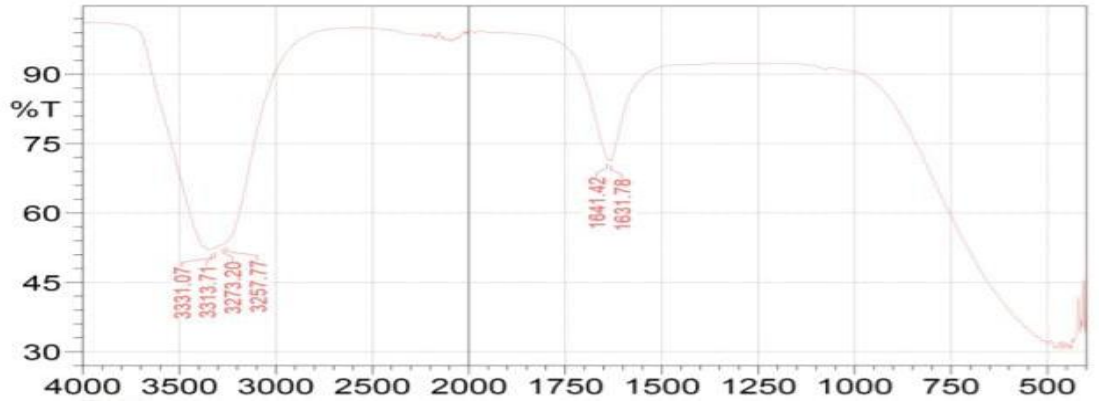
Sr. no	Absorbance (Å)	Wavelength(nm)
1.	0.932	201.30

Fig no.5 UV

Sr. no	Absorbance (Å)	Wavelength (nm)
1.	0.909	252.40

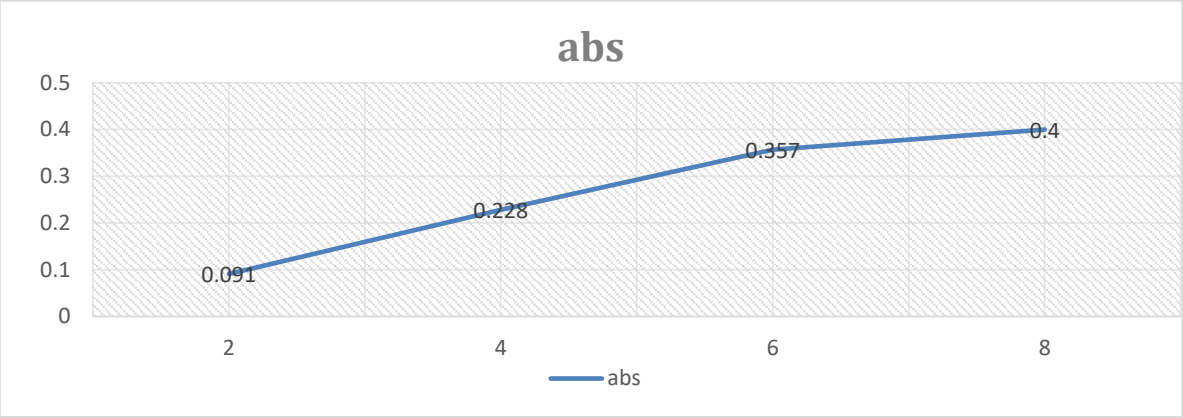
Maximum wavelength of Cream

Infrared Spectroscopy:



Sr. no	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1.	1631.78	71.181	0.712	1633.71	1483.26	9.941	0.017
2.	1641.42	71.584	0.661	1805.37	1637.54	8.508	0.015
3.	3257.77	53.574	0.25	3259.7	2808.36	42.773	0.225
4.	3273.2	53.159	0.062	3275.13	3261.63	3.676	0.001
5.	3313.71	52.408	0.168	3313.56	3304.06	3.771	0.011
6.	3331.07	52.144	0.149	3336.85	3319.49	4.889	0.01

Table No. 2 FTIR Measurement



Linearity:

Sr.no	Concentration µg/mL	Absorbance Å
1.	2	0.091
2.	4	0.228
3.	6	0.357
4.	8	0.400

Table No.3: Linearity

Precision:

Interday Precision:

Sr no.	Absorbance Å	Mean
1.	0.094	
2.	0.106	
3.	0.086	0.092
4.	0.084	
5.	0.097	
6.	0.086	

Intraday Precision:

Sr no.	Absorbance Å	Mean
1.	0.212	
2.	0.198	
3.	0.269	0.213
4.	0.244	
5.	0.170	
6.	0.189	

Accuracy:

	covery level	mple µg/ml	ndard µg/ml	sorbance	ecovery	an SD)	SD
1	50	1.5	0.75	0.598	99.8	0.0022	0.37
				0.599	100		
				0.602	100		
2	100	1.5	1.5	0.813	100	0.0029	0.36
				0.796	99.8		
				0.804	100		
3	150	1.5	2.25	0.995	99	0.0134	1.34
				1.014	101.5		
				0.988	98.89		

Table No.4: Accuracy

Robustness:

Sr. No.	Conc. µg/ml	Wavelength (nm)	Absorbance (x)	$\bar{x}$ (Mean x)	$(x - \bar{x})$	$(x - \bar{x})^2$
1	4	252	0.228	0.227	0.001	0.000001
2	4		0.226		-0.001	0.000001
3	4		0.227		0	0
4	4		0.228		0.001	0.000001
5	4		0.230		0.003	0.000009
6	4		0.232		0.005	0.000025
7	4		0.226		-0.001	0.000001
8	4		0.225		-0.002	0.000004
9	4		0.224		-0.003	0.000009

Table No.5: Robustness

Ruggedness:

Sr. No	Conc. µg/ml	Wavelength (nm)	Absorbance (x)	$\bar{x}$ (mean)	$x-\bar{x}$	$(x-\bar{x})$
1	4	252	0.330	0.329	0.001	0.000001
2	4		0.325		-0.004	0.000016
3	4		0.328		-0.001	0.000001
4	4		0.326		-0.003	0.000009
5	4		0.330		0.001	0.000001
6	4		0.328		-0.001	0.000001
7	4		0.329		0	0
8	4		0.330		0.001	0.000001
9	4		o.338		0.009	0.000081

Table No.6: Ruggedness

LOD &LOQ:

The limit of detection was calculated by standard deviation of the sample and the slope from the Calibration graph.

The limit of quantization of an analytical procedure is the lowest concentration of an analyte in a sample that can be determined with suitable precision and accuracy under the stated experimental condition.

V. CONCLUSION

This study has highlighted the importance of herbal plants.

This study focuses on different extraction and preparation methods of plant extract.

The primary goal of this study is to determine whether plant extracts have any promise for use in medicine.

The cream was found to be safe and effective in treating fungal infection.

The prepared formulations showed good Spreadability, no evidence of phase separation and good consistency during the study period. Stability parameters like visual appearance, nature variations during the period and fragrance of the formulations showed that there were no significant changes during study period.

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