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# Phytochemical analysis of green synthesised *S. platensis* mediated $TiO_2$NPs through Fourier transform infrared spectroscopy (FT-IR)

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## Abstract
The current study involves the phytochemical analysis of green-synthesized *S. platensis*-mediated $TiO_2$ nanoparticles ($TiO_2$NPs) using Fourier Transform Infrared Spectroscopy (FT-IR). The aim of this research is to characterize the nanoparticles, focusing on the FT-IR spectrum. Aqueous extracts of *S. platensis*, a solution of Titanium dioxide (0.01 mM), and *S. platensis*-mediated $TiO_2$NPs were used in the study. The results confirmed the formation of nanoparticles through the analysis of the FT-IR spectrum. The FT-IR spectrum for *S. platensis* was recorded in the range of 3338 to 528 cm-$^1$, highlighting the functional groups and their respective peak values. Similarly, the FT-IR spectrum for $TiO_2$ alone was recorded in the range from 3339 to 528 cm-$^1$, providing insights into the functional groups present and their corresponding peak values. Finally, the FT-IR spectrum for *S. platensis*-mediated $TiO_2$NPs was recorded in the range of 3339 to 539 cm-$^1$, offering information on the functional groups and their identified peak values in the synthesized nanoparticles. This comprehensive FT-IR analysis contributes to the understanding of the chemical composition and structure of *S. platensis*-mediated $TiO_2$NPs, providing valuable insights for further applications and research in nanotechnology.

**Keywords:** *Spirulina*, $TiO_2$, FT-IR, nanoparticles

## Introduction
Silkworm obtain required nutrients entirely from mulberry leaves because mulberry silkworm is monophagous in nature. Generally, vitamins and other essential nutrients present in the mulberry leaves fulfils the minimum needs of silkworms but the amount of nutrients present in the mulberry leaves diverged on the basis of environmental conditions, usage of fertilizers, mulberry varieties and other cultivation practices. *B. mori* takes essential sugars, amino acids, proteins and vitamins for its normal growth and development.

Recently many researchers have made attempts to increase raw silk production in various ways like silkworm hybridization, usage of artificial diet and application of phytojuvenoids. Breeding of silkworm races has been a key strategy to improve silk production, little improvement in silk production has been achieved to date. As a result, the development of sericulture economy has not progressed well, pointing to the need of new ways for improvement of silk production (Ni *et al.*, 2015) [7].

Application of nanotechnology in sericulture for improving the silk yield made a great avenue in the last decade. Nanotechnology deals with the most advanced applications in multidisciplinary fields including targeted drug delivery, molecular diagnosis and electronic imaging (Sankar *et al.*, 2014) [11]. Recently nanotechnology created a greater impact in agriculture and allied sciences including sericulture. Many researchers were made various attempts in increasing silk production, midgut flora assessment and enhancement of reproduction ability in silkworms through nanotechnology (Kumar *et al.*, 2013; Ahmad *et al*, 2013) [6, 1]. In sericulture among these usage of various nanoparticles for different purposes, increase the silk production is gaining momentum. Nanoparticles are synthesized by several methods of which green synthesis of nanoparticles through plants, microorganisms and algae.

Which is considered environmentally safe, clean, efficient and profitable. In the past few years, the synthesis of nanoparticles using cyanobacteria has become an active research field. Cyanobacteria are a diverse group of photoautotrophic prokaryotes that exist in wide range of ecosystems possessed sustainable resources for various bioactive metabolic products and has high therapeutical application, one such is *Spirulina platensis*. The blue-green algae *S. platensis* contains various minerals, 18 amino acids and vitamins. In sericulture, *S. platensis* mediated NPs act as an activation of tissue metabolism and seems to be an essential factor for promotion of biological parameters of silk gland of silkworm larvae (Dharanipriya and Thangapandiya, 2019) [4].

## Materials and Methods

### Preparation of aqueous extraction of *S. platensis*

The aqueous extract of *S. platensis* was obtained by heating, around 10 g of finely ground *S. platensis* powder in 100 ml of deionized water at 90 °C for 45 mins, then the solution was filtered through Whatman filter paper No.1 to remove the debris. The resultant clear green coloured solution was stored at 4-8 °C for further experiments
(Some *et al.*, 2019) [9].

### Synthesis of titanium dioxide nanoparticles

*Spirulina* mediated $TiO_2$NPs were synthesised utilising 0.01 mM titanium dioxide and aqueous extract of *S. platensis* as bio-reductant and capping agent in a green synthesis. Aqueous extract of 20 ml was added to 80 mL of 0.01 M $TiO_2$ solution, which was kept at room temperature for 6 hours with continuous stirring in a hotplate magnetic stirrer. A change in colour to confirmed the production of $TiO_2$NPs. FTIR was used to characterize the green synthesised *Spirulina*-mediated $TiO_2$NPs.

### Characterization

### Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FT-IR) analysis was used to investigate the binding characteristics of *S. platensis* mediated $TiO_2$NPs. The Nicolet TM spectrometer was used to examine the FT-IR spectroscopy of green synthesised *Spirulina* $TiO_2$NPs. The sample was stored in an FT-IR sample chamber, and the spectra was collected with 16 scans in the mid-IR range 4000-400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$. To eliminate spectrum interference caused by ambient carbon dioxide and water vapour, the interferometer and detection chamber were purged with dry nitrogen. Before each sample was analysed, the air background spectrum was recorded (Devi *et al.*, 2017) [3].

## Results

### Synthesis of $TiO_2$NPs using aqueous *S. platensis* extract

The aqueous extract of *S. platensis* was obtained by heating 10 g of finely ground *S. platensis* powder in 100 ml of deionized water at 90 °C for 45 mins, then the solution was filtered through Whatman filter paper No. 41 to remove debris. The resultant clear-coloured solution was stored at 4 to 8 °C.

The *S. platensis* mediated $TiO_2$NPs were prepared using titanium dioxide and aqueous extract of *S. platensis* as a bioreductant and capping agent. The aqueous extract of *Spirulina* 20 ml was added to 80 ml of 0.01 mM $TiO_2$ solution and the mixture was kept at ambient temperature with continuous stirring at 200 rpm. The formation of nanoparticles was confirmed by the change of colour (pale yellow) within an hour.

### Characterization of nanoparticles

The structural and particle size characterization of *S. platensis* mediated $TiO_2$NPs were investigated by FTIR analysis. The results are presented here.

### Aqueous extracts of *S. platensis*

FT-IR spectrum was recorded for aqueous extracts of *S. platensis* in the range from 3338 to 528 $cm^{-1}$ and the functional groups with their identified peak values (Table 1, Figure 1). The functional groups peaked at the frequency of bands *viz.*, amines N-H stretching (3338 and 3331 $cm^{-1}$), alkenes C-H (3031 $cm^{-1}$), alcohols O-H (2292 $cm^{-1}$) alkynes and cycloalkanes C-H (2971 $cm^{-1}$), aromatic compound Ar-H (3032 $cm^{-1}$), alkanes C-H stretching (2949, 2851, 1439 and 1368 $cm^{-1}$), aldehydes C-H (2851 $cm^{-1}$), lactones C=O (1740 $cm^{-1}$), carboxylic acid O-H (2851$cm^{-1}$), phenols C-O stretching (1368 $cm^{-1}$), halogen compound C-F stretching (1368 and 528 $cm^{-1}$), ether C-O (1230, 1211, 1114 and 1069 $cm^{-1}$).

**Table 1:** Characteristic absorbance frequencies of functional groups for *S. platensis*

| Functional groups | Vibration and intensity | Frequency in ($cm^{-1}$) | Peak value |
|---|---|---|---|
| Alkanes | C-H str, m, s | 2960-2850 | 2949.00 |
| | | | 2851.00 |
| | C-H b, m | 1485-1440 | 1439.00 |
| | C-H b, w | 1485-1340 | 1368.00 |
| Alkenes | C-H, m | 3040-3010 | 3031.00 |
| Alkynes and Cycloalkanes | C-H str, m | 3100-2920 | 2971.00 |
| Aromatic compound | Ar-H str, v | 3050-3000 | 3031.00 |
| Halogen compound | C-F str, s | 1400-1000 | 1368.00 |
| | C- str, s | 600-500 | 528.00 |
| Alcohols | O-H str, s | 3000-2000 | 2292.00 |
| | | | 2119.00 |
| Phenols | C-O str,s | 1400-1310 | 1368.00 |
| Ether | C-O str,s | 1270-1200 | 1230.00 |
| | | | 1211.00 |
| | C-O str,s | 1150-1070 | 1114.00 |
| | | | 1069.00 |
| Aldehydes | C-H str, w | 2900-2820 | 2851.00 |
| Lactones | C=O str, s | 1760-1740 | 1740.00 |
| Carboxylic acid | O-H str, w, b | 3000-2500 | 2851.00 |
| Acid anhydrides | C=O str, s | 1790-1740 | 1740.00 |

| | | | |
|---|---|---|---|
| Amines | N-H str, m | 3500-3300 | 3338.00 |
| | | | 3331.00 |

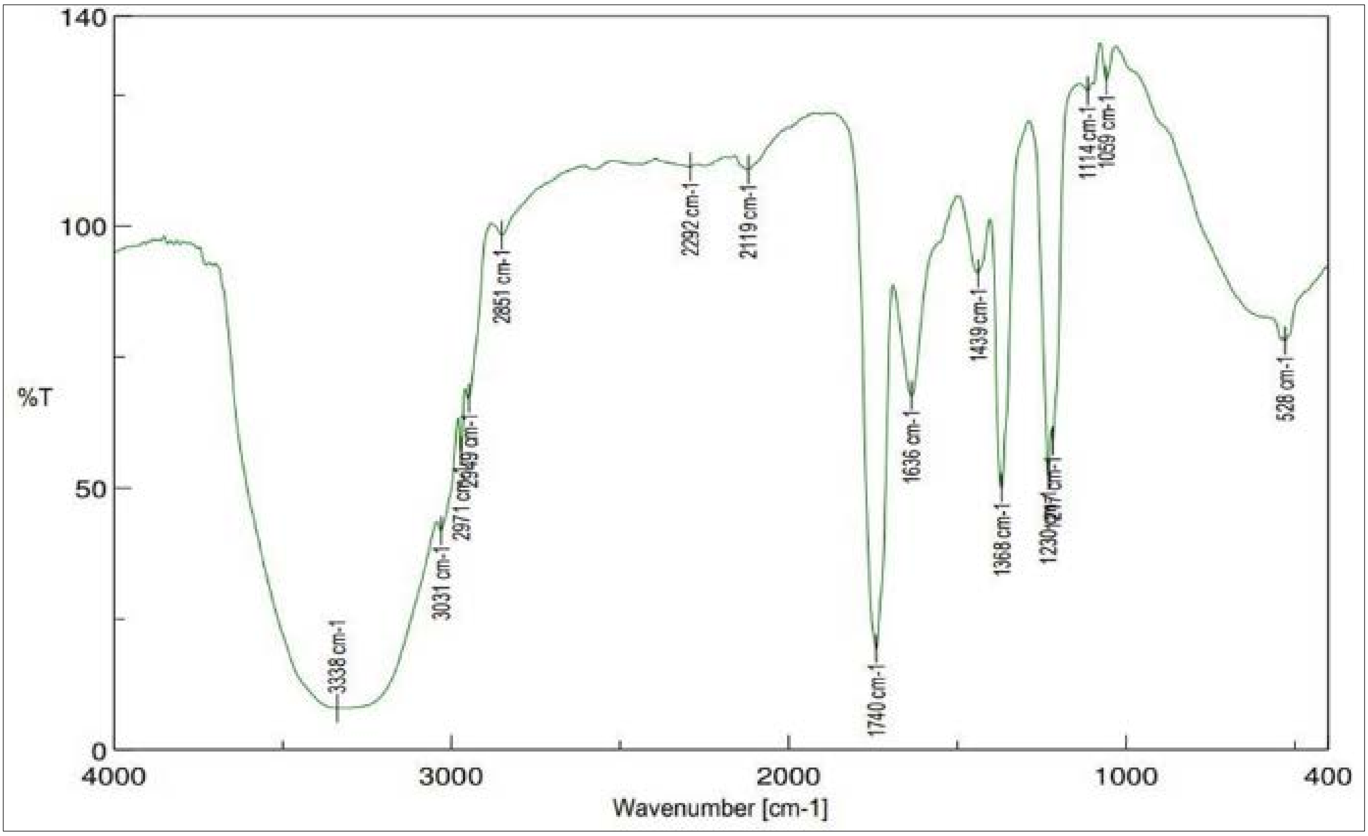


**Fig 1:** FT-IR spectra of *S. platensis*

**Titanium dioxide solution (0.01 mM)**

$TiO_2$ sample alone was subjected to FT-IR spectrum for identification of functional groups and the range of 3339 to 528 $cm^{-1}$ was found. The functional groups peaked at the frequency of bands *viz.*, amines N-H stretching (3339 $cm^{-1}$), alcohols O-H (2294, 2535, 2575 and 2120 $cm^{-1}$), alkynes and cycloalkanes C-H (2971 and 3029 $cm^{-1}$), alkanes C-H stretching (2949, 1439 and 1636 $cm^{-1}$), acid anhydrides and lactones C=O (1740 $cm^{-1}$), carboxylic acid O-H (2854 and 2575 $cm^{-1}$), phenols C-O stretching (1369 $cm^{-1}$), halogen compound C-F stretching (1111 and 1058 $cm^{-1}$), ether C-O (1231 and 1211$cm^{-1)}$ were recorded (Table 2, Figure 2).

**Table 2:** Characteristic absorbance frequencies of functional groups for $TiO_2$ alone

| Functional groups | Vibration and intensity | Frequency in ($cm^{-1}$) | Peak value |
|---|---|---|---|
| **Alkanes** | C-H str, m, s | 2960-2850 | 2949.00 |
| | C-H b, w | 1485-1340 | 1439.00 |
| | C=C str, m | 1662-1626 | 1636.00 |
| Alkynes and Cycloalkanes | C-H str, m | 3100-2920 | 2971.00 |
| | | | 3029.00 |
| Halogen compound | C-F str, s | 1400-1000 | 1111.00 |
| | | | 1058.00 |
| | C- str, s | 600-500 | 528.00 |
| Alcohols | O-H str, s | 3000-2000 | 2294.00 |
| | | | 2535.00 |
| | | | 2575.00 |
| | | | 2120.00 |
| Phenols | C-O str,s | 1400-1310 | 1369.00 |
| Ether | C-O str,s | 1270-1200 | 1231.00 |
| | | | 1211.00 |
| | C-O str,s | 1150-1070 | 1111.00 |
| Aldehydes | C-H str, w | 2900-2820 | 2854.00 |
| Lactones | C=O str, s | 1760-1740 | 1740.00 |
| Carboxylic acid | O-H str, w, b | 3000-2500 | 2854.00 |
| | | | 2575.00 |
| Acid anhydrides | C=O str, s | 1790-1740 | 1740.00 |
| Amines | N-H str, m | 3500-3300 | 3339.00 |

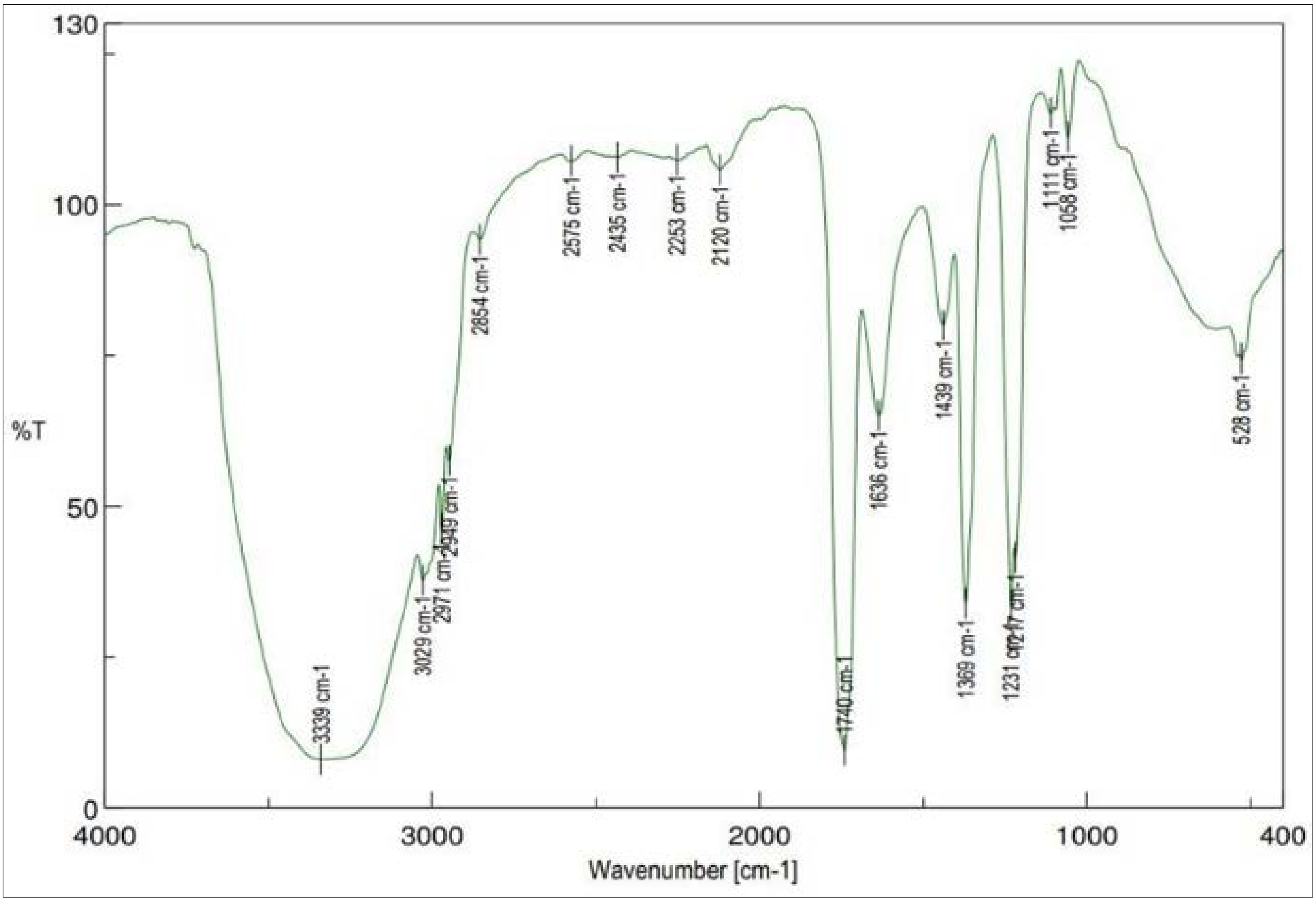


**Fig 2:** FT-IR spectra of aqueous $TiO_2$

### *S. platensis* mediated $TiO_2$NPs solution

FT-IR spectrum recorded *S. platensis* mediated $TiO_2$NPs were in the range from 3339 to 539 cm-1. The functional groups were identified (Figure 3 and Table 3). The frequency of bands was identified as functional peaks *viz.*, amines N-H stretching (3339 $cm^{-1}$), alcohols O-H (2294 and 2118 $cm^{-1}$), alkynes and cycloalkanes C-H (2971 $cm^{-1}$), alkanes C-H stretching (2949 and 1441 cm-1), acid anhydrides and lactones C=O (1740 $cm^{-1}$), carboxylic acid O-H (2846 $cm^{-1}$), phenols C-O stretching (1368 $cm^{-1}$), halogen compound C-F stretching (1368 and 539 $cm^{-1}$), ether C-O (1239and 1211$cm^{-1)}$ were registered.

**Table 3:** Characteristic absorbance frequencies of functional groups for *S. platensis* mediated $TiO_2$NPs

| Functional groups | Vibration and intensity | Frequency in ($cm^{-1}$) | Peak value |
|---|---|---|---|
| Alkanes | C-H str, m, s | 2960-2850 | 2949.00 |
| | C-H b, m | 1485-1440 | 1441.00 |
| Alkynes and Cycloalkanes | C-H str, m | 3100-2920 | 2971.00 |
| Halogen compound | C-F str, s | 1400-1000 | 1368.00 |
| | C- str, s | 600-500 | 539.00 |
| Alcohols | O-H str, s | 3000-2000 | 2294.00 |
| | | | 2118.00 |
| Phenols | C-O str,s | 1400-1310 | 1368.00 |
| Ether | C-O str,s | 1270-1200 | 1239.00 |
| | | | 1211.00 |
| | C-O str,s | 1150-1070 | 1069.00 |
| Aldehydes | C-H str, w | 2900-2820 | 2846.00 |
| Lactones | C=O str, s | 1760-1740 | 1740.00 |
| Carboxylic acid | O-H str, w, b | 3000-2500 | 2846.00 |
| Acid anhydrides | C=O str, s | 1790-1740 | 1740.00 |
| Amines | N-H str, m | 3500-3300 | 3339.00 |

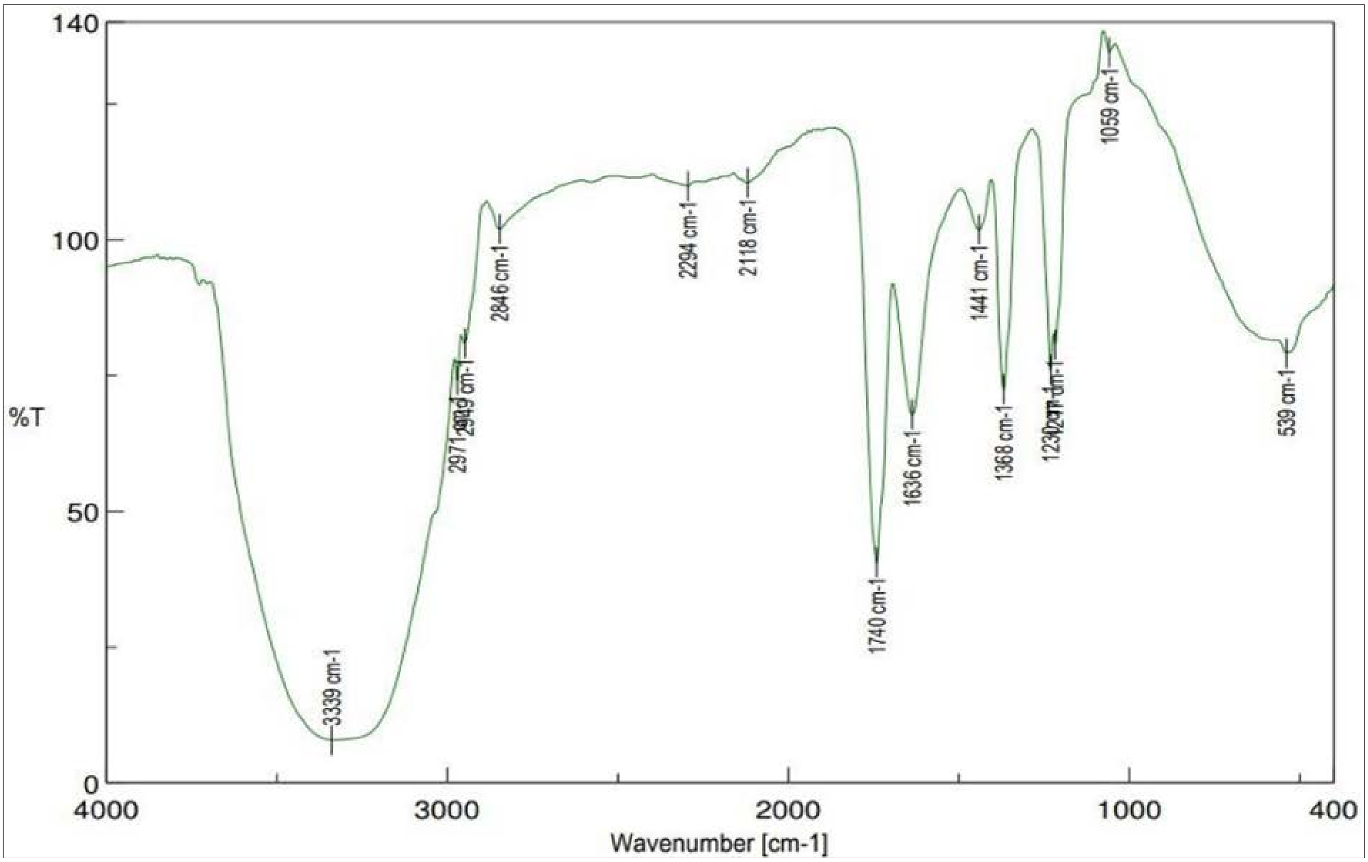


**Fig 3:** FT-IR spectra of *S. platensis* mediated $TiO_2$NPs

## Discussion

### Synthesis of $TiO_2$NPs using *S. platensis* aqueous extract

In the present investigation, the formation of *S. platensis* mediated $TiO_2$NPs was confirmed by the change of colour from white to pale yellow. This result corroborates with the findings of Gunasundari *et al.* (2014) [12] who reported that the formation of *S. platensis* mediated with various metal nanoparticles (Silver, Chromium, Zinc, Lead and Iron) were confirmed by the change of colours such as AgNps the solution colour was changed from yellow to brown, CrNPs colour was changed from orange to green, PbNPs changed from white to blue, ZnNPs changed from green to white and the colour changed from yellow to green for FeNPs.

### Characterization of Titanium dioxide nanoparticles

In the present experiment, *S. platensis* mediated $TiO_2$NPs were characterized through FTIR analysis.

### Fourier transform infrared spectroscopy

FTIR was used to determine the chemical composition of biocoats surrounding biogenic NPs. The present study analyzed the functional groups of *S. platensis* alone, $TiO_2$ alone and *S. platensis* mediated $TiO_2$NPs. The analyzed result indicated the presence of the following functional groups such as alkanes, alkenes, cycloalkanes and alkynes, alcohol, aldehydes, lactones, acid anhydrides, halogen compounds, aromatic compounds, phenols, ether, carboxylic acids and amines. The present result falls in the line of Ahmad *et al.* (2020) [13] who analyzed the functional groups of green synthesised *Mentha arvensis* mediated $TiO_2$NPs showed the presence of alcohols, non-cyclic compounds, carboxylic group, aldehyde and acid anhydrides.

The results corroborates with Sankar *et al.*, (2014) [11] who revealed the FTIR analysis of *Azadirachta indica* $TiO_2$NPs showed the functional groups of amides and nitro compounds. Similarly, Yedurkar *et al.*, (2016) [10] documented the peak at 533 $cm^{-1}$ represents the characteristic absorption of the Zn-O bond, while the broad absorption peak at 3398 $cm^{-1}$ represents the characteristic absorption of a hydroxyl group.

The metal oxide bonds like Ti-O-Ti and Ti-O confirmed the existence of $TiO_2$ in the prepared $TiO_2$NPs. The presence of the Ti-O-Ti bond is due to the strong interaction (capped) of biomolecules with $TiO_2$NPs which results in the presence of phenols. These phytochemicals are responsible for reducing the bulk of titanium dioxide to stable $TiO_2$ in green synthesis (Aravind *et al.*, 2021) [2].

## Conclusion

In the current research, the FT-IR spectrum for S. platensis was recorded in the range from 3338 to 528 $cm^{-1}$, showcasing the identified functional groups along with their respective peak values. Additionally, the FT-IR spectrum for $TiO_2$ alone was recorded within the range from 3339 to 528 $cm^{-1}$, highlighting the functional groups and their corresponding peak values. Furthermore, the FT-IR spectrum for S. platensis-mediated $TiO_2$NPs was recorded in the range from 3339 to 539 $cm^{-1}$. This analysis provides valuable information about the identified functional groups and their associated peak values in each case.

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