CASE REPORT

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FTIR Spectroscopy-based Examination of *Cascabella thevetia* (L.) Lippold: Preliminary Studies on Geographical Variations

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ABSTRACT

Plant poisons such as *Cascabella thevetia* are encountered in toxicological cases in the form of vomit, froth, or the gastric lavage of the victim. This pilot study sought to effectively use Infrared spectroscopy to cater to rapid, unprejudiced and precise identification of *C. thevetia* which will help conclude various forensic investigations soon. In the present study, powdered leaves of this poisonous plant procured from two distinct geographical regions of India were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. The FTIR spectrum revealed the structural composition of this plant poison in the form of major bands, thereby leading to its successful characterization. The spectra obtained for both samples were distinct in terms of the intensity of the peaks present in their constitutional components. This model can be considered reliable and it could provide an outstanding level of confidence during the conduction of 'questioned versus known' comparisons of plant poisons, confirming the cause of death and the origin of the plant parts which will eventually help in rounding off the suspects.

Keywords: FTIR Spectroscopy, *Cascabella thevetia,* Forensic Science, Plant Poison, Forensic Toxicology, Characterization.

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INTRODUCTION

For centuries, millions of rural families all over the world have been reaping the benefits of the medicinal properties of various plants for self-care.[1] The WHO (World Health Organization) reports claim that some countries continue to use plant-based medications as their primary source of medicine.[2] *Cascabella thevetia* (often known as yellow oleander) is one of the many medicinal plant species used in traditional medicine systems as it contains naturally occurring alkaloids and synthetic derivatives that possess antibacterial, antispasmodic, analgesic, and antioxidant properties.[3]

In addition to its medicinal uses, the *C. thevetia* plant also possesses poisonous properties. All parts of this plant are poisonous as they contain cardiac glycosides. They are most abundant in the seed and kernels, followed by leaves, fruit, and sap. The active components of *C. thevetia* include the most dangerous cardenolides- thevetin A and thevetin B; others include peruvoside, neriifolin, thevetoxin, and ruvoside.[4,5] Secondary metabolites include alkaloids, flavonoids, unsaturated steroids, triterpenoids, coumarins, phenols, essential oils, glycosides, terpenes, and tannins.[6,7,8,] The cardiac glycosides cause immense harm to the cardiovascular system (CVS) and abdomen by affecting the heart muscles and the autonomic nervous system (ANS).[9] Death usually occurs within 6-24 hours of ingestion.[10]

Commonly encountered plant poisons used in suicidal and homicidal cases involve aconitum, conium, matura, cerium, oleander and, ricans. Therefore, studying the chemical composition and the poisonous effects of the aforementioned poisonous plants and their characterization may prove to be helpful in such cases.[11]

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Previously, researchers used several methods including chemical tests, chromatographic techniques, and various assays and spectroscopic analysis to analyse *C. thevetia* namely Thin layer chromatography (TLC),[12-16] Column chromatography,1[3] UV-visible spectroscopy,[19-25] Fourier transform near-infrared spectroscopy (FT-NIR),[20-22] scanning electron microscope-energy dispersive X-ray spectroscopy (SEM-EDX),[22] Xray powder diffraction (XRD),[19,23] scanning electron microscope (SEM),[22,25] transmission electron microscopy (TEM),[28] and many more. All of these techniques successfully produced precise results with minimum sample requirements. However, the majority of them were highly expensive, excessively time-consuming and required high precision while handling.

Some studies have also 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.[10,12,26-29] Unfortunately, none of the researchers attempted to characterize the components present in the *C. thevetia* plant or its identification as an unknown sample for that matter.

Literature shows that numerous forensic specialists have already employed spectroscopic methods for analyzing various physical, chemical, and biological samples. Therefore, the present study focuses on the characterization of *C. thevetia* leaves using a robust technique, i.e. FTIR spectroscopy, which will help identify the different functional groups present in this plant species. The use of FTIR spectroscopy has a great diagnostic value and it ensured remarkable results owing to its speed, simplicity, specificity, applicability, precision and low cost as it permitted a greater level of transparency in the decision-making process

MATERIALS AND METHODS

Sample collection and analysis

In the present study, two samples of *C. thevetia* were subjected to analysis using an 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, temperature-34.4° or 93.92°F) and Punjab (Mandi Gobindgarh, Fatehgarh Sahib district, Punjab, Northern Region of India-30.6510°N, 76.3293°E, temperature-27.5°C or 81.5°F).

Sample preparation

For preparing the sample for analysis, approximately 300 grams of fresh leaves along with the petioles were collected from random shrubs of *C. thevetia*. The leaves showing mottled yellow, green leaves which were curled or distorted, and fungal-infected leaves were excluded from the present study. The collected leaves were then cleaned with running tap water to completely remove contaminants, dust particles and other impurities. The cleaned leaves were air-dried for 40 days in the shade to preserve vibrant green colour and stop the breakdown of beneficial chemical substances by photo-degradation. Further, they were pulverized using mortar and pestle for further analysis. The completely dried powdered samples were then stored separately with their sample IDs in sealed zip-lock bags to avoid contamination.

The powdered samples were then sent to CIL (Central Instrumentation Laboratory) at Panjab University, Chandigarh for instrumental analysis. At CIL, the K-Br pellet technique was used to prepare the sample for further analysis. Precautionary measures such as sterilizing all the pieces of equipment before and after each use, cleaning glassware, etc; were carefully taken to avoid contamination of sample which might lead to compromised results.

FTIR parameters

The sample pellets were analyzed using a “spectrum 400” FTIR spectrometer manufactured by Perkin Elmer, which was equipped with a K-Br window. The spectral range selected for the analysis was 400–4000 cm^{-1}, the mid-infrared region (MIR region). Each sample was scanned three times with a constant resolution of 4 cm^{-1}, and the average spectrum value was recorded for analysis. Before running each analyte, the sample surface was cleaned by sterilizing it with ethyl alcohol and fuzz-free tissue. A scan for subtracting the background of the clean crystal surface from the actual spectra was taken between consecutive sample testing. The repeatability of the FTIR spectrometer was checked by analyzing the sample S2 five times, as shown in Figure 1. The resultant values of transmittance obtained for the plant samples gave a deviation of ± 0.005 from the standard values.

Both spectra were truncated to remove the 2248–2381 cm^{-1} spectral for analysis of the data obtained as it contained no relevant peaks. A 0% transmittance baseline was set off of all the spectra.

RESULTS AND DISCUSSION

Plant parts, extracts or regurgitated matter/vomit residues can be easily found at a crime scene which may serve as the main clue in poisoning cases for the identification of the perpetrator and providing justice to the victim in the court of law. Some of the commonly encountered plant poisons used in suicidal and homicidal cases involve aconitum, conium, datura, cerium, oleander and, riccinus. Therefore, studying the variations in the chemical composition of the *C. thevetia* plant procured from two different geographical regions will aid in rounding off the suspects and finding clues concerning an ongoing forensic investigation.

Previously, researchers used several methods to analyze *C. thevetia* namely the disc diffusion method,[30] Agar well diffusion technique,[31] TLC,[12-16] Column chromatography,[13] UV-visible spectroscopy,[19-25] FT-NIR,[20-22] RP-HPLC,[32] SEM-EDX,[22] XRD,[19-23] SEM,[22-25] TEM,[28] Superoxide radical assay,[33] Hemolytic activity assay,[34] and many more. All of these techniques successfully produced precise results with minimum sample requirements. They rendered various advantages but had some disadvantages as well, for instance, the Disc diffusion method was cheap and highly flexible but required a lot of human effort, agar well diffusion technique was cost-effective but was highly prone to contamination and errors, TLC was rapid and yielded accurate results but its resolution was limited and its usage was only limited to non-volatile compounds, column chromatography was robust and useful for complex mixtures but it is highly time-consuming and expensive, SEM allowed high

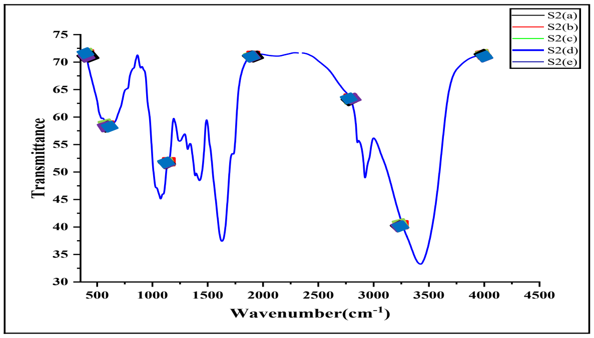

Figure 1: Spectra showing repeatability test for sample S2

resolution and a large depth of field but was a very expensive method. Overall, these techniques had more disadvantages than their advantages. This indicated the need to formulate a new way to investigate the exhibits related to this plant poison. Fortunately, this methodology offered numerous advantages like high sensitivity, reduced time requirement for data acquisition and rendered greater component specificity.

Moreover, the literature shows that spectroscopic methods have already been employed by numerous forensic specialists[35-41] for analyzing various physical, chemical as well as biological samples. Therefore, the present study focuses on the characterization of *C. thevetia* leaves using a robust technique, i.e. FTIR spectroscopy, which will help identify the different functional groups present in this plant species. FTIR spectroscopy has a great diagnostic value and ensured remarkable results owing to its speed, simplicity, specificity, applicability, precision and low cost as it permitted a greater level of transparency in the decision-making process.

Spectral features

The chemical structure, namely the organic components of the thevetia leaves can be ascertained by using FTIR spectroscopy. The FTIR spectra of leaf extracts of sample 1: Kerala and sample 2: Punjab is shown in Figure 2. The prominent peaks for the studied samples were observed in the fingerprint region from 400 to 1800 cm^{-1}. The less significant peaks which were featureless were excluded from the characterization. Comparative representation of peaks about sample 1 and sample 2 concerning their transmittance peaks with the peak assignments that are already mentioned in the literature as given in Table 1 and 2.

Elaborating the table of contents, the peak at 535.89, 537.78 cm^{-1} indicated the C-I stretch, possibly due to aliphatic Iodo-compound/alkyl halide. The characteristic absorption band at 894.59 cm^{-1} may be due to single vinyl compound $-C=CH_2$, 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 1240.64, 1246.59 cm^{-1} indicated aryl-O stretch may be due to phenol and the P-O-C stretch may be because of aromatic phosphates. The peak at 1318.84, 1383.13 cm^{-1} may be due to "iso"-(doublet) and carboxylate group. The significant peak at 1426.24 cm^{-1} indicated that C-H out-of-plane bending may be due to an aromatic compound, carbonate or ammonium ion. 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, 2125.71 cm^{-1} is attributed to C≡C which may be due to the 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 characteristic band at 3425.83, 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.

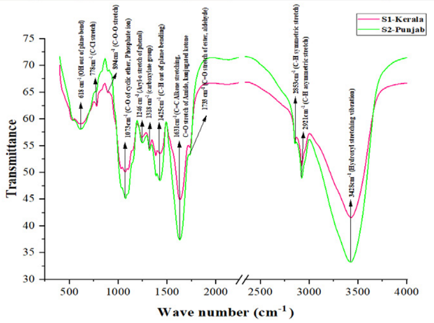

Figure 2: Spectra showing variations in the composition demonstrated are S1 and S2

Based on the supplementary table T2, the samples could be easily differentiated using the extra peaks. For instance, the peaks in the range 535-545 cm^{-1} were present in both samples which may be due to the presence of C-I stretch (alkyl halide). However, peaks in the range 605–615 cm^{-1} were only present in sample S2, indicating Disulphides (C-S stretch), Sulphates (S-S stretch) or C-Br stretch. A major discriminating peak at 775–785 cm^{-1} was seen in the only S1, probably attributed to the cis-C-H out-of-plane bending or C-l stretch. A distinct peak was recorded in both samples at around 894 cm^{-1}, which may be attributed to aromatic phosphates, C-H out-of-plane bend, or $C=CH_2$ stretch. A prominent peak between 1025 and 1035 cm^{-1} was observed in S1, respectively. However, in sample 2, no prominent band was observed in the same peak range. Another characteristic peak near 1235 cm^{-1} was present in S1, possibly because of phenols or aromatic phosphates. Distinct peaks were observed in sample S1 at 618.09 and 2117.43 cm^{-1}, whereas in sample S2 at 772.60, 1024.65 and 1246.59 cm^{-1}.

Apart from this, a major difference was observed in both spectra in terms of the intensity of the peaks, possibly due to the respective concentration differences in both samples. It was also observed that Sample 2 exhibited a slightly stronger peak and a weaker absorption peak was observed in sample 1. When comparing both plant samples, the peak of sample 2 was more prominent and distinguishable than that of sample 1. This way, discrimination was fairly possible using this method. However, the author would like to add here that since no extraction method was used to extract the active components of *C. thevetia* in this preliminary study, there is a fair possibility that some of the peaks obtained might be due to the other components of the leaf of this plant poison such as pigments, tannins, alkaloids, etc. Therefore, it is recommended that future researchers, while taking reference may consider this and experiment accordingly. For future research, the authors will ensure to use one or the other suitable extraction methods.

Overall, it can be said that the results of this study signified the invaluable discriminating power of FTIR spectroscopy for the characterization and differentiation of the plant poison samples. Therefore, the discriminating power was favorably significant, considering the respective spectral absorbance peaks in *Cascabella* samples.

In comparison to the previous experiments,[12-34] it can be said that the current study has multiple advantages like greater

Table 1: Range with their band assignment[41-44]

Spectral range (cm^{-1})	*Band assignments*
535.89,537.78	C-I stretch (alkyl halide)
611.99,618.09	PO_4, Alkyne C-H bend, C-Br stretch, Alcohol, -OH out of plane bend, Disulphides (C-S stretch), Disulphides (S-S stretch), Sulphate ion
772.60,778.68	Cis-C-H out of plane bend, C-H 1,3-disubstitution (meta), C-Cl stretch
829.58	C-H 1,4-disubstitution (para), peroxides, C-O-O stretch, Nitrate ion
894.59	Single vinyl compound $C{=}CH_2$, vinylidene C-H out of a plane bend, Aromatic phosphates (P-O-C stretch)
1024.65,1029.53	Cyclo hexane ring vibration, Primary amine, C-N stretch, Aliphatic phosphates (P-O-C stretch), Asymmetric stretching vibrations of organic siloxane or silicone (Si-O-Si), Phosphate ion, Silicate ion
1072.91	Cyclic ether, large ring compound, C-O stretch, Phosphate ion, Silicate ion
1099.17,1101.91	C-O stretch, Secondary alcohol, Sulfonates, Organic siloxane or silicone (Si-O-C)
1145.20	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	Phenol, C-O stretch, Aromatic ether, aryl-O stretch, Aromatic phosphates (P-O-C stretch)
1318.84	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	Di methyl or iso-doublet group, Carboxylate group
1426.24	Aromatic compound, C-H out of plane bending, Carbonate ion, Ammonium ion
1627,1631.96	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	C=O, Ketone, Carbonyl group, aldehyde, esters, carboxyl group, six-membered ring lactone
2117.43,2125.71	C≡C, transition metal carbonyls, iso thio cyanate (-NCS), Cyanide ion, Thio cyanate ion and related ions
2853.12	C-H symmetric stretch, Aldehyde
2921.46	Methylene (CH_2) group, C-H asymmetric/symmetric stretch, Aliphatic compound
3425.83,3428.82	Hydroxyl group, OH stretching, Aromatic ring, Aromatic secondary amine, >NH stretch

Table 2: The characteristic transmittance peaks of samples are given in the table shown below. The tick (✓) markings indicate that a peak exists at the specific wavenumbers (nm) and additional peaks mentioned

Peak Range (in cm^{-1})	*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, low cost, quick, accurate results, and unbiased and reliable outputs. Contrary to the numerous applications, FTIR spectroscopy has some shortcomings. This technique requires sample preparation and cannot reveal non-polar molecules' spectral features or those show dipole moment changes. Moreover, this method is tedious and can only be considered a good option for the individualization of limited samples as it is laborious and cumbersome.

Study based on Geographical Region

The *C. thevetia* plant from two different geographical regions was analyzed and the resultant spectra obtained were almost similar yet visually distinct in terms of the intensity of the constitutional components. Slight variations were observed in the peak intensities. As seen in Figure 2, the peaks at 618, 1074, 1333, 1631, 2921, and 3428 cm^{-1}, are more prominent for S1, whereas the peaks at 778 and 894 cm^{-1} are more prominent for S2. These variations may be because of the change in concentration of constituents of the plant according to different geographical regions like the weather, humidity, soil type, atmospheric pressure, etc.

CONCLUSION

The use of Infrared (IR) 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 K-Br 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 *C. 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 maybe in the form of a trace present in vomit or froth 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 analysed using FTIR spectroscopy and the resultant spectrum can then be compared with the standard spectrum of *C. thevetia*. If the spectrum is identical, the questioned plant sample can be confirmed as *C. thevetia*.

In the present study, it was observed that there were distinct 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, and the use of ATR-FTIR spectroscopy instead of K-Br method is recommended. Moreover, 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 this study's scope and prove the training dataset's competency.

REFERENCES

1. Patil HSR, Kekuda TRP, Makari HK, Gurumurthy H. In vitro antimicrobial activity of ethanol extract of Thevetia peruviana, Biotechnology an Indian. J BTAIJ. 2008;2(1):05-7.
2. Greenwell M, Rahman PKSM. Medicinal plants: their use in anticancer treatment. Int J Pharm Sci Res. 2015;6(10):4103-12. doi: 10.13040/IJPSR.0975-8232.6(10).4103-12, PMID 26594645.
3. Vij K. Textbook of Forensic Medicine and Toxicology Principles and Practice fifth edition; 2011.
4. Phuse SS, Khan ZH. Antioxidant and Hemolytic quantification of Thevetia flowers in different extract. Int J Pharm Biol Sci. 2018;8(2):110-6.
5. Redha A. Phytochemical investigations of nerium oleander L. Leaves Flowers Int J Sci Res Chem Sci. 2020;7(4):1-4.
6. Seetharaman S, Indra V, Sundar N, Geetha S. Phytochemical profiling, antibacterial activity and antioxidant potential of Cascabela Thevetia (L.) whole plant extracts. J Pharmacogn Phytochem. 2017;6(3):93-7.
7. Rupiasih NN, Aher A, Gosavi S, Vidyasagar PB. Green synthesis of silver nanoparticles using latex extract of Thevetia peruviana: a novel approach towards poisonous plant utilization. J Phys Conf S. 2013;423:012032.
8. Solomon S, Muruganantham N, Senthamilselvi MM. Antimicrobial activity of Cascabela Thevetia (Flowers). J Pharmacogn Phytochem. 2016;5(5):335-8.
9. Sunshine coast committee. Cascabela thevetia. https://keyserver.lucidcentral.org/weeds/data/media/Html/cascabela_thevetia.htm (Accessed on 14th may 2022).
10. National library of medicine. Thevetin A. https://pubchem.ncbi.nlm.nih.gov/compound/Thevetin-A (Accessed on 15th May 2022).
11. National Institute of drugs. Peruvoside. https://drugs.ncats.io/drug/CT36KGC6A6 (Accessed on 16th 2022).
12. Kishan S, Kumar AK, Vimlesh M, Mubeen US, Alok S. A review on: Thevetia peruviana. Int Res J Pharm. 2012.
13. Sowjanya KM, Narendra K, Swathi J, Satya AK. Phytochemical extraction and antimicrobial efficiency of crude leaf extract of medicinal plant Cascabela Thevetia. Int J Res Pharm Biomed Sci ISSN. 2016;3701:2229.
14. Gogoi N, Bhuyan B, Deka T. Pharmacognostic standardization, preliminary phytochemical screening and TLC fingerprinting of the bark of Cascabela Thevetia L. Int J Pharm Pharm Sci. 2019;11(7):125-30. doi: 10.22159/ijpps.2019v11i7.29218.
15. Arasaretnam S, Prasadini HRP, Mathiventhan U. Qualitative phytochemical screening, anti-bacterial activity and TLC profiling of different parts of three medicinal plants. Int J Multidiscip Stud. 2021;8(1):37-58. doi: 10.4038/ijms.v8i1.132.
16. Save SA, Padmanabhan U, Kothari ST, Lokhande RS, Chowdhary AS. Chemical and pharmacological study of the twigs of Thevetia peruviana plant and its bio- activity profiles. J Eng Appl Sci. 2015;5(6).
17. Rao YV, Raju M. Antioxidant and protease activities of seven native plant sources. Int J Bio-Resource Stress Manag. 2016;7(6):1346-50, 10.5958/0976- 4038.2016.00220.7. doi: 10.23910/IJBSM/2016.7.6.1646.
18. Sahil K, Sudeep B, Akanksha M. Standardization of medicinal plant materials. Int J Res Ayurveda Pharm. 2011;2(4):1100-9.
19. Oluwaniyi O, Adegoke HI, Adesuji ET, Alabi AB, Bodede SO, Labulo AH et al. Biosynthesis of silver nanoparticles using aqueous leaf extract of Thevetia peruviana Juss and its antimicrobial activities. Appl Nanosci. 2015;6(6). doi: 10.1007/s13204- 015-0505-8.
20. Ghavri K, Adhav M. Phytochemical screening, ultra violet and infrared spectroscopy of ethanolic leaf extract of Thevetia peruviana (Pers.) Thevetia yellow, The Pharma. Innov J. 2018;7(9):314-8.
21. Mendoza D, Arias JP, Cuaspud O, Ruiz O, Arias M. FT-NIR spectroscopy and RP- HPLC combined with multivariate analysis reveals differences in plant cell suspension cultures of Thevetia peruviana treated with salicylic acid and methyl jasmonate. Biotechnol Rep (Amst). 2020, Aug 12;27:e00519. doi: 10.1016/j.btre.2020.e00519, PMID 32874946.
22. Medhi H, Chowdhury PR, Baruah PD, Bhattacharyya KG. Kinetics of Aqueous Cu(II) biosorption onto Thevetia peruviana Leaf powder. ACS Omega. 2020;5(23):13489-502. doi: 10.1021/acsomega.9b04032, PMID 32566814.
23. Usman MA, Momohjimoh I, Adeniyi AS. Characterization of Thevetia peruviana (yellow oleander) shell ash powder as a possible filler in polymer composites, mechanics of advanced. Compos Struct. 2021;8:109-17.
24. Dubey NK, Dwivedy AK, Chaudhari AK, Das S. Common toxic plants and their forensic significance. Natural Products and Drug Discovery section. Available from: https://doi.org/10/1016/B978-0-08-102081-4.00013-7. Vol. 2; 2018..
25. Bawazeer S. Thevetia peruviana roots extract medicated gold

nanoparticles and its urease inhibitory activity. Int J Appl Pharm. 2020;13:79-82. doi: 10.22159/ijap.2021.v13s1.Y1026.

26. Nandiyanto ABD, Oktiani R, Ragadhita R. How to read and interpret FTIR spectroscope of organic material. Indonesian J Sci Technol. 2019;4(1):97-118. doi: 10.17509/ijost.v4i1.15806.
27. Hemmalakshmia S, Priyangaa S, Devakia K. Fourier transform infrared spectroscopy analysis of Erythrina variegata L. J Pharm Sci Res. 2017;9(11):2062-7.
28. Sahayaraj PA, Gowri J, Dharmalingam V, Shobana R, Prema AA. Phytochemical screening by FTIR spectroscopic analysis of leaf and stem Extracts of Wedelia biflora, International Conference on Chemical and Environmental Research. 2015;2(5):322-34.
29. Pakkirisamy M, Kalakandan SK, Ravichandran K. Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of Curcuma caesia Roxb (black turmeric). Pharmacogn J. 2017;9(6):952-6. doi: 10.5530/pj.2017.6.149.
30. Zhang J, Li B, Wang Q, Wei X, Feng W, Chen Y et al. Application of Fourier transform infrared spectroscopy with chemometrics on postmortem interval estimation based on pericardial fluids. Sci Rep. 2017;7(1):18013. doi: 10.1038/s41598-017-18228-7, PMID 29269843.
31. Alkhuder K. Attenuated total reflection-Fourier transform infrared spectroscopy: a universal analytical technique with promising applications in forensic analyses. Int J Legal Med. 2022;136(6):1717-36. doi: 10.1007/s00414-022-02882-2, PMID 36050421.
32. Mitu B, Cerda M, Hrib R, Trojan V, Halámková L. Attenuated total reflection Fourier transform infrared spectroscopy for forensic screening of long-term alcohol consumption from human nails. ACS Omega. 2023;8(24):22203-10. doi: 10.1021/acsomega.3c02579, PMID 37360459..
33. Angrish A, Kumar R, Chauhan R, Sharma V. On the IR spectroscopy and chemometric based rapid and non-destructive method for the investigation of sunscreen stains: application in forensic science. Spectrochim Acta A Mol Biomol Spectrosc. 2020;242:118708. doi: 10.1016/j.saa.2020.118708, PMID 32731143.
34. Wong JXW, Sauzier G, Lewis SW. Forensic discrimination of lipsticks using visible and attenuated total reflectance infrared spectroscopy. Forensic Sci Int. 2019;298:88-96. doi: 10.1016/j.forsciint.2019.02.044, PMID 30884439.
35. Chophi R, Sharma S, Singh R. Discrimination of vermilion (sindoor) using attenuated total reflectance fourier transform infrared spectroscopy in combination with PCA and PCA-LDA. J Forensic Sci. 2021;66(2):594-607. doi: 10.1111/1556-4029.14609, PMID 33137213.
36. Sharma A, Chauhan R, Kumar R, Mankotia P, Verma R, Sharma V. A rapid and non-destructive ATR-FTIR spectroscopy method supported by chemometrics for discriminating between facial creams and the classification into herbal and non-herbal brands. Spectrochim Acta A Mol Biomol Spectrosc. 2021;258:119803. doi: 10.1016/j.saa.2021.119803, PMID 33933939.
37. Xu W, Xia J, Min S, Xiong Y. Fourier transform infrared spectroscopy and chemometrics for the discrimination of animal fur types. Spectrochim Acta A Mol Biomol Spectrosc. 2022;274:121034. doi: 10.1016/j.saa.2022.121034, PMID 35248857..
38. Bhattacharya A, Ghosal S, Bhattacharya SK. Anti-oxidant effect of Withania somnifera glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. J Ethnopharmacol. 2001;74(1):1-6. doi: 10.1016/s0378-8741(00)00309-3, PMID 11137343.
39. Puhan S, Vedaraman N, Ram BVB, Sankarnarayananb G, Jeychandran K. Mahua oil (Madhuca indica seed oil) methylester as biodiesel-preparation and emission characteristics. Biomass Bioenergy. 2005;28:87.e93.
40. Kos G, Lohninger H, Krska R. Development of a method for the determination of Fusarium fungi on corn using mid-infrared spectroscopy with attenuated total reflection and chemometrics. Anal Chem. 2003;75(5):1211-7. doi: 10.1021/ac0260903, PMID 12641243.
41. Wilson RH, Smith AC, Kačuráková M, Saunders PK, Wellner N, Waldron KW. The mechanical properties and molecular dynamics of plant cell wall polysaccharides studied by Fourier-transform infrared spectroscopy. Plant Physiol. 2000;124(1):397-405. doi: 10.1104/pp.124.1.397, PMID 10982452.
42. Zareef M, Chen Q, Ouyang Q, Kutsanedzie FYH, Hassan MM, Viswadevarayalu A et al. Prediction of amino acids, caffeine, theaflavins and water extract in black tea using FT-NIR spectroscopy coupled chemometrics algorithms. Anal Methods. 2018;10(25):3023-31. doi: 10.1039/C8AY00731D.
43. Baiyi L, Jianyang C, Weisu H, Di W, Wei X, Qing X et al. Determination of flavonoids and phenolic acids in the extract of bamboo leaves using near-infrared spectroscopy and multivariate calibration. Afr J Biotechnol. 2011;10(42):8448-55. doi: 10.5897/AJB11.320.
44. Moser BR, Knothe G, Vaughn SF, Isbell TA. Production and evaluation of biodiesel from Field pennycress (Thlaspiarvense L.) oil. Energy Fuels. 2009;23:4149e55.