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On the Characterization of Plant Poison : Cascabella Thevetia (L.) Lippold, using FTIR Spectroscopy-Application in Forensic Science

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ABSTRACT

Poisonous plant parts or extract of C. thevetia may be encountered as vital forensic evidence in criminal investigations involving intentional, accidental or homicidal poisoning cases. The pilot study sought to effectively use infrared spectroscopy to unprejudiced, precise and high-speed characterization of C. thevetia in order to support various forensic investigations. The dried and powdered leaves of this plant were analyzed for a variety of bioactive functional chemical components by Fourier Transform Infrared (FTIR) spectroscopy using KBr disc method. The FTIR spectrum revealed the presence of several functional groups of chemical constituents such as aromatics, amide, ketone, aldehydes, alcohols (primary, secondary or tertiary) alkyl halides compounds, aliphatic amines (1°, 2° or 3°), aromatic amines (1°, 2° or 3°), ether, aromatic ether, phosphate ion, organic nitrate, phenols, carboxylic group, esters, six membered ring lactone, peroxides, siloxane, cyanide ion, thiocyanate ion alkane, alkene and alkyne. The spectra showed 19 major distinctive bands of bioactive chemical components. C. thevetia plant from two different geographical regions were analyzed using FTIR, which successfully distinguished the variations in the concentration of constituents of the plant according to difference in the geographical region. This model can be considered reliable and can be foreseen to provide a good level of confidence during the conduction of 'questioned versus known' comparisons of Cascabela thevetia plant.

Keywords: FTIR Spectroscopy, Characterization, C. thevetia, Plant Poison and Toxicology

I. INTRODUCTION

In India, approximately 8000 medicinal plant species are found; of which, 50% are the higher flowering plants. These plant species have medicinal properties which have been used for self- care by millions of rural families for decades [1]. The World Health Organization (WHO) reports that some countries continue to use plant-based treatments as their primary source of medicine. Moreover, the developing countries are making use of therapeutic benefits of substances derived from naturally occurring sources as

they have several advantages, like being inexpensive, easy availability, greater efficiency, easy handling and fewer side effects [2].

C. thevetia is one of the numerous medicinal plant species which is used in traditional medicine systems as it possesses antibacterial and antioxidant properties. An earlier study by Geetha et.al. (2017) reported that the naturally occurring alkaloids and the synthetic derivatives of Cascabela plant have analgesic, antispasmodic and antibacterial properties [10]. The plant has been used for centuries to treat a variety of diseases, including diabetes, malaria, jaundice and many more [11].

The dicotyledonous plant Cascabela thevetia (L), often known as yellow oleander, is a member of the Apocynaceae family [5]. It is mostly found in Australia as well as the subtropical and tropical regions. Foreigner's tree, Be-still tree, dicky plant, still tree, cook tree, captain cook tree, currant-tree, lucky nut, yellow oleander and Mexican oleander are some common names used for this plant [6].

In addition to its medicinal uses, the C. thevetia plant also possess poisonous properties. All parts of this plant are poisonous since they contain cardiac glycosides. They are most abundant in the seed and kernels, followed by leaves, fruit, and sap. The active components of Cascabela thevetia include the most dangerous cardenolides-Thevetin A and Thevetin B; others include Peruvoside, Neriifolin, Thevetoxin, and Ruvoside [17,18]. These cardenolides can be termed as effective as digoxin of D. purpurea and are not damaged by heating or drying. They cause harm to the cardiovascular system and abdomen [7].

In northern as well as southern India, consuming yellow oleander is a common form of self-harm, and is considered as a major public health issue. The kernels or leaves of yellow oleander can be associated with serious poisoning and even death. It is consumed either raw, or after being ground with molasses or jaggery, or in a curry in case of suicidal poisoning. The consumption of "herbal tea" or "traditional medicine" containing specific parts of this plant can lead to accidental poisoning. There are many incidents of children accidentally poisoning themselves with this plant, through misidentification or exploring activities. There have also been incidents of homicide involving the use of this plant poison [19]. Heart muscles and the autonomic nervous system both are affected by cardiac glycosides present in it. Death usually occurs within 6-24 hours of ingestion. There are several poisonous secondary metabolites found in this plant which can be fatal to humans and may also cause a variety of diseases in animals. Alkaloids, flavonoids, coumarins, phenols, essential oil, glycosides, terpenes, unsaturated steroids, tannins and triterpenoids are a few of them [12].

Previously, researchers used several methods to analyze C. thevetia namely Disc diffusion method [1,5], Agar well diffusion technique [12], Thin layer chromatography [10,27,28], Column chromatography [13], DPPH (2,2-diphenyl-2-picrylhydrazyl) assay [29,30,31], UV-visible spectroscopy [4,31,32,33], Fourier transform near-infrared spectroscopy(FT-NIR) [34], Reverse phase coupled with high performance liquid chromatography (RP-HPLC) [34], Scanning Electron Microscope-Energy Dispersive X-ray spectroscopy (SEM-EDS) [11,32,35,36], X-ray powder diffraction (XRD) [11,32], High resolution-transmission electron microscopy (HR-TEM) [11], Scanning Electron Microscope (SEM) [32], Transmission Electron Microscopy (TEM) [32], Superoxide radical assay [30], Hemolytic activity assay [30] etc. All of these techniques produced precise results with minimum sample requirements but the majority of them were expensive, time-consuming and required accurate handling.

Various other studies [10,12,27,28,31,33] revealed the presence of tannins, carbohydrates, alkaloids, saponins, steroids, phenol or cardio glycosides in C. thevetia through phytochemical screening utilizing chemical tests along with TLC. Okafor et.al (2000) investigated the toxicity of yellow oleander extracts from the leaves, bark or kernel in albino rats. Apart from these, the

analysis of leaves using IR spectroscopy has been done for the synthesis of Ag nanoparticles and their antibacterial properties through leaf extract of thevetia plant. Unfortunately, none of the researchers attempted to characterize the components present in C. thevetia plant.

In criminal cases involving homicide, suicide, burglary and other offences, sometimes plant evidences are found at the crime scene which may serve as the main clue for identifying the perpetrator and providing justice to the victim in the court of law. Criminals commonly employ botanical weapons or plant-based weapons to attempt criminal offences as they are cheap, easily available and hard to identify. Some commonly encountered plant poisons in suicidal and homicidal cases are Oleander, Nerium, Datura, Conium, Ricinus and Aconitum. Therefore, the characterization of the leaves of this poisonous plant may be helpful in such cases. Due to this reason, the chemical composition and the poisonous effects of this plant poison needs to be characterized [26].

Previously, several chemical tests, chromatographic techniques, and various assays and spectroscopic analysis have already been employed by numerous forensic specialists to examine different sections of C. thevetia, including plant twigs, seed, bark, latex and flower; but they were not focused on identification using characterization. Therefore, the current study focuses on the characterization of C. thevetia leaves using FTIR spectroscopy, which is a powerful and robust technique. It helps in determining the functional groups present in bioactive compounds. FTIR spectra ensure remarkable value due to their speed, simplicity, specificity, applicability, precision and low cost. The identification of specific organic components in plants via infrared spectroscopic analysis has great diagnostic value [14].

II. METHODS AND MATERIAL

i. Sample collection and analysis

In the present study, two samples of Cascabela thevetia were subjected to analysis using FTIR spectrophotometer. The specimens were collected from two distinct geographical regions namely Kerala (Muthalamada, Palakkad district, Kerala, Southern Region of India-10.6358° N, 76.7987° E) and Punjab (Mandi Gobindgarh, Fatehgarh Sahib district, Punjab, Northern Region of India-30.6510° N, 76.3293° E). The samples with their sample IDs are shown in Fig. 1 & 2.

The plant samples were collected during the summer season. In Kerala, the temperature during the collection of the sample was 34.4°C (93.92°F). While the Punjab region's average maximum temperature was around 27.5°C (81.5°F).

For analysis, a total of 300 grams of leaves along with the petioles were collected from random shrubs of C. thevetia and they were cleaned with running tap water for the complete removal of contaminants, dust particles and soil. In particular, fresh, young, completely developed leaves were chosen. The leaves showing mottled yellow color, green leaves which were curled or distorted plus fungal infected were excluded from the present study. The cleaned leaves were air dried for 40 days in the shade to preserve vibrant green color and stop the breakdown of beneficial chemical substances by photodegradation. They were then pulverized using mortar and pestle for further analysis. The completely dried powdered samples were then stored separately in sealed zip-lock bags to avoid contamination as shown in Fig. 3 (a) & (b).

Figure 1 : Sample 1-Kerala (C. thevetia plant collected from Kerala).

Figure 2 : Sample 2-Punjab (C. thevetia collected from Punjab).

(a) (b)

Figure 3 : Powdered samples of C. thevetia - (a) Sample 1: Kerala (b) Sample 2: Punjab.

The samples were then sent to CIL (Central Instrumentation Laboratory) at Panjab University, Chandigarh for instrumental analysis. The KBr pellet technique was used to prepare the sample. KBr pellets were prepared by grinding 1mg sample in a mortar pestle. Added to it, 150 mg potassium bromide crystals, and ground again. The pellet die system was assembled, and the die was then fitted inside the cavity. Using a sterilized metal spatula, the powdered material was then transferred into the chamber. By pressing and rotating the plunger, the sample was spread evenly across the cavity. The whole entity was then moved into a hydraulic press setup and placed between the piston and ram. It was made sure the die was securely held in the press. The lever was then pulled repeatedly until the meter read 10 metric tonnes, then waited for two minutes. After two minutes, the pressure was released. Following which, the press's upper wheel was raised, and the die was removed. The bottom plunger was then removed from the pellet die system and the die was placed in the ejector. The pellet was released from the die by applying pressure. The KBr pellet was then cautiously transferred with forceps to the sample holder. Before transferring the disc to the spectrometer, its homogeneity and translucency were checked to prevent the formation of a poor-quality spectrum. All the equipments used were sterilized properly before starting.

ii. FTIR parameters

The fragments were analyzed using “spectrum 400” FTIR spectrometer manufactured by Perkin Elmer, which was equipped with KBr window. The spectral range selected for the analysis was 400-4000 cm^{-1}, that is the mid infrared region (MIR region). Prior to running each analyte, the sample surface was sterilized with ethyl alcohol and fuzz-free tissue. Following that, the KBr pellet was inserted into the sample chamber and scanned. Each sample was scanned three times with a constant resolution of 4 cm^{-1}, and the average spectrum value was recorded for statistical analysis. This was done to exclude any possibility of noise/disturbance or an error in the spectra that might cause false interpretations. A scan for subtracting the background of the surface from the actual spectra was taken between consecutive sample testing. The pellet's FTIR spectra were recorded and the FTIR spectrometer's repeatability was checked by evaluating the sample 1: Kerala five times. The resultant values of transmittance obtained for both the samples gave a deviation of $\pm$0.005 from the standard values.

III. RESULTS AND DISCUSSION

i. Spectral features

The chemical structure, namely the organic as well as inorganic components of the thevetia leaves can be ascertained by using FTIR spectroscopy. The FTIR

spectrum of leaf extracts (sample 1: Kerala, sample 2: Punjab) is shown in Fig. 4 & 5. The prominent peaks for the samples studied were observed in the fingerprint region from 400 cm^{-1} to 1800 cm^{-1}. Comparative representation of peaks of two samples to their absorbance peaks with the peak assignments that are already mentioned in the literature as given in Table 1.

Elaborating the table, the peak at 535.89 cm^{-1} indicated the C-I stretch which may be due to aliphatic Iodo-compound/ alkyl halide. The peak at 618.09 cm^{-1} marked the presence of C-Br stretch which may be due to the presence of aliphatic bromo compounds and -OH out of plane bend may be due to alcohol, S-S stretch may be due to Disulphides as well as sulphate ion or C-H bend may be because of alkyne. The characteristic absorption band at 894.59 cm^{-1} may be due to single vinyl compound -C=CH2, vinylidene, C-H out of plane bend, and P-O-C stretch which may indicate the presence of aromatic phosphates.

The peaks at 1029.53, 1072.91, 1101.91 and 1145.20 cm^{-1} indicated the presence of organic siloxane or silicone (Si-O-Si), phosphate ion. The band at 1029.53 cm^{-1} indicated the presence of primary amine and cyclohexane ring vibration. The characteristic peak at 1072.91 cm^{-1} marked the presence of C-O stretch which may be observed due to the presence of cyclic ether or a large ring compound. The band at 1101.91 cm^{-1} may be due to secondary alcohol. The characteristic peak at 1145.20 cm^{-1} indicated C-F stretch and C-O stretch which may be due to primary and tertiary alcohol, secondary amine, CN stretch or carbohydrate. The band at 1240.64 cm^{-1} indicated aryl-O stretch may be due to phenol and the P-O-C stretch may be because of aromatic phosphates. The characteristic band at 1318.84 cm^{-1} indicated CN stretch which may be due to the presence of aromatic primary, secondary as well as tertiary amine, also OH in-plane bend could be due to the carboxylate group, and tertiary alcohol. The peak at 1383.13 cm^{-1} may be due to gem-Dimethyl or "iso"-(doublet) and carboxylate group. The significant peak at 1426.24 cm^{-1} indicated C-H out of plane bending may be due to aromatic compound, carbonate ion or ammonium ion.

The strong intense band was identified at 1631.96 cm^{-1} which indicated that C≡C, C=C bonds may be observed due to conjugated ketone and olefinic compound as well as the NH bend may be due to amide, protein open-chain imino (-C=N-), organic nitrate, primary or secondary amine. The significant peak at 1733.79 cm^{-1} could be assigned to the carbonyl group, C=O which may be due to ketone, aldehyde, esters, carboxyl group or six-membered ring lactone. The peak at 2117.43 cm^{-1} attributes to C≡C which may be due to alkyne, transition metal carbonyls, isothiocyanate (-NCS), cyanide ion, thiocyanate ion and related ions. The band at 2853.12 cm^{-1} may be due to aldehyde as well as lipids. The distinct peak that was recorded in the samples around 2921.46cm^{-1} was indicating the Methylene (CH2) group or C-H symmetric/asymmetric stretch which may be due to aliphatic compounds, alkene or alkane. The characteristic band at 3428.82 cm^{-1} indicated OH stretching as well as >NH stretch it may be observed due to the hydroxyl group, aromatic ring also aromatic secondary amine.

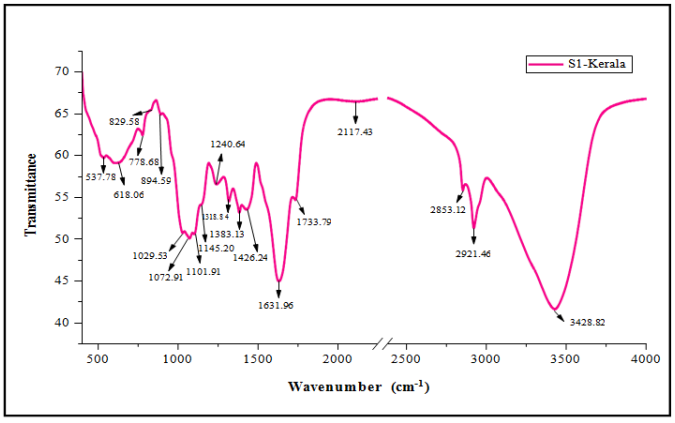

Figure 4: Spectra of Sample 1: Kerala

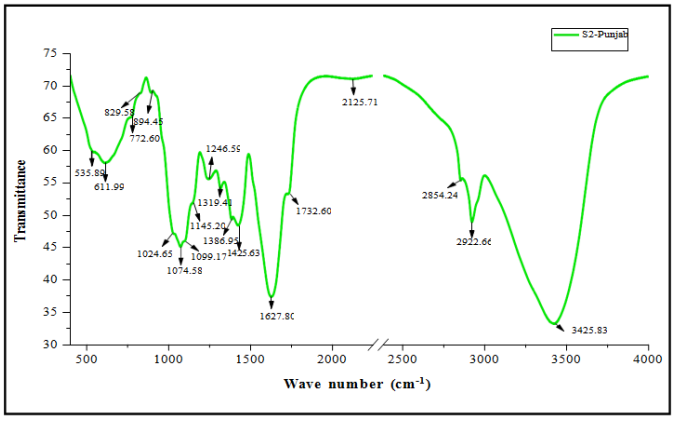

Figure 5: Spectra of Sample 2: Punjab

Table 1: Range with their band assignment [41,42,43,44,]

Spectral range	Band assignments
535.89, 537.78 cm^{-1}	C-I stretch (alkyl halide)
611.99, 618.09 cm^{-1}	PO4, Alkyne C-H bend, C-Br stretch, Alcohol, -OH out of plane bend, Disulphides (C-S stretch), Disulphides (S- S stretch), Sulphate ion
772.60, 778.68cm^{-1}	Cis-C-H out of plane bend, C-H 1,3-disubstitution(meta), C-Cl stretch
829.58 cm^{-1}	C-H 1,4-di substitution (para), Epoxy and Oxirane rings, peroxides, C-O-O stretch, Nitrate ion
894.59 cm^{-1}	Single vinyl compound C=CH2, vinylidene C-H out of plane bend, Aromatic phosphates (P-O-C stretch)
1024.65, 1029.53 cm^{-1}	Cyclohexane ring vibration, Primary amine, CN stretch, Aliphatic phosphates (P-O-C stretch), Asymmetric stretching vibrations of organic siloxane or silicone (Si-O-Si), Phosphate ion, Silicate ion
1072.91 cm^{-1}	Cyclic ether, large ring compound, C-O stretch, Phosphate ion, Silicate ion
1099.17, 1101.91 cm^{-1}	C-O stretch, Secondary alcohol, Sulfonates, Organic siloxane or silicone (Si-O-C)
1145.20 cm^{-1}	C-F stretch, Primary and tertiary alcohol, C-O stretch, secondary amine, CN stretch, sulfonates, Organic siloxane or silicone (Si-O-C)
1240.64, 1246.59 cm^{-1}	Phenol, C-O stretch, Aromatic ether, aryl-O stretch, Aromatic phosphates (P-O-C stretch)
1318.84 cm^{-1}	Carboxylate group, Phenol, Tertiary alcohol, OH in plane bend, Aromatic primary amine, Aromatic secondary amine, Aromatic tertiary amine, CN stretch, Organic phosphates (P=O stretch), Di alkyl/aryl sulfones
1383.13 cm^{-1}	gem -Dimethyl or iso -doublet group, Carboxylate group
1426.24 cm^{-1}	Aromatic compound, C-H out of plane bending, Carbonate ion, Ammonium ion
1627, 1631.96 cm^{-1}	C≡C, C=C, C=O, Amide, Olefinic compound, Primary amine, Secondary amine, NH bend, Conjugated ketone,Open-chain imino (-C= N-), Organic nitrate
1733.79 cm^{-1}	C=O, Ketone, Carbonyl group, aldehyde, esters, carboxyl group, six membered ring lactone
2117.43, 2125.71 cm^{-1}	C≡C, transition metal carbonyls, isothiocyanate (-NCS), Cyanide ion, Thiocyanate ion and related ions
2853.12 cm^{-1}	C-H symmetric stretch,

	Aldehyde
2921.46 cm^{-1}	Methylene (CH_2) group, C-H asymmetric/symmetric stretch, Aliphatic compound
3425.83, 3428.82 cm^{-1}	Hydroxyl group, OH stretching, Aromatic ring, Aromatic secondary amine, >NH stretch

As it can be seen from the Table 2, minor variations were present in sample 1 and 2 (as shown in figure 6). The intensity variations in peaks may be due to the respective concentration differences. The less significant peaks present in the range of 2248-2381cm^{-1} were excluded from the characterization. The peaks in the range 535-545 cm^{-1} were present in both the samples which may be due to the presence of C-I stretch (alkyl halide). Sample 2 exhibited slightly stronger peak and weaker absorption peak was observed in sample 1. A discriminating peak between 605-615 cm^{-1} was observed in sample 2 which may probably be due to Disulphides (C-S stretch), Sulphates (S-S stretch) or C-Br stretch. However, in sample 1 the peak in the range 605-615 cm^{-1} was not observed. Another characteristic peak observed at the peak range 775-785 cm^{-1} was present in sample 1 but not sample 2, which may be because of cis-C-H out of plane bending or C-l stretch. A distinct peak was recorded in both the samples at around 894 cm^{-1} which may probably be attributed to aromatic phosphates, C-H out of plane bend or C=CH_2 stretch.

When comparing the peaks of each sample, the stronger peak was observed in sample 1 and in sample 2 the peak was weaker. A characteristic peak between 1025-1035 cm^{-1} was observed in sample 1 which may indicate the presence of CN stretch, Primary amine, Cyclohexane ring vibration, Aliphatic phosphates (P-O-C stretch), Phosphate ion, Asymmetric stretching vibrations of organic siloxane or silicone (Si-O-Si) and Silicate ion. However, in sample 2 no prominent band was observed in the same peak range. An identical peak was observed in both the samples at around 1065-1075 cm^{-1} which may be present due to C-O stretch, large ring compound, Cyclic ether, Phosphate ion as well as Silicate ion. The characteristic peaks in the range 1095-1105 cm^{-1} were observed in both samples, in sample 2 the peak (1099.17 cm^{-1}) was more distinguishable than the peak (1101 cm^{-1}) of sample 1 which may be due to the presence of a varying concentration of constituents. The peak observed at this range may be because of C-O stretch, Secondary alcohol, Sulfonates, Organic siloxane or silicone (Si-O-C). Sample 1 shows an absorption band around 1240 cm^{-1} which may be observed due to the presence of Phenol, C-O stretch, Aromatic ether or aryl-O stretch. A peak around 1318 cm^{-1} was recorded in both the samples which indicated the presence of the Carboxylate group, Tertiary alcohol, OH in-plane bend, CN stretch Aromatic amine or Organic phosphates. This significant peak was quite broader in sample 2 and fairly pointed in sample 1. Similarly, a distinct peak was recorded in almost all the samples at around 1425 cm^{-1} which may be due to Aromatic compound, C-H out of plane bending, Carbonate ion or Ammonium ion. When comparing both plant samples, the peak of sample 2 was more prominent and distinguishable than that of sample 1.

A characteristic peak between 1625-1635 cm^{-1} was observed in sample 1 and 2, which may be due to C≡C, C=C, C=O, Amide, Olefinic compound, Primary and secondary amine, NH bend, Conjugated ketone, Open-chain imino (-C= N-) or Organic nitrate. As compared to sample 1 the peak was slightly shifted towards the right in sample 2, and sample 2 exhibited a more intense peak than sample 1. Similarly, a peak around 1733 cm^{-1} was observed in both the samples which may be due to the presence of C=O stretch, Ketone, Carbonyl group, aldehyde, esters, carboxyl group and six-membered ring lactone. Both the samples show a characteristic peak in the range of 2845-2855 cm^{-1} which may indicate the presence of

an aldehyde. Sample 2 shows a stronger peak at 2854.24 cm^{-1} and sample 1 shows a slightly weak peak at 2853.12 cm^{-1}. The peak at 2922 cm^{-1} was recorded in both the samples which may be present due to the Methylene group, asymmetric/symmetric stretch of C-H or Aliphatic compounds.

The peak (2922.66 cm^{-1}) of sample 2 was more intense than the peak (2921.46 cm^{-1}) of sample 2. A significant peak was observed in both the samples in the range of 3425-3435 cm^{-1} which may probably be attributed to the OH stretching or Hydroxyl group. Sample 2 shows the stronger peak at 3425.83 cm^{-1} and sample 1 shows a comparatively weaker peak in this manner, the minor variations of the samples were able to fairly discriminate.

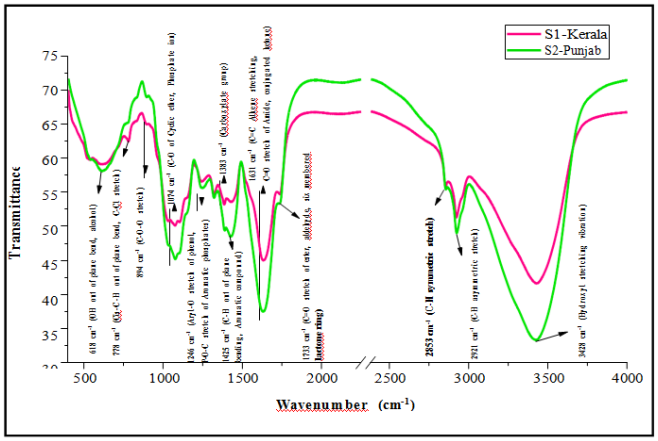

Figure 6: Spectra demonstrating variation among samples from two differentgeographical regions.

Table 2: The characteristic absorbance peaks of the plant samples are in the table shown below. The tick (✓) marks signify the presence of peaks at the particular wave numbers (cm^{-1}) along with some extra peaks mentioned:

Peak Range	Sample 1	Sample 2
535-545	✓	✓
605-615		✓
775-785	✓	
885-895	✓	✓
1025-1035	✓	
1065-1075	✓	✓
1095-1105	✓	✓
1235-1245	✓	
1315-1325	✓	✓
1625-1635	✓	✓
1725-1735	✓	✓
2845-2855	✓	✓
2915-2925	✓	✓
3425-3435	✓	✓
Extra Peaks	618.09, 829.58, 1383.13, 1426.24, 2117.43	772.60, 829.58, 1024.65, 1246.59, 1386.95, 1425.63, 2125.71

Discrimination:

To achieve meaningful differentiation, a forensic expert should look at the differences in the chemical compositions of the sample so obtained. The manual peak-to-peak comparison is relatively a tedious task. However, according to Table 2, majority of the peaks were similar in both the samples leaving behind some peak range 775 – 765 cm^{-1}, 1025 -1035 cm^{-1} and 1235 – 1245 cm^{-1} which was observed only in sample 1. Also, the peak range 605 – 615 cm^{-1} was only seen in sample 1. Some characteristic peaks in the range of 535-545 cm^{-1}, 1025-1035 cm^{-1}, 11380-1390 cm^{-1}, 1235-1245 cm^{-1}, 1420-1430 cm^{-1}, 1625-1635 cm^{-1}, 2915-2925 cm^{-1}, 3425-3435 cm^{-1} were observed in both the sample, nevertheless showing minor variations with the intensity of peaks as shown in Figure 19. To give an example, the peak in the range of 885-895 cm^{-1} was present in two plant samples but the peak was weaker in sample 2 and more intense in sample 1. Additionally, when comparing the spectra of the samples in the range of 1625- 1635 cm^{-1}, shows variations in the intensities. Along with this, the samples may also distinguish based on the additional peaks. These findings demonstrated the invaluable discriminating ability of FTIR spectroscopy along with the KBr disc method for the differentiation of the same plant from two different geographical regions.

Study based on Geographical Region: The C. thevetia plant from two different geographical regions were analyzed and the resultant spectra obtained were almost similar. Slight variations were observed in the peak intensities. This variation may be because of the change in concentration of constituents of the plant accordingto different geographical regions.

IV. CONCLUSION

The use of Infrared spectroscopy has been demonstrated to be an excellent method for sensitive, rapid, precise and objective characterization of plant samples as it resulted in reliable outputs. FTIR spectroscopy along with the KBr disc method yields spectra with high resolution. This study can be considered reliable and foreseen to provide a good level of confidence during the conduction of 'questioned versus known' comparisons of the Cascabela thevetia plant.

These plant evidence may be encountered in various crime scenes such as rape, suicide, homicide burglary etc. It may be discovered in its natural form next to the corpse or may be in the form of a paste which is partially consumed in case of suicidal poisoning. In homicidal cases, it may be ingested and found in the intestine of the victim in an undigested or partially digested state. In all these scenarios, the plant evidence may be analyzed using FTIR and the resultant spectrum can then compare with the standard spectrum of C. thevetia. If the spectrum is found identical then the questioned plant sample can be confirmed as C. thevetia.

In the present study, it was observed that there were minor variations in the peak intensity of C. thevetia collected from two different geographical regions (southern and northern regions) of India. The pilot study indicates that it can provide an opportunity for future research in FTIR analysis of various plant parts of the C. thevetia from other geographical regions as well. However, it is suggested another model with an integrated study of numerous spectroscopy techniques on the same plant could result in greater efficiency. In addition to this, incidents of poisoning cases also involved fruit, seed or bark along with leaves of the C. thevetia plant. A more detailed experiment could be conducted to analyze all parts of the C. thevetia plant, which would also enhance the scope of this study and will prove the competency of the trained dataset.

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