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FTIR Analysis of *Raillietina tetragona* (Chicken Cestode)

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Abstract

FTIR analysis is robust technique which can be used to identify functional groups of given material. In the present study an attempt was made to profile the cestode powder of *Raillietina tetragona* for the first time. The study was quite successful and FTIR analysis of the cestode powder yielded interesting results. The spectrum ranged from 1000-3500cm^{-1}. A total of 19 major peaks were identified, of which five were the sharp peaks. The highest sharp peak was found at 1539cm^{-1}, followed by 1641cm^{-1}, 1026cm^{-1}, 2920cm^{-1} and 2851cm^{-1}. The functional groups were identified using https://chem.libretexts.org/ and Thermo fisher databases. The identification and interpretation of these peaks, yielded interesting results. The highest peak at 1539, refers to alpha (α) elastin, which seems to be abundant in the sample and probably justifies with the fact that the parasite may be often dependent upon this protein for apolysis. Peak at 1641cm^{-1} suggest a strong c=c stretching and refers to the presence of amide group, similarly 1026cm^{-1} refers to cellulose, followed by 2920cm^{-1} for suberin and 2851cm^{-1} for lignin, indicating that the cestode may have ingested these plant components from the host animal (chicken) and accumulated in its body. Apart from these peaks some minor peaks were observed at 721cm^{-1} and also at 1081cm^{-1}. The peak at 721cm^{-1} suggests accumulation of iron oxide hematite and the peak at 1081cm^{-1} suggesting the presence of cadmium oxide. In summary it may be said that, functional group profiling of *Raillietina tetragona* using FTIR was successful in giving us an in-depth understanding about the composition of dead parasite and also about the dietary constituents of the host animal in particular, which might help us to develop newer strategies and drugs to control the helminthic infections.

Key words : *Raillietina tetargona,* FTIR, alpha elastins, cestode powder, functional groups.

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It is a well-known fact that, helminth infections, cause substantial damage to poultry and also to economy. According to global statistics, 66 % of helminth infections are caused due cestodes and *Raillietina tetragona* being the most prevalent of all helminth infections[18]. There are some stray instances where in this infection has been reported in humans, rats and also in monkeys in the past. However in recent times, no such report has been published. To treat this infections, Quite a number of anthelminthic drugs such as Niclosamide, Praziquintel, Albendazole are currently being used. Though the synthetic drug strategy to treat infections, is successful to certain extent, there is an ever growing evidence of developing resistance against these drugs. To overcome the problem of drug resistance, more and more farmers, and breeders are indulging in use of natural agents such as plant sources as anthelminthics. In this context, several authors, have investigated quite a number of plant varieties, and their extracts for their anthelminthic properties[3,16]. Though this approach seems to be successful to certain extent, but not efficient enough to eliminate the parasites. This may be because the parasites seem to develop new strategies to evade host immune system or seem to undergo certain physiological changes. In this connection, there are few evidences available where in trace elements seems to have played a role in modifying the tegument physiology, sequestration of free radicals, and also in evading host defense mechanism[10]. Based upon these studies, it may be said that certain radicals and also certain trace elements present in the parasites seem to have an impact on the parasite physiology and also might impact its association with the host animal (chick). Therefore the present study was aimed to learn about the chemical composition of the cestode in general and also to detect the presence of any trace elements if any. To achieve this, FTIR analysis of the *Rallientina tetragona* parasite as a powder was performed.

The cestode parasites *Raillietina tetragona*, were collected from the chicken intestines. The Discarded chicken intestines, were brought from the source *i.e.* from slaughter house. The intestines were thoroughly washed and were dissected using dissection tray and forceps. The parasites were collected in to petridish using water. The petridish with the collected parasites were placed in the incubator at a temperature of 30^{o}C for a period of 30 minutes. The parasites were thoroughly dried and were later powdered using mortar and pestle. The parasite powder was white in colour and was collected in to the test-tube and was later used for FTIR analysis. The prepared sample was used as a sample for the FTIR Analysis at C.F.R.D. Osmania University, Hyderabad.

Fourier Transform Infrared Spectroscopy (FTIR) is an important technique that provides an easy way to identify the presence of certain functional groups in an organic molecule. Functional groups have vibration frequencies that are characteristic of that functional group. These vibration frequencies fall with the infrared (IR) frequency range. As such, passing an IR signal through the organic compound causes the functional groups to vibrate at specific frequencies. In other words, an infrared signal that passes through an organic compound will be absorbed at these characteristic frequencies, which can be transformed into a

unique spectrum. An IR beam goes through a partially silvered mirror, which splits the beam into two beams of equal intensity. One portion of the beam goes through the sample. The other is guided through the machine with mirrors. The sample will absorb some of the energy of the IR source depending on the functional groups that are present. A wave pattern is created from the constructive and destructive interference that occurs when the two beams meet. This resulting wave is known as an interferogram.

FTIR analysis resulted in 19 peaks. 5 of them are major peaks and their functional groups are as follows.

Fourier transform changes the data from intensity as a function of time into intensity as a function of frequency (more commonly wavenumber) to reveal an IR spectrum. The IR spectrum can be presented either as absorbance or transmission. The absorption is typically presented as downward peaks in an IR spectrum.The interferogram, is plotted with Transmittance on the "Y axis and wave number on the "X" axis.

Table-1. All the functional groups and the nature of the bonding has been illustrated in the table

S. no	Peak value	Peak Range	Functional group	Nature	Name of the compound
1.	1539	1500-1550	N-O	Stretching	Nitrocompound
2.	1641	1640-1690	C=N	Stretching	Imine/ oxime
3.	2920	2840-3000	C-H	stretching	Alkane
4	1026	1020-1075	C-O	Stretching	Vinyl ether
5	2851	2830-2695	C-H	stretching	aldehyde
6	3286	3200-3550	O-H	Stretching	alcohol
7	1574	-	-	-	-
8	1741	1735-1750	C=O	Stretching	ä-lactone
9	1465	1465	C-H	Bending	Alkane
10	1414	1380-1415	S=O	Stretching	Sulphate
11	1233	1020-1250	C-N	Stretching	Amine
12	1151	1120-1160	S=O	stretching	Sulfone
13	1081	-	-	-	-
14	931	-	-	-	-
15	859	-	-	-	-
16	762	780+/- 20	C-H	bending	1,3 dissubstituted
17	721	655-730	C=C	Bending	Alkene
18	675	515-690	C-Br	Stretching	Halo compound
19	622	515-690	C-Br	Stretching	Halo compound

The functional group identification was done using
https://chem.libretexts.org/Ancillary_Materials/Reference/Reference_Tables/Spectroscopic_Reference_Tables/Infrared_Spectroscopy_Absorption_Table

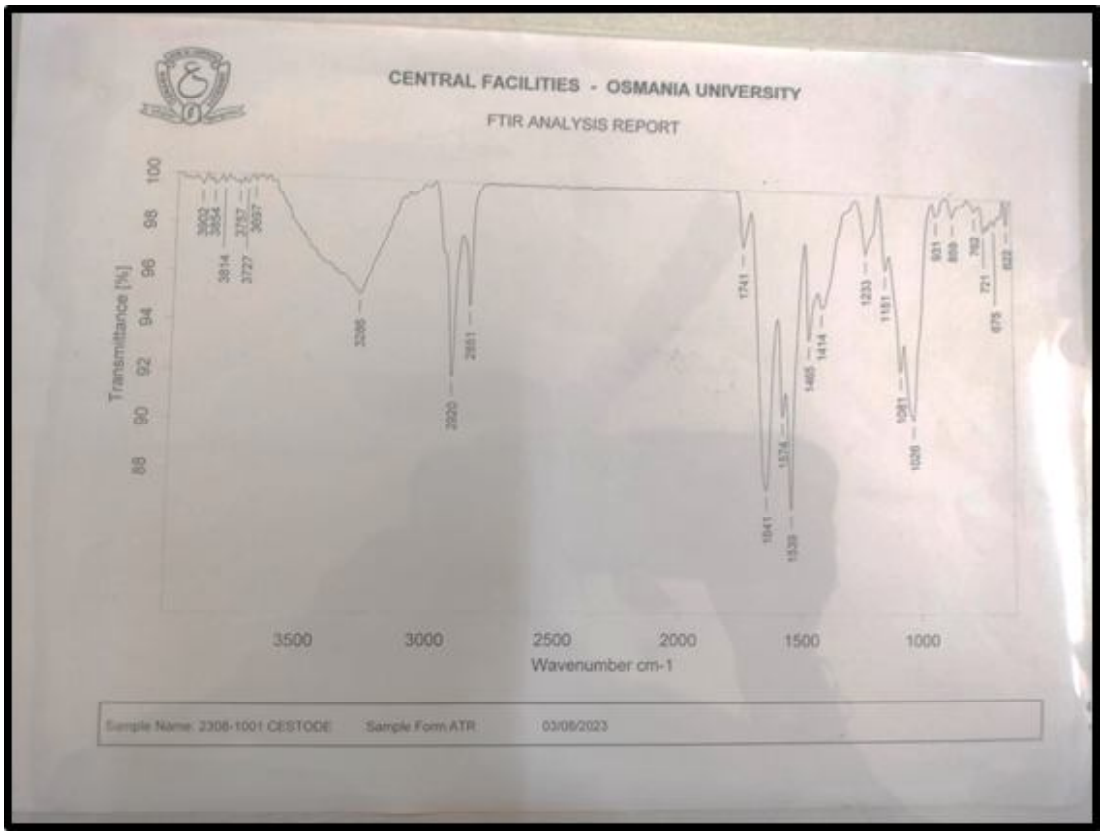

Fig. 1

In the present study, it was interesting to study the FTIR analysis of the Chicken cestode, *Raillietina tetragona*. As sated earlier, the rationale behind, subjecting cestode, as a powder to FTIR analysis was to identify major functional groups, and also to know about the presence of any trace elements or any other metals of biological importance. FTIR analysis of the cestode, revealed nineteen prominent peaks, of which 5 peaks are of major importance. A tallest t or a highest of all the peaks was formed at 1539 cm^{-1}, followed by 1641 cm^{-1}. 2920cm^{-1}, 2851cm^{-1} and lastly a broad area at 3286cm^{-1} was found. As 1531 was found to be sharpest and abundant of all the peaks, it was quite evident that this component seemed to be the main ingredient of the parasite. **1531** peak falls in the range of 1500-1550, which suggest, a N-O stretching (Libre text.org) and is known to be an alkene (Thermo fisher) This peak may be attributed to soluble alpha (α) elastins of amide II type

as suggested by Popescu *et.al*,[13]. **This is an interesting observation, which has been made for the very first time in parasite biology**. Elastins are structural proteins found in the extracellular matrix in mammals[11] and are known to provide elasticity and resilience to the tissues[9]. Additionally they are known to exhibits coacervation when found in solution as suggested by Elena Ragnon *et. al*,[4]. .Presence of this peak in the cestode, samples, clearly indicates the presence of alpha elastins, and these proteins seem to be beneficial for the parasite as it is often known to shed its proglottids and so needed to restructure its tissues. The next peak which was prominently noticed was at **1641** cm^{-1}, this peak represents, that it has a strong C=C stretching and it may be an alkene. It falls under the category of 1638-1648 (libre text.org) and also it may be an amide belonging to class1. These observations coincides with that of Li QB, *et.al*,[12] who have observed almost a similar peak at 1645 in their gastric biopsy samples and attributed it to amide of class1. In another study by Jamil *et. al.*[8] have reported a similar vibrational peak at 1641cm^{-1} in their study about essential oils. Presence of this peak representing an amide in the present study, definitely suggests that cestodes may steal the proteins (polyamides) from the host animal depriving them of protein nourishment.In addition to these major peaks, it was interesting to see another peak at **2920** cm-^{1}. This peak falls in the category of 2800-3000cm^{-1}, and shows the presence of strong N-H stretching and could be an amine salt. A similar peak was observed by authors like Ferreira *et al.*[5], and they attributed this peak to aliphatic chains of suberin, plant cell wall polymer[20]. This vibration at 2920cm^{-1}, in the present cestode powder, seems to suggests that probably the diet of chicken consisted certain plant source containing corn or some plant roots and so has been reflected as a peak in the FTIR analysis. Apart from these, there were some sharp peaks noticed at 1026 cm^{-1} and 2851cm^{-1} and broad peak is seen at 3286cm^{-1}. Among these peaks the peak at **1026c**m^{-1} is quite significant. This peak falls under the region of 1020-175, representing a strong C-O stretching. This peak represents cellullose as suggested by Kim *et.al.* (2018), who have observed almost a similar peak at 1028cm^{-1}, in their analyses when they had studied different natural polymers using FTIR analysis. The relevance of this peak in the present study is that, the host animal feed may have contained large quantities of plant derived material containing cellulose and this may have been either absorbed or accumulated by the parasite from the host intestine. The vibrational peak at 2851 cm^{-1}, also seems to be relevant to cestode and its host chicken. This peak closely resembles the peak of lignin as suggested by Salim *et.al,* (2021), who have observed a similar peak in their studies about the constituents of *Leucaena leucocephala* bark. They had observed a strong peak of lignin in their studies, at 2855cm^{-1}. Moreover studies by Sahoo *et al.*[17] suggest that peaks at 2927 and 2855 cm^{-1} could possibly correspond to the C–H stretching in the lignin molecules. Therefore it may be suggested that, in the present study, the cestode may have absorbed this heavy carbohydrate in to its body from the host diet. Another cellulose peak can be identified at 3286cm^{-1} in the cestode sample. This peak was broad and resembles that of intra molecular cellulose bonding of cellulose fibres[19] Apart from these major peaks, some minor peaks can be seen.

These peaks are at 721cm^{-1}, 1081cm^{-1}. Peak at 721 is peak which might be formed due formation of iron oxide, heamatite. A peak at this range illustrates Fe-O vibrations, suggesting the formation of iron oxide.[7] This may be because the cestode may have ingested the iron from the diet of the host and could have formed an iron oxide. Similarly the peak at 1081may be that of a trace element cadmium oxide[14] which is a trace element. Presence of iron and cadmiuon oxide in the cestode powder of *Raillietina tetragona*, seems to suggest, that the parasite may be in the process of developing new strategies, to maintain tegument physiology or synthesize new compounds so as to evade the host immune system and thereby survive in the host intestines. These observations, apparently give an in-depth understanding about the composition of cestode morphology and physiology. FTIR analysis seems act as an index, detailing the components ingested or synthesized by the parasite when present inside the host intestine.

In summary it may be said that the main ingredient of the cestode powder was alpha (α) elastin, followed by amide, suberin, lignin and cellulose. The minor peaks represent ingested iron, and trace element like cadmium oxide. **A study of this nature is first of its, kind and opens up a whole new avenue of research in the field of helminthology**.

The FTIR analysis of the *Raillientina tetragona* as cestode powder was very successful. The main ingredient was found to be alpha elastin and amide. Other plant cell wall constituents such as lignin, suberin and cellulose were also reported. Trace elements such as iron and cadmium oxide were present in the sample. This study is a first of its kind, and in future similar analytical techniques such as GC-MS, or UV-ViS may be employed for further profiling of the parasites so as to learn more about their unknown secrets.

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