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Research Article

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Evaluation of Antioxidant and Spectroscopic analysis of *Psoralea corylifolia* and *Nigella sativa* Plant Extract

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Abstract:

Background: Analytical methods of UV spectroscopy, FTIR, TLC and antioxidant activity are used to test the drug and active constituent in the laboratory to determine how much active constituent is present in the test drug through these methods.

Aim: In UV estimation, the test at 254 nm has a very sharp peak, which represents that the test drug is pure, which shows that this nm is present in the psoralene active constituent that is present in the *psoralene corylifolia* and In FTIR analysis of the sample, how many functional groups are present in the test sample.

Methods: In TLC analysis, a different mobile phase was prepared, and all the samples were run, but in one phase, the test samples ran excellently, and separation was good (hexane: ethyl acetate) in a ratio of 8.5:1.5 v/v. Compared to the test drug with an active constituent, the RF value is the same.

Result: In antioxidant activity, antioxidant properties with DPPH and compare them to the standard antioxidant drug, Gallic acid. In this activity we prepare the different concentrations of the solution and check which concentration shows the high antioxidant activity and compare it with the standard drug concentration activity.

Conclusion: The test drug antioxidant property is high in the 100 μ L concentration. The sample is run in UV-visible spectroscopy at 517 nm, and the standard is run at 270nm for the absorbance of the drug.

Major Finding: The study revealed that both *Psoralea corylifolia* and *Nigella sativa* extracts exhibit strong antioxidant activity, with *Nigella sativa* showing slightly higher free radical scavenging potential. Spectroscopic analysis confirmed the presence of phenolic and flavonoid compounds contributing to their antioxidant properties.

Key Words: UV Spectroscopy, FTIR, DPPH activity, Herbal drug Analysis, Analysis, Vitiligo, Inflammatory bowel disease, Thymoquinone, Bakuchiol.

Introduction:

Bakuchi (*psoralea corylifolia*) is an herbal drug that is used in vitiligo and inflammatory bowel disease, as well as different autoimmune diseases such as thyroid disease, RA, and other diseases. The bakuchiol seeds are black in color, in which various active constituents are present, like *bakuchiol*, *bavachinin*, *isobavachalcone*, *psoralen*, and *psoralidin*. The *bakuchiol* is useful in the treatment of vitiligo and skin diseases such as psoriasis, vitiligo, and other diseases. *Isobavachalcone* has to show that its activity in the treatment of autoimmune diseases and skin-related diseases can help to treat IBD. *Psoralen* has an activity to treat the autoimmune disease and skin diseases psoriasis and other diseases. *Psoralidin* has activity in the treatment of various diseases, such as skin, immune system-related, and rheumatoid arthritis. They have antioxidant activity, antimicrobial activity, antibacterial activity, anti-inflammation, neuroprotective activity, and immunoprotective activity. It is common language known as Bakuchi. *Psoralea corylifolia* belongs to the *Leguminosae* family. Bakuchi has been used for a long time in traditional medicine; it has various activities for curing diseases like psoriasis, leukoderma, leprosy, vitiligo, IBD, and other autoimmune diseases. We have studied the seed part of the plant, such as UV spectroscopy, FTIR, antioxidant activity, TLC, and HPTLC. In this study, we found that this drug is very effective for the treatment of vitiligo, inflammatory bowel disease, and other autoimmune diseases. All the parameters have been done for a positive result for the treatment of the inflammatory bowel disease, vitiligo.¹ *Nigella sativa* Linn is also known as black cumin/kalajira/kalonji. *Nigella*

sativa is broadly cultivated in India, southern Europe, Saudi Arabia, Pakistan, and Turkey. In India, there are West Bengal, Punjab, Himachal Pradesh, Assam, Bihar, and Uttar Pradesh. The seeds of the *N. sativa* have a lot of the active constituents, such as linoleic acid, oleic acid, arachidonic acid, and thymoquinone. In the seed, *thymoquinone* contains 30 to 48% of this active constituent, which has been responsible for the treatment of vitiligo, IBD, and other autoimmune diseases. They have a lot of antioxidant activity, anti-inflammatory activity, and antibacterial activity. *Nigella sativa* has a lot of antioxidant activity, which is helpful in the treatment of all autoimmune diseases because when ROS increases in the body, it causes various types of diseases, including IBD, vitiligo, and other autoimmune diseases. The mixture of the solution is very helpful for the autoimmune disease because they have a double-effect property to cure the disease.²

Method and Materials:

Plant collection:

Psoralea corylifolia (*Bakuchi*) dry plant seeds collected from Parul Institute of Ayurveda and authenticated by Parul Institute of Ayurveda, Parul University, Vadodara, Gujarat 391760, India. *Nigella sativa* (*black cumin*) dry plant seeds collected from Market Vadodara, Gujarat, India.

Extraction of Drug: After the collection of the crude drug, we crush the plant seed in an electric grinder. After grinding, we take powdered drug: 100 gm of Bakuchi and 25 gm of Bakuchi + 25 gm of *N. Sativa*. We use the maceration process for the extraction of two drugs by using a hydro alcoholic solution in the ratio of 50:50. By proper stringing, we add powdered drug in solution. When the powdered drug is immersed in

the solution, the solution should be three parts above the powder. Then we put this solution in for seven days, and we stir the solution twice a day for proper mixing. On the 7th day, we filter out the hydroalcoholic solution using Whatman paper no. 42. After the filtration of the drug, the crude drug is dried and weighed. After that, we take the hydroalcoholic solution, which is the behavior of the weight of the dry crude drug, and mix it with the solution and keep it for 3 days. After the 3 days, again, this process is repeated 2 times for the proper extraction of the active constituent of the crude drug. After the filtration process was done, then we were going to concentrate the extract in the water bath at 600°C. After that, we calculated the yield. The yield calculation formula is given below.³

Formula:

$$\text{Yield (\%)} = (\text{Weight of Concentrated} / \text{Weight of Initial Extract}) \times 100$$

Preparation of Standard: Bring the standard drug from the yukata, then prepare the standard solution. Take 0.1 gm of *psoralene* in 1 ml of methanol and shake it continuously until it gets dissolved fully. Take the 0.16 gm of *Thymoquinone* in 1 ml of the Methanol and shake it continuously until it gets dissolved fully.⁴

Preparation of UV spectroscopy solution: First, we prepare the different concentrations of solution for the UV estimation. After this, we take the extract of *Bakuchi and N. Sativa*, 1 ml in a measuring cylinder, and then we make up the volume to 10 ml with methanol. Then, again, take 2 ml of sample in 10 ml of solution and make up the volume up to 8 ml with methanol. Then again, take 3 ml of sample from the 2nd sample and make up the volume up to 7 ml with methanol. Then again, take a 4 ml sample from the 3rd sample and make up the volume up to 6 ml with methanol.

Again, take a 5 ml sample from the 4th sample and make the volume up to 5 ml with methanol. After that, go to the UV spectroscopy for the absorbance of the sample in different sample concentrations in UV spectroscopy. The solution of the test drug has a concentration of $\mu\text{g/l}$. Then we have taken 1 ml of the solution in the stock solution and made volume up to 10 ml with methanol, the dilution having the concentration of 0.1 $\mu\text{g/ml}$. the 2nd sample having a concentration of ions of 0.01 $\mu\text{g/ml}$. The 3rd sample has a concentration of 0.001 $\mu\text{g/ml}$. The 4th sample has a concentration of 0.0001 $\mu\text{g/ml}$. The absorbance of the test drug was scanned for full wavelength to find out the maximum absorption of the sample for the presence of psoralene, a naturally occurring phytochemical compound found in the seeds of the psoralea corylifolia or certain fruits like figs and citrus fruits. The chemical structure of the compounds has fused furan rings. On the basis of the literature survey, it was first found that the compounds were 8-methoxypsoralen; hence, to validate the standard UV, a descriptive scan was taken. ⁵

Preparation of Solution for IR: For the estimation of IR, we double filter the extract with Whatman filter paper no. 42, and then we take 2 2ml of filtrate for the IR estimation.⁶

Preparation of TLC plate: The TLC plate is coated with silica gel F60F254, which is obtained from Parul Institute of Pharmacy and Research, Parul University, Limda, Waghodia, Vadodara, Gujarat. The thickness of the silica gel is 0.2 mm of the plate.

Preparation of Standard Galic acid Solution: Gallic acid is a natural phenolic compound that is widely used to evaluate antioxidant activity as a standard drug at 1 mg/ml dissolved in ethanol.⁷

Preparation of the DPPH Solution: Take the DPPH (2, 2-diphenyl-1-picrylhydrazyl) 0.04 gm in a volumetric flask and dissolve in 100 ml of methanol. DPPH is a light-sensitive chemical. When preparing the solution, the dark place and volumetric flask are covered by the aluminum foil and put in the dark and cool place.⁸

Results and Discussion:

Estimation of UV Spectroscopy: After the preparation of the test drug in different concentrations, then estimate the sample in the UV-Visible Spectroscopy in 200-400 nm. (Figures no. 1, 2)

Improvements for Accuracy and Reliability of UV Spectroscopy:

- Proper calibration and regular calibration of the instrument that is being used with the reference standards to minimize the baseline. Optical wavelength selection ensures that the correct λ_{max} maximum absorbance wavelength is used to avoid the measurement of the errors.
- Blank Corrections: It is used to appropriate the blank solution, the blank solution to correct for the solvent and cuvette absorbance.
- Minimize stray light and noise by keeping the instrument in a vibrant, dust-free environment and using light shields to minimize the light interference.

Additional Validation:

- In UV spectroscopy, the additional validation such as fluorescence spectroscopy and Fourier transform infrared, colorimetric assays, and stability studies.
- In the fluorescence spectroscopy methods, complementary absorption data with

higher sensitivity are provided.

- The colorimetric assays are responsible for the biological sample such as antioxidants.

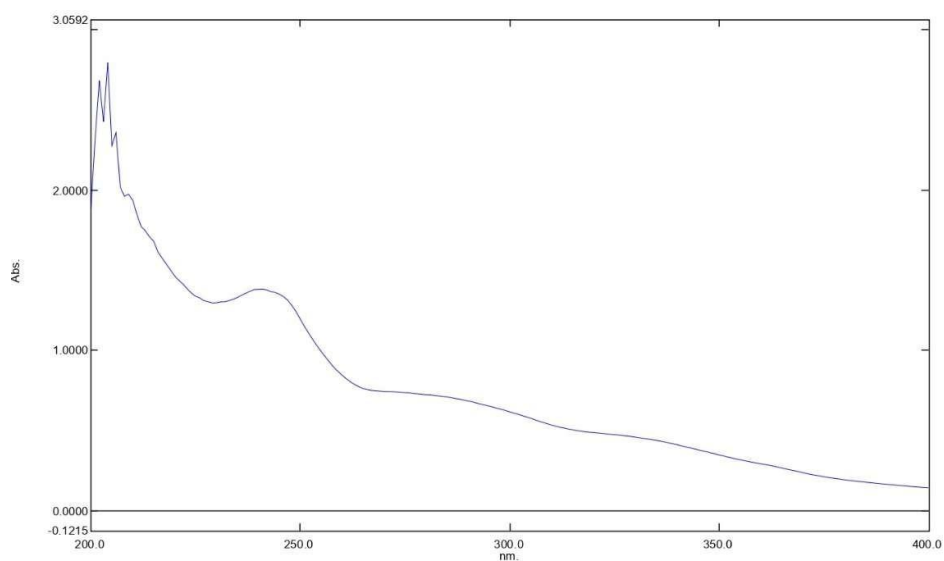


Fig no. 1 UV Spectroscopy of *Psoralea Corlylifolia* absorbance at 240nm.

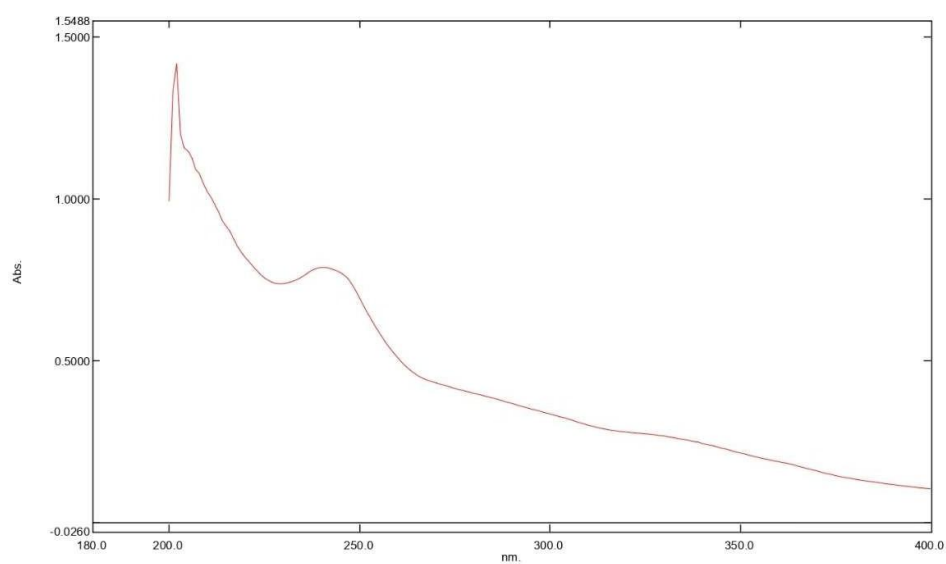


Fig no. 2 UV Spectroscopy *Nigella sativa* absorbance at 254nm

Estimation of FTIR Spectrophotometer: After the extraction of the test drug, the sample is taken for the estimation of the functional group present in the sample through the FTIR spectrophotometer, and the wavelength is set between 1000 and 3500 cm^{-1} .
(Figure no. 3, 4, 5) (Table no. 1, 2, 3)

Improvements for Accuracy and Reliability of FTIR:

- Sample preparation It ensures homogenous sample preparation, which uses high- quality KBr pellets for the solid samples.
- Instrument calibration and regulations calibration using a polystyrene standard to verify the wavelength and accuracy.
- The dilution of sample concentration is within the linear range of the Beer-Lambert law to avoid the saturation effects.

Additional Validation:

- NMR spectroscopy that confirms the molecular structure of the drug with high accuracy.
- Mass spectrometry helps to validate the molecular weight and composition of the compounds analyzed by the IR.

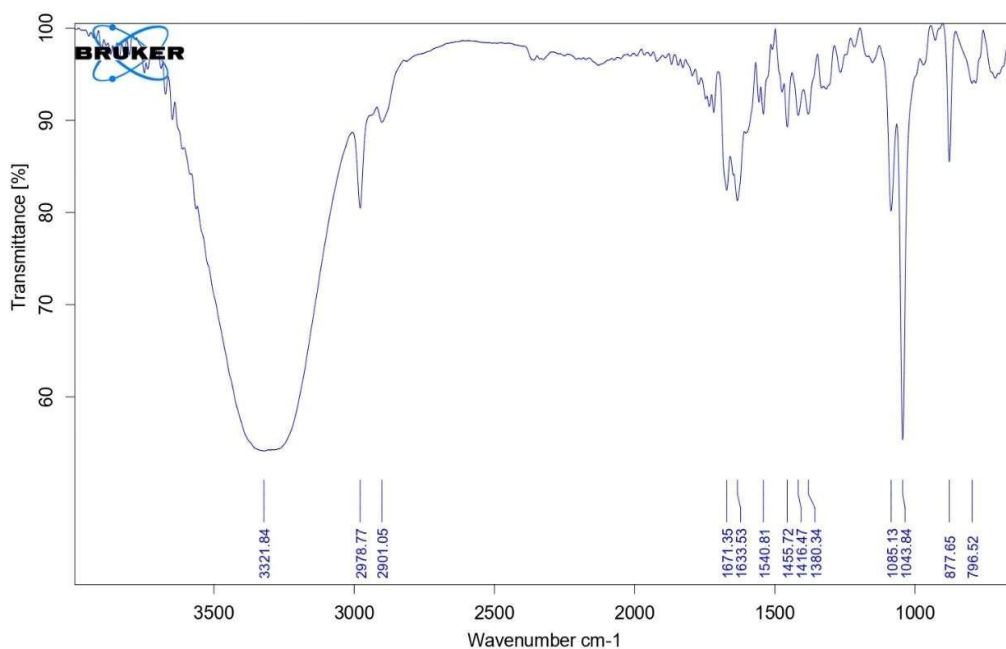


Fig no. 3 IR Spectrophotometer *Psoralea corlylifolia*

Table no. 1 Identification of the Functional group in the *Psoralea corlylifolia*

Sr. No	Wavenumber	Group	Interpretation	
1	3321	≡C-	Stretching	Mode
		H N-	Stretching	mode
		H	Stretching	mode
		OH	(COOH)	
		NH	(Primary amide 2 bond Secondary amide 1 bond)	

2	2978	O-H	Stretching mode (COOH)
3	2901	OH	Stretching (COOH)
4	1671	C=O	Stretching mode (Amide)
5	1633	C=	Stretching (Amide)
		O	Bending (Amide)
		N-H	
6	1540	-NO2	(Nitro)
		NH	Bending in Secondary amide
7	1455	N-H	Bending in Secondary amide
8	1416	C-O-H	Bending Mode
9	1380	C-O-H	Bending
		NO2	Symmetric Stretch (Aliphatic nitride)
10	1085	C-O	(Alcohol)
		C-O	(Ether)
		C-O	(Stretching)
		C-F	(Stretching)
11	1043	C-O	(Alcohol)
		C-F	(Ether)
12	877	=C-H	Out of plane Bending (Aromatic)
13	796	=C=	Out of plane bending
		H N-	Out of plane bending mode
		H C-	Stretching mode
		C1	

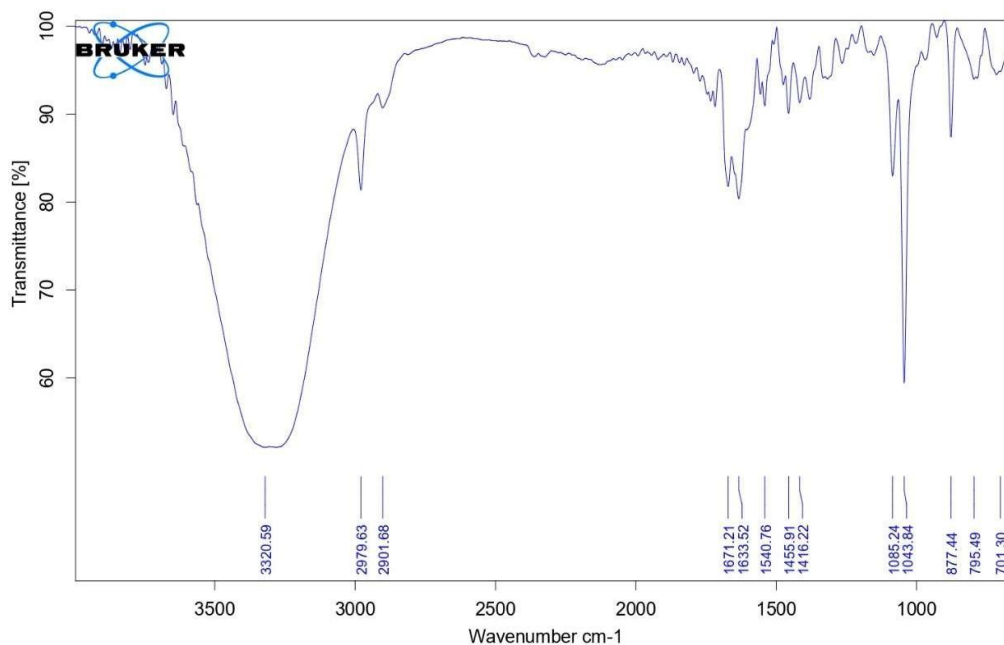


Fig. 4 IR Spectrophotometer *Nigella sativa*

Table no. 2 Identification of the Functional group in the *Nigella sativa*

Sr. No	Wavenumber	Group	Interpretation
1	3320	$\equiv$ C-H	(Hydrogen bonded O-H bond Alcohol)
		N-H	Stretching mode
		O-H	Stretching (COOH)
		N-H	Stretching (Primary amide-2bond)
			(Secondary amide- 1 bond)
2	2979	O-H	Stretching mode (COOH group)
3	2901	O-H	Stretching mode (COOH group)
4	1671	C=O	Stretching mode
		C=O	Stretching (amide)

5	1633	C=O	(Amide)
		N-H	(Amide)
		N-H	(amide (1))
6	1540	N-H	Bending in secondary amine
		-NO ₂	Aliphatic nitric
		-NO ₂	Aliphatic nitric
7	1455	N-H	Bending in 2 amines
8	1416	C-O-H	Bending mode
10	877	=C-H	(Aromatic ring)

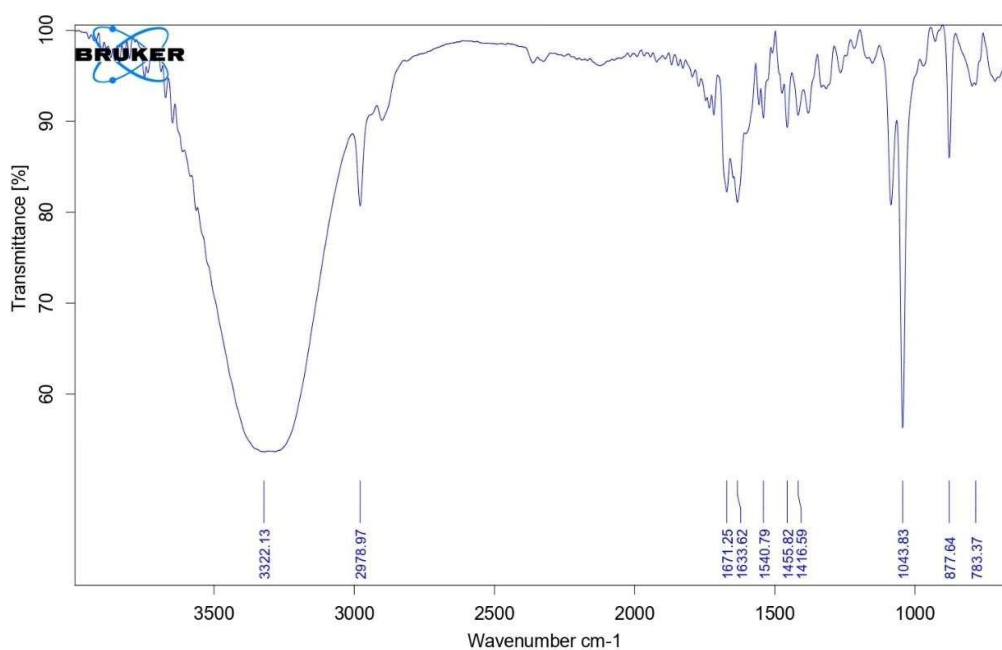


Fig no. 5 IR Spectrophotometer *Psoralea corlylifolia* & *Nigella sativa*

Table no. 3 Identification of the Functional Group in the *Psoralea corlylifolia* & *Nigella sativa*

Sr. No	Wavenumber	Group	Interpretation
1	3322	$\equiv\text{C}-$	Stretching (O-H Alcohol)
		H N-	Stretching
		H	Stretching (COOH)
		OH	Stretching (1 amide-2)
		N-H	(2 amide-1)
2	2978	O-H	Stretching mode (COOH Carboxylic acid)
3	1671	C=O	Stretching (Conjugation of aldehyde with phenol)
		C=O	Stretching mode (amide)
4	1633	C=C	Stretching
		N-H	Bond in Primary amine
		C=	Stretching (amide)
		O	Bonding (amide)
		N-H	
5	1540	C=	Stretching
		C	Bonding in 2 amino
		N-H	Asymmetric Stretch (aliphatic as well as aromatic)
		NO ₂	
6	1455	N-H	Bending in secondary amino
7	1416	C-O-H	Bonding mode
8	1043	C-O	Stretching (alcohol)
		C-O	Stretching (alcohol)
9	877	C-H	Out of plane bending mode
		C-H	Out of plane aromatic

10	783	C-H	Out of plane bending
		N-H	mode Out of plane
		C-	bending mode Stretching
		Cl	mode

Estimation of Antioxidant Activity of Test Drug: when we start the anti-oxidant activity, we prepared the test drug different concentration 20 µg, 40 µg, 60 µg, 80 µg, 100 µg in volumetric flask make up the volume up to 3ml with methanol for all test concentration. After then we mix the DPPH solution of 1 ml in all the sample and put all the sample for 30mins in dark area. After, 30mins we get absorbance in UV visible spectroscopy in 517nm. The standard drug absorbance in 270nm after getting absorbance we compare the test and standard drug antioxidant activity. The IC₅₀ values, inhibitory concentration for the 50% effect, depend on the targeted disease and cell type. The *Psoralea corlifolia* IC₅₀ values to inhibit the IL-6 and TNF-α are 25 µM, and the *Nigella sativa* IC₅₀ values for the inhibiting of the oxidative stress and inflammatory pathways are 10 µM. (Figure no. 6, 7, 8, 9)

Improvements for Accuracy and Reliability of DPPH:

- Always prepare a fresh DPPH solution that can improve the accuracy and reliability of the methods, and the DPPH solution is light sensitive, so it is stored in an amber-colored bottle and can be kept and wrapped in aluminum foil because it is light sensitive and can be used within 24 hours.
- Proper wavelength selection to the absorbance corrects for 517nm.
- Multiple readings were taken; the first reading is taken with a blank (methanol) to avoid any background impurities.

Additional Validation:

- Ferric reducing antioxidants power it can measure the antioxidants capacity by assessing ferric ion reduction.
- Oxygen Radical Absorbance Capacity: It can measure the antioxidant activity against peroxy radicals and give the antioxidant profile data of the drug.

Comparison of the test drug with standard drug formula:

The comparison of the antioxidant activity of the test drug with the standard drug at 100 $\mu\text{L/mL}$ concentration needs to analyze the DPPH radical scavenging activity of both samples.

$$\% \text{inhibition} = (\text{Acontrol} - \text{ASample} / \text{Acontrol}) \times 100$$

Where,

Acontrol = Absorbance of DPPH solution without the drug

ASample = Absorbance of the test or standard drug at 100 $\mu\text{L/mL}$ concentration.

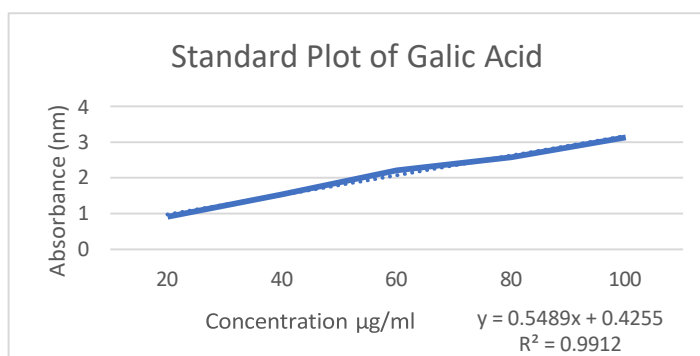


Fig no. 6 Standard Plot of Galic Acid

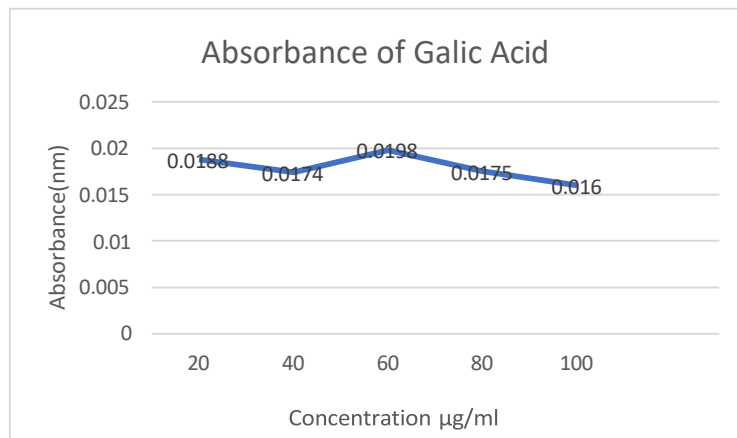


Fig no. 7 Absorbance of Gallic acid

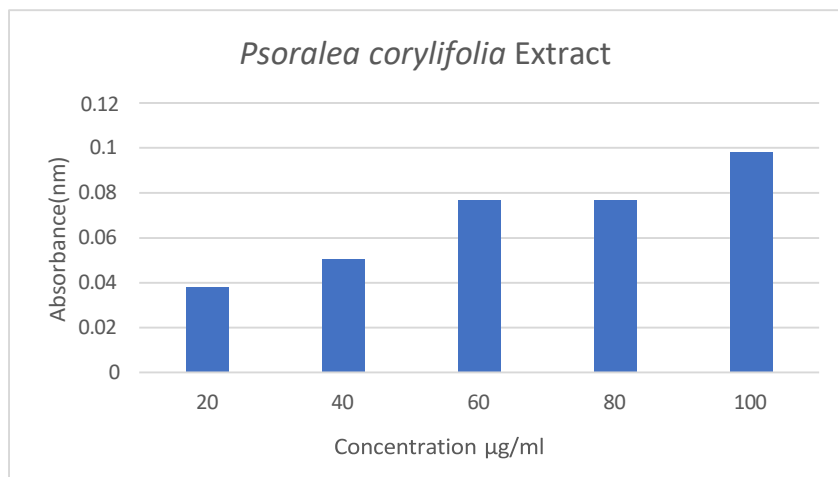


Fig no. 8 Absorbance of *Psoralea corylifolia* Extract

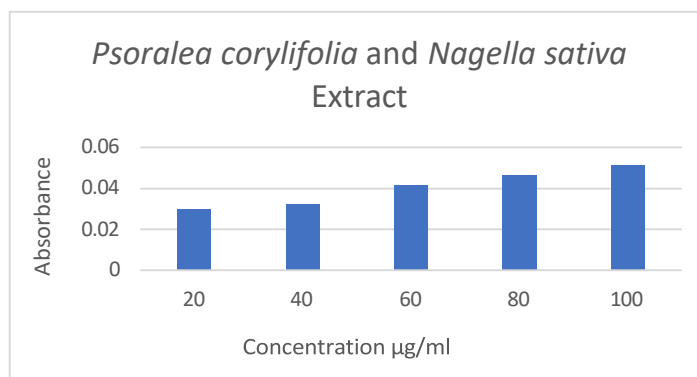


Fig no. 9 Absorbance of *Psoralea corylifolia* and *Nagella sativa* Extract

Conclusion: In this research article, we performed the various activity tests for the drug and standard TLC, UV, FTIR, and antioxidant. The test drug preparation used the bakuchi dry seed and *N. sativa*, also a dry seed, used after the extraction process. We are doing the analysis in the lab for the confirmation of the drug purity and how much active compound is present in the test drug, which is responsible for the treatment of inflammatory bowel disease and vitiligo. They have a lot of antioxidant activity, which is helpful for curing various autoimmune diseases such as thyroid, IBD, vitiligo, RA, and other diseases. The bakuchi absorbance in UV 240nm shows that the psoralenes are present in the test drug, which are responsible for the treatment of IBD and skin disease, and the *N. sativa* absorbance at 254 nm shows that the thymoquinone is present in the test drug, which is responsible for the treatment of IBD and skin disease. During analysis, we observed that bakuchi and kalajeera are very helpful in the treatment of autoimmune diseases, which are responsible for the immunity enhanced in the body, and they act on the TNF alpha receptors 1 and 2, which are responsible for the causes of IBD and other autoimmune diseases. The drug helps to decrease the inflammation mediators like the TNF- alpha and IL-6 in our body and increase the body's immunity system.

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Competing interests: Not Applicable.

Funding: Not Applicable.

Author's Contributions:

Rohit Agrahari, G S Chakraborty design the study.

Rohit Agrahari conducted all the experimental Analysis and interpreted the data.

G S Chakraborty was in the preparation of manuscript and its alignment with the findings.

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