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FT-IR Spectroscopic Characteristics of *Ganoderma lucidum* Secondary Metabolites

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Authors' contributions

This work was carried out in the collaboration among all authors and experiment design, the guidance of this study was designed and discussed by each author. All the authors read and approved the final manuscript.

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ABSTRACT

Ganoderma lucidum is an important medicinal mushroom widely used in pharmaceuticals for their antiviral, antibacterial, antifungal, anticancer and immunoregulatory properties and also used in agriculture as an antiviral and antibacterial agent. Fourier Transform Infrared (FT-IR) spectroscopy is a tool widely used in the researches for the identification of organic compounds in the organism. In our study, we used FT-IR Spectroscopy for determination the chemical nature of *Ganoderma lucidum*, and their standards Squalene and Ganoderic acid A. In the FT-IR spectrum for the *G. lucidum* obtained in the region of 3782.69 cm^{-1} to 568.898 cm^{-1} the absorptions peak represents the alcohols, halogens, silicon and phosphorus groups present in the sample. In the squalene standard, ketones, amides, and guanidine, nitrile and azine stretches were found in the wavelength

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1655-1550 cm^{-1}. In the Ganoderic acid A sulfur compounds with weak stretching intensity were obtained in the wavelength 500-400 cm^{-1}. We have concluded that FT-IR spectroscopy is an effective method to analyze the chemical nature of the organic groups present in the samples.

Keywords: Ganoderma lucidum; Ganoderic acid A; squalene; Fourier transform infrared spectroscopy.

1. INTRODUCTION

Ganoderma lucidum (also called ‘‘Lingzhi’’) used as a medicinal herb, belongs to the family Agaricomycetidae, division Basidiomycota, is highly appreciated as a traditional Chinese herb. It contains a lot of biologically constituents *viz.,* polysaccharides, proteoglycans, proteins, triterpenoid, nucleotides, fatty acids, glycol-proteins, sterols, steroids, alkaloids, enzymes, proteins and peptides and which is widely used for the medicinal purpose [1]. Anti-microbial [2], anti-HIV activities [3], anti-tumor, immune-modulating effect [4], anti-inflammatory and antiperoxidative [5], was reported by in the *Ganoderma lucidum*. Because of these compounds in *Ganoderma lucidum* has the potential of antifungal, antibacterial, antiviral properties. Some of the researchers reported the antiviral, antibacterial and antifungal activities against plant pathogens. The commercial products of *G. lucidum* are available in various forms, such as powders, dietary supplements, and tea to improve the longevity of human life. These products were prepared from different parts of the mushroom, including mycelia, spores, and fruit body. The peculiar health benefits of lingzhi comprise of control of blood glucose levels, modulation of the immune system, hepatoprotection, bacteriostasis, etc.,

Bioactivity studies have shown that *G. lucidum* possesses various biological properties, such as antihypertensive, anticancer, antiviral and immunomodulatory activities. The screening of *G. lucidum* and *Ganoderma applanatum* strains for antiviral properties of their metabolites has shown that all of them were able to inhibit TMV development. The activity of preparations increased with an increase in concentration. *Ganoderma lucidum* and *G. applanatum* at a concentration in 1000 µg/mL inhibited viral infection to 65–70% [6].

In this study, we aimed to evaluate the chemical nature of *Ganoderma lucidum.* Its fruiting bodies, mycelia, and spores have been traditionally used for the prevention and treatment of various types of diseases, such as hepatitis, hepatopathy, hypertension, nephritis, bronchitis and cancers [1].

Triterpenoids and polysaccharides present in medicinal mushrooms which is responsible for bioactive properties against pathogens. Meanwhile, more triterpenoids were produced from secondary metabolites. This is the reason behind that, we have chosen the FT-IR techniques to the identification of the chemical nature of the compound in the basidiomycete fungus *Ganoderma lucidum*.

Antibacterial activity of *Ganoderma lucidum* and *Laetiporus sulphureus*, against phytopathogenic bacteria *viz., Agrobacterium rhizogenes*, *Agrobacterium tumefaciens*, *Erwinia carotovora* subsp. *carotovora*, *Pseudomonas syringae* pv. *syringae*, and *Xanthomonas campestris* pv. *campestris* was reported [7]. Similarly, another basidiomycete fungi *Lentinula edodes* reported against bacterial spot of tomato (*Solanum lycopersicum*) caused by *Xanthomonas campestris* pv. *vesicatoria* [8].

Fourier transform infrared spectroscopy is a method for quick surface characterizing tool, which provides chemical information about a substrate up to several micrometers deep. FTIR spectra reveal the composition of solids, liquids, and gases. The most common use is in the identification of unknown materials and used for the confirmation of production materials (incoming or outgoing). The information content is most specific in many cases, permitting fine discrimination between like materials. The speed of this analysis very important techniques useful in many advanced research applications. This spectroscopy is proven to be a useful tool for elucidating structures and the composition of chemical compounds. Moreover, this technique used in quality assessment and it is used for several agricultural research for the identification of the chemical nature of the particular compound [9,10].

FT-IR spectroscopic analysis was used in *G. lucidum* and reported the distribution of functional group within organic fractions. The spectrum confirms the stability of these nanoparticles due to the presence of amide linkages and protein capping in the extracellular suspension filtrate of the mycelia of *G. lucidum* with silver nitrate nanoparticles shown strong bactericidal activity

against test pathogens *Staphylococcus aureus* and *Escherichia coli* was demonstrated [11].

The anti-oxidant compound present in the purified polysaccharides extracted from the fruiting body of *Ganoderma atrum* using infrared spectroscopic analysis and they were found carboxyl group and C-H groups compounds from their samples [12].

The characteristic organic groups identified in the 288 polysaccharides, especially O-H, N-H, and C=O, in the wavelength range between 3600 and 3200 cm−1 by FT-IR spectroscopy [13]. *G. capense* mycelia powder of the homogeneous polysaccharide organic groups were character-ized using FT- IR [14].

2. MATERIALS AND METHODS

2.1 Extraction and Purification of *Ganoderma lucidum* Metabolites

A strain of *Ganoderma lucidum* was obtained from the Mushroom research and Training centre, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. Cultures were grown for 20 days at 25ºC on orbital shaker in a Mushroom complete medium Broth (MCMB) containing glucose, 20 g; peptone, 2 g; yeast extract, 2 g; K_2HP0_4, 1 g; $MgSo_4$. 7H2O, 0.5 g, KH_2PO4, 0.46 g for 1 litter and the broth were sterilized in autoclave 121ºC for 20 min. After that *Ganoderma lucidum* were inoculated on this MCM broth and kept in orbital shaker incubator 25ºC. After 20 days the cultures were filtered through Whatman No.4 filter paper and centrifuged at 7000 rpm at 4ºC for 10 min to separate cell mass from the mushroom complete medium broth. The supernatant was taken to conical flasks and equal volume of ethyl acetate were added and kept in shaker for 24 hours. After that, solvents were separated through separating funnel. Separated solvents were concentrated in Vacuum flash evaporator at 40ºC. Then the solvents were evaporated at overnight. The metabolites were scrapped using HPLC- grade methanol and filtered using 0.2µm syringe filter and obtained 2 ml of metabolite for FT-IR analysis [15].

The standard compound Squalene (≥ 98%) (CAT. No# S3626) and Ganoderic acid A (CAT. No# SMB00445) was purchased from Sigma-Aldrich (St Louis MO, USA) and the compounds were dissolved in HPLC grade methanol and given for FT-IR analysis in Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India.

3. RESULTS AND DISCUSSION

3.1 FT-IR Spectra for *G. Lucidum*

FT-IR techniques were widely used for characterization of functional groups of particular compounds and [16] for evaluated the storage stability of the polysaccharides in the *Ganoderma lucidum*. In our study, result showed in the FT-IR spectrum for the *G. lucidum* obtained the region of 3782.69 cm^{-1} to 568.898 cm^{-1}. The peak absorptions were mentioned in Fig. 1 with different wavelengths. The alcohols, halogens, silicon, and phosphorus compound were identified in the FT-IR analysis and results were given in Table 1. In the same way, band between 3600 and 3200 cm^{-1} was reported as hydroxyl groups and 2923 cm^{-1} reported as C-H stretching in the crude extracts of *Ganoderma lucidum* using FTIR spectroscopy analysis [13]. In our study, we have obtained the hydroxyl bond in the *Ganoderma lucidum*. Correspondingly, [17] reported the stretching mode of hydroxyl group between the absorption value of 4000 and 1800 cm^{-1} and 750–950 cm^{-1} interval, assigned to α- and ß- glucans, are provided for all species studied.

The presence of silicon and phosphorus compounds were responsible for the nature of the biological activity of *G. lucidum*. Alcohol R-CH2-OH group in strong stretches, C-O bond was observed in the wavelength 1075-1000 cm^{-1} and variable stretches with OH bond were obtained, between 3400-3200 cm^{-1} and 1480-1410 cm^{-1} medium to weak intensity deformation stretches was also obtained. Furthermore, the main absorptions of C-O stretching (1155.04 cm^{-1}, 1079.61 cm^{-1}, and 1022.59 cm^{-1}) reported as the characteristics of sugar structures the band at 899.10 cm^{-1} was attributed to *β*-configuration in the polysaccharide [14].

Alcohol Ph-CHR-OH group was found C-O bond 1075-1000 cm^{-1} strong, OH 3400-3200 cm^{-1} variable and 1350-1260 strong deformation were obtained. Phosphorus compound P-O-P bond, stretches were observed in 1025-870 cm^{-1} wavelength and strong stretches of phosphorus compound were found in the wavelength 580-440 cm^{-1}. Also, scientist was used the mid-infrared and near-infrared spectroscopy to the characterization, detection and quantification of bands of carbohydrates in the wavelength

1425 cm^{-1}, 1316 cm^{-1} from the polysaccharide extraction of *Ganoderma lingzhi* (*G. lingzhi*), *G. sinense G. applanatum* [18].

Silicon compound 1020-1010 cm^{-1} variable-strong and 1100-1000 cm^{-1} variable- strong stretching with the open-chain intensity of silicon compound were found in the secondary metabolite of *G. lucidum*. Halogens also characterized in the sample; strong intensity observed in the wavelength C-Br 600-500 cm^{-1}, C-I bond strong and C-F bond 1300-900 cm^{-1} wavelength variable- strong intensity stretches. The chitin representative bands were reported at the wavelength of 3,430 and 894 cm^{-1} in *Ganoderma lucidum* submerged mycelium cultures [19]. In addition, the scientists used the FT-IR spectroscopy technique for the characterization organic groups amides, water, alcohol, phenol groups in the wheat varieties [10]. Similarly, researchers [20] used the FTIR diffuse reflectance spectroscopy with the combination of the chemometric method for differentiate the wild-grown and cultivated *G. lucidum* for medicinal value (anticancer effect).

3.2 FT-IR Spectra for Squalene

Ketones content were determined by FT-IR, C-(C=O)-C=C-OH group peak was observed on 1640-1540 cm^{-1} strong intensity (Beta diketones (enolic) C=O in the functional group, C-(C=O)-Ph-ßOH strong stretches 1655-1635 cm^{-1} (Beta-hydroxy aryl ketone), R-(C=O)-C=C-NH2 group 1640-1540 cm^{-1} (α ß unsaturated, beta-amino), C-(C=O)-Ph-ßNH2 group 1655-1635 cm^{-1} (Beta-amino aryl ketone), Naphthoquinones 1655-1635 cm^{-1} (Fused ring) were obtained (Fig. 2). The broad band spectrum at 3,430 cm^{-1}, which represented the stretching of the hydroxyl groups reported [21] and [22]. Also, the researcher was found the functional group alkali lignin in brown-rot fungus *Fomitopsis* sp. by FTIR spectroscopy [23]. The region between 1800 and 1500 cm^{-1}, is reported as the carbonyl and C=C double bond group. The region between 1500 and 750 cm^{-1}, is associated with the vibration of proteins, lipids, and carbohydrates. The absorption bands in the mid-infrared region 1200 to 800 cm^{-1} are useful for the identification of polysaccharides with different structures and compositions [24]. In the same way, [25] reported the carboxylic compounds such as aldehydes, esters, ketones, and lactones present in the high oleic sunflower oils formed during oxidation shown higher the intensities at 1739 cm−1.

In our results, we were found the amide groups in the wavelength 1670-1630 cm^{-1}. R-CO-NR2 (Tertiary amide) and Guanidines RNH-(C=NH)-NHR group 1680-1550 cm^{-1} strong (Structure shows one possible resonance form) were obtained. Nitriles C-N=C 1650-1550 cm^{-1} (Isonitriles) were observed. Azines RCH=N-N=CHR group in the wavelength of 1680-1600 cm^{-1} medium-strong stretches were observed (Table 2). Similarly, absorption bands of polysaccharide, sterols, proteins, fatty acids in the *Ganoderma lucidum* spores by using the FT-IR spectrum. 3290 cm^{-1} (N H stretching of amide and OH stretching of triterpene, polysaccharide, sterols), 3000–2800 cm^{-1}

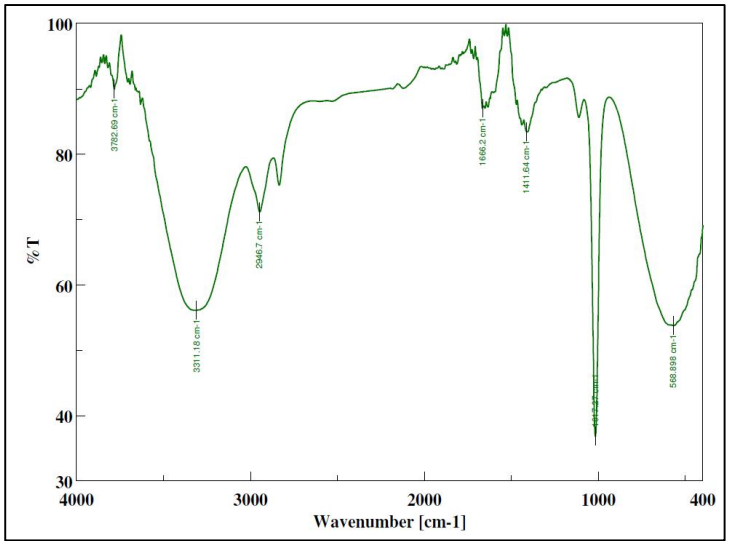

Fig. 1. Fourier Transformed-Infrared (FT-IR) spectroscopy analysis for *Ganoderma lucidum*

Table 1. FT-IR spectroscopy analysis of *Ganoderma lucidum*

S. No	Classification	Group	Bond	Wavelength cm^{-1}	Intensity	Mode	Notes
1.	Alcohols	R-CH_2-OH	OH	3400-3200	Variable	Stretching	Hydrogen
			OH	1480-1410	Medium- weak	deformation	bonded, broad
			C-O	1075-1000	Strong	Stretching	peak
2.	Halogens	C-F	C-F	1300-900	Variable- Strong	Stretching	-
3.	Halogens	C-Br	C-Br	600-500	Strong	Stretching	-
4.	Halogens	C-I	C-I	610-485	Strong	Stretching	-
5.	Phosphorus compound	P-O-P	P-O-P	1025-870	Strong	Stretching	-
6.	Phosphorus compound	P-Cl	P-Cl	580-440	Strong	Stretching	-
7.	Silicon compound	Silicon compound	Si-O-Si	1020-1010	Variable- Strong	Stretching	Cyclic trimer
8.	Silicon compound	Si-O-Si	-	1100-1000	Variable- strong	Stretching	Open chain
9.	Alcohols	Ph-CHR-OH	OH	3400-3200	Variable	Stretching	Stretching
			OH	1350-1260	Strong	deformation	-
			C-O	1075-1000	Strong	Stretching	-

Table 2. FT-IR spectroscopy analysis of squalene standard purchased from sigma- aldrich

S. No	Classification	Group	Bond	Range	Intensity	Mode	Notes
1.	Ketones	C-(C=O)-C=C-OH	C=O	1640-1540	Strong	Stretching	Beta diketones (enolic)
2.	Ketones	C-(C=O)-Ph-ßOH	C=O	1655-1635	Strong	Stretching	Beta- hydroxy aryl ketone
3.	Ketones	R-(C=O)-C=C-NH_2	C=O	1640-1540	Strong	Stretching	α ß unsaturated, beta amino
4.	Ketones	C-(C=O)-Ph-ßNH_2	C=O	1655-1635	Strong	Stretching	Beta- amino aryl ketone
5.	Ketones	Naphthoquinones	C=O	1655-1635	Strong	Stretching	Fused ring
6.	Amides	R-CO-NR_2	C=O	1670-1630	Strong	Stretching	Tertiary amide
7.	Guanidines	RNH-(C=NH)-NHR	C=N	1680-1550	Strong	Stretching	Structure shows one possible resonance form
8.	Nitriles	C-N=C	C=N	1650-1550	Strong	Stretching	Isonitriles
9.	Azines	RCH=N-N=CHR	C=N	1680-1600	Medium- strong	Stretching	
10.	Hydrazones	CH=N-NH_2	C=N	1680-1580	Medium- strong	Stretching	

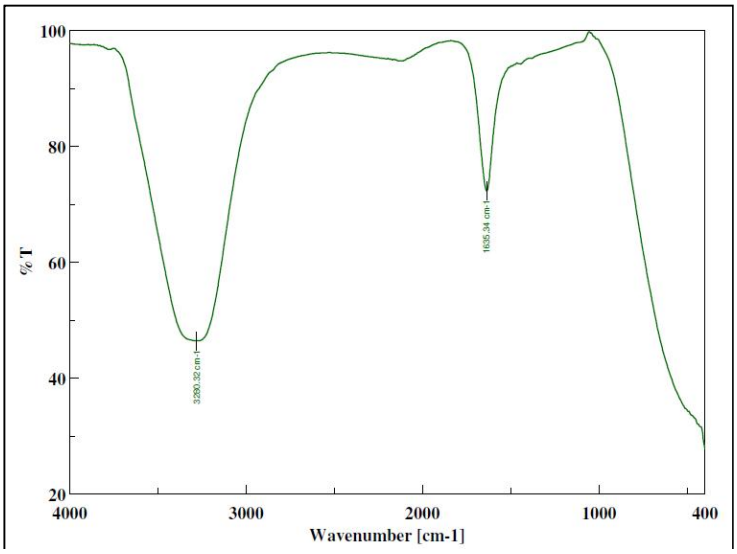

Fig. 2. Fourier Transformed- Infrared (FT-IR) spectroscopy analysis for squalene

Table 3. FT-IR spectroscopy analysis of ganoderic acid A standard purchased from sigma-aldrich

S. No	Classification	Group	Bond	Range	Intensity	Mode
1.	Sulfur compound	S-S	S-S	500-400	Variable- weak	Stretching

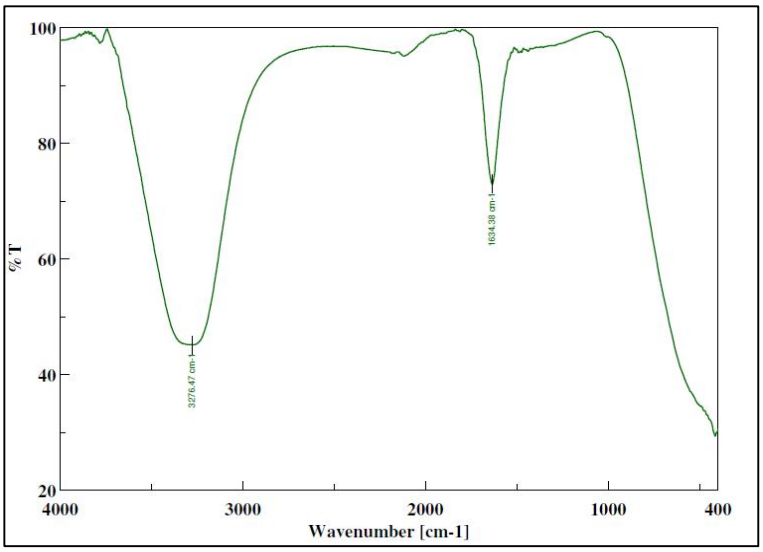

Fig. 3. Fourier Transformed- Infrared (FT-IR) spectroscopy analysis for ganoderic acid

(asymmetric and symmetric stretching of CH3 and CH2, 1155 cm^{-1} (C-O stretching in proteins and carbohydrates) [15], [26] and [12].

3.3 FT-IR Spectra for Ganoderic Acid A

The spectra of the ganoderic acid compound were taken which showed peaks on the 500-400 cm^{-1} wavelength (Fig. 3). The absorption peak represents the S-S group, sulfur compound variable- weak intensity stretching was observed (Table 3). The peaks are representative of the chemical group of the components present in the samples. Similarly, [26] used the Attenuated Total Reflection-Fourier Transform Infrared spectroscopy to identify the functional groups of the molecules in the *Ganoderma* sample.

FTIR spectrum measurement in the frequency range of 400 to 4000 cm^{-1} *G. lucidum* strain mycelial biomass and fruit body along with the standard DXN capsule were tested in the bands in the range of 1200 to 800 cm^{-1} indicated the presence of polysaccharides in the sample [27].

4. CONCLUSION

FTIR spectroscopy analysis were widely used for the characterization of organic compounds present in the samples in the different wavelength of infrared ranges. We have characterized the organic compounds such as ketones, silicon, phosphorus, halogens, guanidines, azides and amides in the *Ganoderma lucidum* and their standard compounds. Further, we have concluded our research, FT-IR is a fast-accurate technique very useful for the organic compounds characterization in the samples.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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