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### *RESEARCH ARTICLE*

# USING FOURIER TRANSFER INFRARED SPECTROSCOPY TO ANALYSIS EFFECTS OF CADMIUM TREATMENT ON RICE (*ORYZA SATIVA* L.) SEEDLINGS

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## *Abstract*

The present study is aimed to analyse the DMSO (Dimethyl sulfoxide) extract of shoots and roots of *Oryza sativa*L. (var IET 4786) through FTIR spectroscopy method. These studies contain different characteristic of the peak values with various functional compounds in the extracts. This analysis revealed the presence of different types of biomolecules in plant extracts. The FTIR analysis of DMSO leaf extracts of *Oryza sativa*L. confirmed the presence of alcohols, phenols, amides, alkanes, carboxylic acids, aldehydes, ketones, alkenes, aromatics, esters, alkyl halides and aliphatic amines compounds; each of the functional group showed major peaks. This method was performed on a spectrophotometer system which was used to detect the characteristics peak values and their functional groups. In this present study, the FTIR spectrum profile for the plant *Oryza sativa* L. is generated andcan be used in the industry.

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## Introduction:-

Cadmium (Cd) is non-essential heavy metal and also it is hazardous for living organism (Bhattacharya, 2015; Aiqing, 2021; Shanmugaraj, 2019). The common symptoms of the cadmium toxicity are chlorosis, growth inhibition and necrosis. In order to the functional groups present in these chemical constituents of plant are usually identified by FTIR. It is used to elucidate the structure of isolated compounds.The presence of phenols, alkanes, alcohol, alkyl halides, carboxylic acid and aromatic compounds in DMSO extracts of *Oryza sativa*L. was also studied by FTIR spectroscopy method. Identify the chemical nature of the constituents that present in the rice plants. And the properties are related to certain compounds such as flavonoids, alkaloids and other things.Thus, FTIR spectroscopy method has become one of the avenues for the identification of compounds. Hence, an attempt is made in the present study to analyse the functional groups of Phyto active compounds present in the shoot and root extracts (in solvent such as, DMSO). This also helps to know the influence of solvents on functional group present in the plant (Raghavendran, et al., 2011)

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## Materials and Methods:-

### Seed collection and treatment

The rice seedlings *Oryza sativa*L. (var. IR 4786) was obtained from the Bidhan ChandraKrishi Vishwavidyalaya (BCKV), which is also known as the Bidhan Chandra AgriculturalUniversity and is located in Mohanpur,West Bengal, India. After being surface sterilizedwithasolutioncontaining1%sodiumhypochlorite,thisvarietywassoakedindistilledwaterat 4$^{o}$C for 24 hours to absorb water. The 15 seeds were then transferred to three petriplates and surrounded by three filter papers. The seedlings are then given Cadmium chloride(CdCl2) treatments (0, 100, 500 µM). For 10 days, the experiment was carried out in thelaboratory under room temperature conditions. By irrigation with distilled water twiceper week, the Cadmium (Cd) treatment was administered. According to [4], three sets weremaintained throughout the entire experiment. Every day, the nutrient solutions were changed[2].After sevendays,theshootsandrootsof three rice plantsampleswere usedfor FTIRexperiments.

### FTIR Spectroscopic Analysis:

Fourier Transform Infrared Spectrophotometer (FTIR) is most significant tool for identifying the types of functional groups that present in chemical compounds. The plant material was dried in hot air oven. The dried plants shoot and root material was ground to powder and stored in air tight container for further use.The dried powdered shoot and root (1 g) of *Oryza sativa*L. was ground in mortal and pestle in order to obtain fine powder and mixed with DMSO (95% v/v) for 24 hours. After 24 hours the plant extract was loaded in FTIR spectroscope ((Shimadzu, IR Affinity 1, Japan) and Scan range from 400 to 4000 cm$^{-1}$ with a resolution of cm$^{-1}$(Nair, Sar, Arora, & Mahaptra, 2019)

## Result:-

The FTIR spectrum of shoot and root extracts of the data on the peak values and the functional groups of *Oryza sativa*L. presented in table 1 to 4

In control, the intense band occurring at 2920 cm$^{-1}$, 2854 cm$^{-1}$, 2226 cm$^{-1}$, 1746 cm$^{-1}$, 1672 cm$^{-1}$, 1437 cm$^{-1}$, 1316 cm$^{-1}$, 1014 cm$^{-1}$, 704.6 cm$^{-1}$ and 610.5 cm$^{-1}$ corresponding to C-H/ O-H/ -C#C-/ C=O/ C=C/ C-F/ C-O/ =C-H/ C-Br stretch and bend are indicating the presence of Alkanes, Carboxylic acids, Alkynes, Amides, Alkenes, Aromatics, Alkyl halides and Esters compounds respectively in shoots and 2919 cm$^{-1}$,1672 cm$^{-1}$,1315 cm$^{-1}$, 1014 cm$^{-1}$, 704.3 cm$^{-1}$ and 617.3 cm$^{-1}$ corresponding to O-H/ C=O/ C-C/ C-O/ =C-H/ C-H stretch and bend are indicating the presence of Carboxylic acids, Ketones, Aromatics, Esters, Alkenes, Alkynes compounds respectivelyin roots IET 4786 variety of *Oryza sativa*L. (Rice).

In (100 µM) the low concentration of cadmium chloride, the intense band occurring at 2919 cm$^{-1}$,1672 cm$^{-1}$,1437 cm$^{-1}$, 1316 cm$^{-1}$, 1013 cm$^{-1}$, 705.1 cm$^{-1}$ and 609 cm$^{-1}$ corresponding to C-H/ C=O/ C-C/ C-O/ C-F stretch, "oop", and bend are indicating the presence of Alkanes, Ketones, Aromatics, Carboxylic acids, Alkyl halides, Alkynes compounds respectivelyin shoots and 2919 cm$^{-1}$,1672 cm$^{-1}$,1437 cm$^{-1}$, 1316 cm$^{-1}$, 1013 cm$^{-1}$, 705.4 cm$^{-1}$ and 609.7 cm$^{-1}$ corresponding to C-H/ C=O/ C-C/ C-N/ C-F, N-H wag and stretch and bend are indicating the presence of Alkanes, Ketones, Aromatics, Carboxylic acids, Alkyl halides, Amines, Alkynes compoundsrespectively in roots the IET 4786 variety of rice(*Oryza sativa*L.).

In (500 µM) the high concentration of cadmium chloride, the intense band occurring at 2919 cm$^{-1}$,1676 cm$^{-1}$,1437 cm$^{-1}$, 1316 cm$^{-1}$, 1014 cm$^{-1}$, 704.8 cm$^{-1}$ and 565.2 cm$^{-1}$ corresponding to C-H/ C=O/ C-C/ C-N/ C-O/ C-Br stretch and "oop" are indicating the presence of Alkanes, Amides, Aromatics, Carboxylic acids, Esters, Alkyl halides compounds respectivelyin shoots and 2920 cm$^{-1}$,1672 cm$^{-1}$,1437 cm$^{-1}$, 1316 cm$^{-1}$, 1014 cm$^{-1}$, 704.6 cm$^{-1}$ and 610.5 cm$^{-1}$ corresponding to C-H/ C=O/ C-C/ C-O/ C-F stretch, "oop", and bend are indicating the presence of Alkanes, Ketones, Aromatics, Carboxylic acids, Alkyl halides, Alkynes compoundsrespectively in roots the IET 4786 variety of *Oryza sativa*L. (Rice).

**Table 1:-** FTIR spectra without $CdCl_2$ concentrations on 10 days old(var. IET 4786) shoot of *Oryza sativa* L. riceseedlings.

| **Peak number** | **Wave number cm$^{-1}$[Test sample]** | **Wave number cm$^{-1}$ [Reference article]** | **Functional groups** | **Phyto compounds** |
|---|---|---|---|---|
| 1 | 2920 | 3000–2850 | **C–H** stretch | Alkanes |
| 2 | 2854 | 3300-2500 | **O–H** stretch | Carboxylicacids |

| | | | | |
|---|---|---|---|---|
| 3 | 2226 | 2260-2100 | **–C#C**– stretch | Alkynes |
| 4 | 1746 | 1750 -1740 | **C=O** stretch | Amides |
| 5 | 1672 | 1680-1640 | **C=C** stretch | Alkenes |
| 6 | 1437 | 1500-1400 | **C–C** stretch (in-ring) | Aromatics |
| 7 | 1316 | 1350 - 1000 | **C-F** stretch | Alkyl halides |
| 8 | 1014 | 1300-1000 | **C–O** stretch | Esters |
| 9 | 704.6 | 1000-650 | **=C–H** bend | Alkenes |
| 10 | 610.5 | 690-515 | **C–Br** stretch | Alkyl halides |

**Table 2:-** FTIR spectra without $CdCl_2$ concentrations on 10 days old (var IET 4786) root of *Oryza sativa* L. riceseedlings.

| **Peak number** | **Wave number $cm^{-1}$ [Test sample]** | **Wave number $cm^{-1}$ [Reference article]** | **Functional groups** | **Phyto compounds** |
|---|---|---|---|---|
| 1 | 2919 | 3300-2500 | **O-H** stretch | Carboxylic acids |
| 2 | 1672 | 1715 | **C=O** stretch, aliphatic ketones | ketones |
| 3 | 1315 | 1500-1400 | **C-C** stretch (in-ring) | Aromatics |
| 4 | 1014 | 1200-1000 | **C-O** stretch | Esters |
| 5 | 704.3 | 1000-650 | **=C–H** bend | Alkenes |
| 6 | 617.3 | 700-610 | **C–H** bend | Alkynes |

**Table 3:-** FTIR spectra low $CdCl_2$ concentration (100 µM) on 10 days old (var IET 4786) shootof *Oryza sativa* L. riceseedlings.

| **Peak number** | **Wave number $cm^{-1}$ [Test sample]** | **Wave number $cm^{-1}$ [Reference article]** | **Functional groups** | **Phyto compounds** |
|---|---|---|---|---|
| 1 | 2919 | 3000–2850 | **C–H** stretch | Alkanes |
| 2 | 1672 | 1685-1666 | **C=O** stretch | Ketones |
| 3 | 1437 | 1500-1400 | **C–C** stretch | Aromatics |
| 4 | 1316 | 1320-1210 | **C–O** stretch | Carboxylic acids |
| 5 | 1013 | 1350 - 1000 | **C-F** stretch | Alkyl halides |
| 6 | 705.1 | 900-675 | **C–H "oop"** | Aromatics |
| 7 | 609 | 700-610 | **C–H** bend | Alkynes |

**Table 4:-** FTIR spectra low $CdCl_2$ concentration (100 µM) on 10 days old (var IET 4786) root of *Oryza sativa* L. riceseedlings.

| **Peak number** | **Wave number $cm^{-1}$ [Test sample]** | **Wave number $cm^{-1}$ [Reference article]** | **Functional groups** | **Phyto compounds** |
|---|---|---|---|---|
| 1 | 2919 | 3000–2850 | **C–H** stretch | Alkanes |
| 2 | 1672 | 1685-1666 | **C=O** stretch | Ketones |
| 3 | 1437 | 1500-1400 | **C–C** stretch | Aromatics |
| 4 | 1316 | 1335-1250 | **C–N** stretch (aromatic amines) | Carboxylic acids |
| 5 | 1013 | 1350 - 1000 | **C-F** stretch | Alkyl halides |
| 6 | 705.4 | 910-665 | **N–H** wag (primary and secondary amines only) | Amines |
| 7 | 609.7 | 700-610 | **C–H** bend | Alkynes |

**Table 5:-** FTIR spectra high $CdCl_2$ concentration (500 µM) on 10 days old (var IET 4786) shoot of *Oryza sativa* L. riceseedlings.

| **Peak number** | **Wave number cm$^{-1}$ [Test sample]** | **Wave number cm$^{-1}$ [Reference article]** | **Functional groups** | **Phyto compounds** |
|---|---|---|---|---|
| 1 | 2919 | 3000–2850 | **C–H** stretch | Alkanes |
| 2 | 1676 | 1685-1666 | **C=O** stretch (free) | Amides |
| 3 | 1437 | 1500-1400 | **C–C** stretch | Aromatics |
| 4 | 1316 | 1335-1250 | **C–N** stretch (aromatic amines) | Carboxylic acids |
| 5 | 1014 | 1300-1000 | **C-O** stretch | Esters |
| 6 | 704.8 | 900-675 | **C–H** "oop" | Aromatics |
| 7 | 565.2 | 700-610 | **C–Br** stretch | Alkyl halides |

**Table 6:-** FTIR spectra high $CdCl_2$ concentration (500 µM) on 10 days old (var IET 4786) root of *Oryza sativa* L. riceseedlings.

| **Peak number** | **Wave number cm$^{-1}$ [Test sample]** | **Wave number cm$^{-1}$ [Reference article]** | **Functional groups** | **Phyto compounds** |
|---|---|---|---|---|
| 1 | 2920 | 3000–2850 | **C–H** stretch | Alkanes |
| 2 | 1672 | 1680-1640 | **C=C** stretch | Alkenes |
| 2 | 1437 | 1500-1400 | **C–C** stretch (in ring) | Aromatics |
| 4 | 1316 | 1350 - 1000 | **C-F** stretch | Alkyl halides |
| 5 | 1014 | 1300-1000 | **C–O** stretch | Esters |
| 6 | 704.6 | 1000-650 | **=C–H** bend | Alkenes |
| 7 | 610.5 | 690-515 | **C–Br** stretch | Alkyl halides |

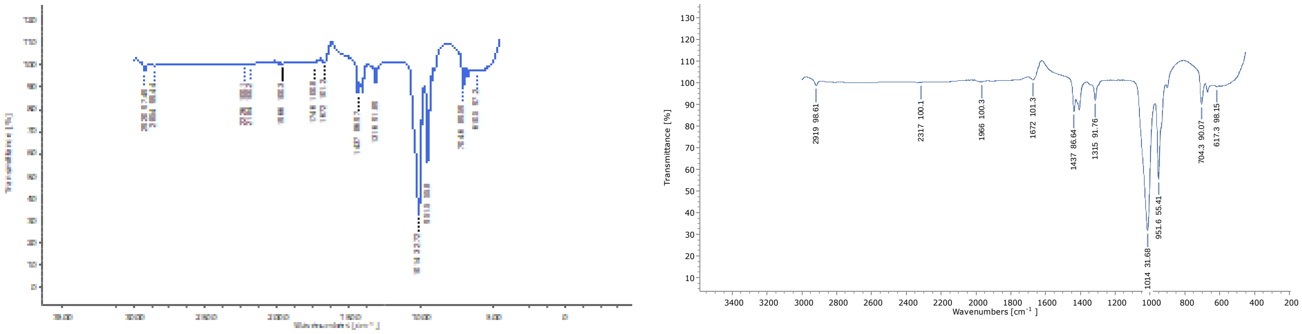

**Figure 1:-** FTIR analysis without $CdCl_2$ concentrations of shoot and root on (var IET 4786) of 10 days old rice seedlings *Oryza sativa* L.

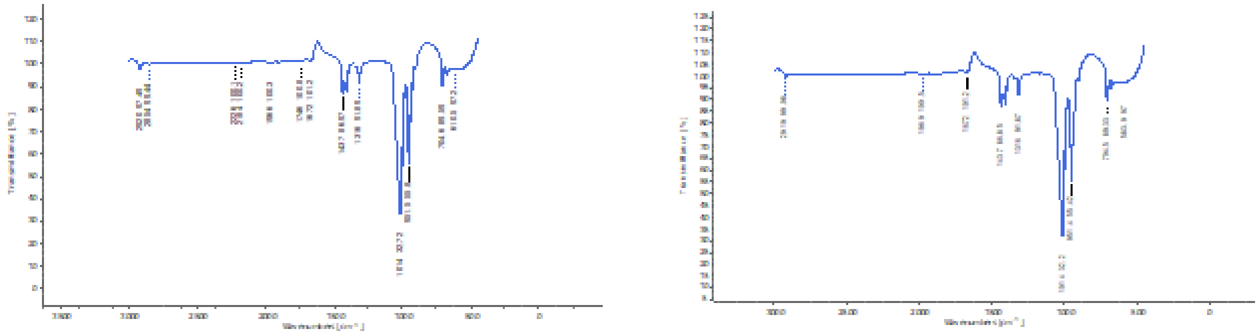

**Figure 2:-** FTIR analysis low $CdCl_2$ concentration (100 µM) on 10 days old (var IET 4786) shoot and root of *Oryza sativa* L. riceseedlings

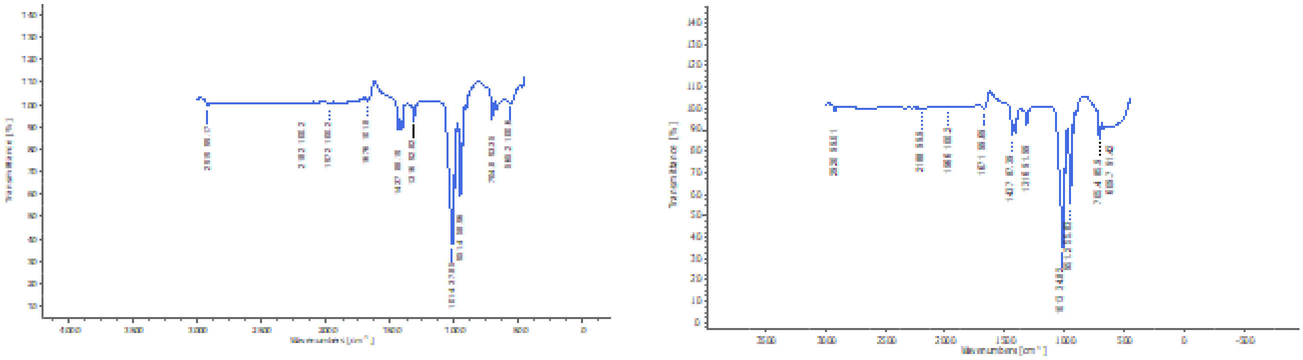

**Figure 3:-** FTIR analysis high $CdCl_2$ concentration (500 µM) on 10 days old (var IET 4786) shoot and root of *Oryza sativa* L. riceseedlings.

## Discussion:-

In this study we identify the functional group that present in the shoot and root in the variety (IET 4786) of Oryza sativa L. with the help of FTIR analysis.In fig 1 to 3 it helps to identify the chemical components, interprets the structure of chemical structure and helps to understand the importance of functional group.

The spectra of simple alkanes are characterized by absorptions due to C–H stretching and bending (the C–C stretching and bending bands are either too weak or of too low a frequency to be detected in IR spectroscopy). In simple alkanes, which have very few bands, each band in the spectrum can be assigned. However, each organic compound has its own unique absorption pattern in this region and thus an IR spectrum can be used to identify a compound by matching it with a sample of a known compound( MEENAMBAL, et al., 2012).

Carboxylic acids show a strong, wide band for the O–H stretch. Unlike the O–H stretch band observed in alcohols, the carboxylic acid O–H stretch appears as a very broad band in the region 3300-2500 $cm^{-1}$, cantered at about 3000 $cm^{-1}$. This is in the same region as the C–H stretching bands of both alkyl and aromatic groups. Thus, a carboxylic acid shows a somewhat "messy" absorption pattern in the region 3300-2500 $cm^{-1}$, with the broad O–H band superimposed on the sharp C–H stretching bands. The reason that the O–H stretch band of carboxylic acids is so broad is because carboxylic acids usually exist as hydrogen-bonded dimers. The carbonyl stretch C=O of a carboxylic acid appears as an intense band from 1760-1690 $cm^{-1}$. The exact position of this broad band depends on whether the carboxylic acid is saturated or unsaturated, dimerized, or has internal hydrogen bonding. (Sharma, et al., 2011). The C–O stretch appears in the region 1320-1210 $cm^{-1}$, and the O–H bend is in the region 1440-1395 $cm^{-1}$ and 950-910 $cm^{-1}$, although the 1440-1395 band may not be distinguishable from C–H bending bands in the same region.

Alkynes are compounds that have a carbon-carbon triple bond (–C#C–). The –C#C– stretch appears as a weak band from 2260-2100 $cm^{-1}$. This can be an important diagnostic tool because very few organic compounds show an absorption in this region. A terminal alkyne (but not an interchain alkyne) will show a C–H stretch as a strong, narrow band in the range 3330-3270 $cm^{-1}$. (Often this band is indistinguishable from bands resulting from other functional groups on the same molecule which absorb in this region, such as the O-H stretch.). A terminal alkyne will show a C–H bending vibration in the region 700-610 $cm^{-1}$(Ncube , et al., 2008).

Amides functional group combines the features of amines and ketones because it has both the N-H bond and the C=O bond. Therefore, amides show a very strong, somewhat broad band at the left end of the spectrum, in the range between 3100 and 3500 $cm^{-1}$ for the N-H stretch. At the same time, they also show the stake-shaped band in the middle of the spectrum around 1710 $cm^{-1}$ for the C=O stretch(Gaurav , et al., 2010). As with amines, primary amides show two spikes, whereas secondary amides show only one spike.

Alkenes are compounds that have a carbon-carbon double bond, –C=C–. The stretching vibration of the C=C bond usually gives rise to a moderate band in the region 1680-1640 $cm^{-1}$. Stretching vibrations of the –C=C–H bond is of higher frequency (higher wavenumber) than those of the –C–C–H bond in alkanes. Absorption peaks above 3000 $cm^{-1}$ are frequently diagnostic of unsaturation. This is a very useful tool for interpreting IR spectra. Only alkenes and aromatics show a C-H stretch slightly higher than 3000 $cm^{-1}$. Compounds that do not have a C=C bond show C-H stretches only below 3000 $cm^{-1}$. The strongest bands in the spectra of alkenes are those attributed to the carbon-hydrogen bending vibrations of the =C–H group. These bands are in the region 1000-650 $cm^{-1}$(THENMOZHI, et al., 2011).

The =C–H stretch in aromatics is observed at 3100-3000 $cm^{-1}$. This is at slightly higher frequency than is the –C–H stretch in alkanes. This is a very useful tool for interpreting IR spectra: Only alkenes and aromatics show a C–H stretch slightly higher than 3000 $cm^{-1}$. Compounds that do not have a C=C bond show C–H stretches only below 3000 $cm^{-1}$. Aromatic hydrocarbons show absorptions in the regions 1600-1585 $cm^{-1}$ and 1500-1400 $cm^{-1}$ due to carbon-carbon stretching vibrations in the aromatic ring. Bands in the region 1250-1000 $cm^{-1}$ are due to C–H in-plane bending, although these bands are too weak to be observed in most aromatic compounds. Besides the C–H stretch above 3000 $cm^{-1}$, two other regions of the infrared spectra of aromatics distinguish aromatics from organic compounds that do not have an aromatic ring(DEEPASHREE, et al., 2012). It is useful to remember that aromatics in general show a lot more bands than compounds that do not contain an aromatic ring.

Alkyl halides are compounds that have a C–X bond, where X is a halogen: bromine, chlorine, fluorene, or iodine. In general, C–X vibration frequencies appear in the region 850-515 $cm^{-1}$, sometimes out of the range of typical IR instrumentation. C–Cl stretches appear from 850–550 $cm^{-1}$, while C–Br stretches appear at slightly lower wavenumbers from 690-515 $cm^{-1}$. In terminal alkyl halides, the C–H wag of the –CH2X group is seen from 1300-1150 $cm^{-1}$. Complicating the spectra is a profusion of absorptions throughout the region 1250-770 $cm^{-1}$, especially in the smaller alkyl halides(JANAKIRAMAN, et al., 2011) All of these bands are in the fingerprint region.

The carbonyl stretch C=O of aliphatic esters appears from 1750-1735 $cm^{-1}$; that of alfa,beta-unsaturated esters appears from 1730-1715 $cm^{-1}$.The C–Ostretches appear as two or more bands in the region1300-1000$cm^{-1}$(MUTHANNA, et al., 2009).

The carbonyl stretching vibration band C=O of saturated aliphatic ketones appears at 1715 $cm^{-1}$. Conjugation of the carbonyl group with carbon-carbon double bonds or phenyl groups, as in alpha, beta-unsaturated aldehydes and benzaldehyde, shifts this band to lower wavenumbers, 1685-1666 $cm^{-1}$.

The most characteristic band in amines is due to the N-H bond stretch, and it appears as a weak to medium. This band is positioned at the left end of the spectrum, in the range of about 3250 - 3400 $cm^{-1}$. Primary amines have two N-H bonds;therefore, they typically show two spikes that make this band resemble a molar tooth. Secondary amines have only one N-H bond, which makes them show only one spike, resembling a canine tooth(MURUGANANTHAM, et al., 2009) Finally, tertiary amines have no N-H bonds, and therefore this band is absent from the IR spectrum altogether. The spectrum below shows a secondary amine.

Secondary amines ($R_2NH$) show only a single weak band in the 3300-3000 $cm^{-1}$ region, since they have only one N–H bond. Tertiary amines ($R_3N$) do not show any band in this region since they do not have an N–H bond. The N–H bending vibration of primary amines is observed in the region 1650-1580 $cm^{-1}$. Usually, secondary amines do not show a band in this region and tertiary amines never show a band in this region. Another band attributed to amines is observed in the region 910-665 $cm^{-1}$. This strong, broad band is due to N–H wag and observed only for primary and secondary amines.

## Conclusion:-

In the current study from the results, after extraction the functional groups have been noticed in the sample. Using FTIR spectrum we can confirm the functional groups are present in the given extract and indicate the presence of phytoconstituents like carbohydrates, flavonoids, carotenoids, amino acids, amides, phosphates, lipids and phenols. We know OH group present in the plant extract that has inhibitory activity against different microorganisms.Many researchers applied the FTIR spectrophotometer as a tool for distinguishing the associated plants.Additionally, it has accurate and sensitive technique for detecting the changing of biological molecular compounds.

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