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Vibrational spectroscopic characterization of tuibur: an indigenous nicotine delivery medium

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ABSTRACT

It is estimated that the use consumption_of tobacco in diverse forms leading to is one of the important causative factors of avoidable yet ever increasing cancer death burden globally every year, with a majority occurring in Asia. Owing to the considerable difference in the patterns of usage of various categories of smokeless tobacco products (STP), it is possible that a broad range of toxicant content exposure among STP users. Habitual consumption of *tuibur*, a peculiar form of tobacco smoke infused saturated aqueous solution is presumed as one of the "safe" nicotine delivery medium. When commercially Commercially available *tuibur* sample was collected and subjected to the vibrational spectral study, it has revealed the molecular vibrational frequencies corresponding to various functional groups of different chemical constituents present in *tuibur*.

Key words: Tobacco smoke; Tuibur; Infrared Spectroscopy; Hydrogen bonding; Nicotine.

INTRODUCTION

Habitual consumption of a broad range of tobacco products has become one of the most serious health hazards and their contribution to the global health burden is incrementally increasing every year. Use of smokeless tobacco products or STPs (snuff, tobacco powder for oral use, gutka, etc.) and/or cigarette smoking is known to cause various forms of cancers as multifarious chemical constituents of tobacco are found to be carcinogenic. The cigarette smoke is a very complex mixture of organic and inorganic compounds that mainly are non-polar and/or polar in nature including water vapour[1-3] that can be studied in a simple and rapid manner, simultaneously in real-time, as admixture of gas-phase compounds using Fourier transform infrared (FTIR) spectroscopy.[4,5]

Identification of various hazardous compounds in tobacco products by employing molecular spectroscopic tools is an important task in order to evaluate the potential bioavailability of insidious compounds of tobacco. The application of vibrational spectroscopy methods reveals important structural and molecular information

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concerning the putative compounds originating due to the thermal/enzymatic degradation of tobacco derived chemicals.[6] In addition, it may also provide an insight into the potential risk of these deleterious chemical species to human health.

Molecular spectroscopy techniques such as infrared (IR) spectroscopy have been employed to gain a better understanding of the chemical nature and unravel the associated molecular composition of materials that possess a wide distribution of different components.[7] IR spectroscopy essentially probes the fundamental molecular vibrations associated with specific molecular functional groups present in specific organic compounds of the sample. In the present study, we have applied high resolution fast scanning Fourier transform infrared (FTIR) spectroscopy for the characterization of various kinds of specific functional groups present within different unknown chemical components of tuibur sample. For this purpose, commercially available tuibur sample was collected and subjected to the vibrational spectral study.

MATERIALS AND METHOD

Commercially available tuibur sample was filtered twice (Whatman filter paper Grade-4 qualitative filter paper 20-25 mm retention) to remove the suspended particles. Fourier transform infrared spectroscopy (FTIR) analysis of tuibur was performed on a Thermo Avatar 370 FTIR spectrometer. A small quantity of the filtered tuibur sample was added to KBr in the ratio 1:100 approximately. The resulting matrix was ground for 3-4 minutes using mortar and pestle. The fine powder was transferred into 13 mm diameter die and made into a pellet using a hydraulic press by applying a pressure of 7 ton/sq. mm. The resulting thin and semi-transparent pellet was subjected to FTIR analysis using a universal pellet holder. Infrared spectral data were collected over a range of 4000-400 cm^{-1} with an interferogram of 32 scans.

RESULTS AND DISCUSSION

The region below 1000 cm^{-1} is normally used for detecting the presence of stretching and bending vibrations of bonds between metal atoms and both inorganic as well as organic ligands,[8] in addition to the deformational vibrations of organic compounds. In a preliminary atomic emission spectral study, it has been observed that metal ions, of Fe, Cu, Ca, Mg, Mn, Ni and Zn besides few other elemental species are present in tuibur samples.[9] Halide group such as aliphatic chloride and iodide normally exhibit vibrations in stretching mode below 850 cm^{-1}, often in the range of 500-600 cm^{-1} and 515-690 cm^{-1}, respectively. Likewise, the vibrational frequencies, in general, arising from aliphatic halides, are observed in the range of < 700 cm^{-1}. The vibrational frequencies of monosubstituted aromatic compounds can be observed with the corresponding C-H deformation bands < 1000 cm^{-1}. For tuibur sample, a weak band at ~ 810 cm^{-1} and ~ 748 cm^{-1} that appear as weak shoulder (Figure 1) could be indicative of the presence of aromatic compound(s) as these band are normally observed for the aromatic C-H out-of-plane (OoP) bending vibrations.[10] In addition, the band of medium intensity observed at 691 cm^{-1} is in agreement with the OoP ring C=C bending corresponding to the aromatic ring.[11] Moreover, a very weak band observed at ~ 650 cm^{-1} may be due to the OoP band arising from the phenolic O-H group. While, the vibrational band of medium intensity observed at 618 cm^{-1} may be attributed to the N-H OoP bands of hetero-aromatic compounds and it is worth noting that the position of the N-H band (occurs at ~ 600 cm^{-1}) varies according to the degree of hydrogen bonding.[10] Alternatively, this low frequency fundamental vibrational band may also be likely due to the free 'inorganic' sulphate ion $(SO_4)^{2-}$ group.[8]

Moreover, a preliminary high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) study of tuibur solution has revealed that there were more than 20 chemical components of tobacco, as exhibited by the LC-MS data, the prominent base ion (the most intense peak) as well as molecular ion peak belongs to nicotine, as $[M-H]^+$ species, with an (m/z) = 163.2.[9] In addition to nicotine, other nicotine-like alkaloidal species observed were: anabasine (163.2), 3-hydroxymyosmine (163.2) along with pro-oxidant species, hydroquinone/ catechol (111). The other ions are currently being analyzed and characterized by comparing available data in the literature.[12,13] It is obvious that further experimentation is certainly needed in this area and further work is currently underway in the authors' laboratory.

The detection of alkaloid species of tobacco in tuibur solution using LC-MS method (*vide supra*) commensurates with the observation of a rather broad and very intense band in the 3800–3000 cm^{-1} region indicating the presence of H-bonded proton attached with electronegative atoms such as oxygen (O–H) or nitrogen (N–H) at the expense of free O-H group vibrations which normally observed as sharp intense peak.[14] Surprisingly, the IR spectrum of tuibur lacks C-H stretching vibrational bands for both sp^3 (below 3000 cm^{-1}) and sp^2 (above 3000 cm^{-1}) hybridized carbon containing moieties. This type of unstructured and rather broad O-H vibrational bands that overlay C-H vibrations in this region is also typical of neat samples of carboxylic acids, alcohols and amines. Moreover, the cigarette smoke consists of various phenolic compounds associated with different thermal degradation conditions.[1,12] This is not unexpected as tuibur is a resultant product of the thermal degradation of tobacco stalk, contains a wide range of volatile and semivolatile compounds, few among them which are polar in nature may be exhibiting significant hydrogen bonding.[15]

Obtaining a well resolved IR spectrum of aqueous solution samples is rather difficult task. Furthermore, in tuibur solution, a broad range of multifarious compounds with varying polarity could be present.[1,2] Yet the analysis of related compounds by applying vibrational spectroscopy methods can be very helpful in the interpretation of the FT-IR spectrum of tuibur sample.[10,11,14,16] Nevertheless, the residual water, typical of polar samples, exhibits high background signals. As a result, the less intense bands are overlaid by those dominant bands that can only be clearly observed in the recorded IR spectrum as vibrational bands of various constituents are connatural and overlapping.[6,7]

Indeed, the neat IR spectrum of nicotine exhibited vibrational frequencies more akin to the vibrational spectrum of pyridine, *viz.*, the slightly split band for the C=C vibration at 1600 cm^{-1} and the deformation bands between 700 and 800 cm^{-1}.[17] The aliphatic moiety of nicotine has also been shown to exhibit very strong C-H stretching vibrations from 3000 to 2800 cm^{-1}, in addition to a relatively broad band at 3475 cm^{-1} indicative of the presence of hydrogen bonding. In the double bond region, one would expect vibrational frequencies corresponding to aromatic C=C as well as aliphatic C=C vibrations. Distinctly strong absorption with sharp band at 1636 cm^{-1} (Fig. 2 & 3) (probably due to the presence of aromatic and olefinic double bonds) in the finger print region and the strong band at 875 cm^{-1} (Fig. 1) indicate the presence of *para*-substituted aromatic ring which commensurates with the preliminary LC-MS detection of hydroquinol (p-hydroxy phenol).[10] It is worthwhile to note here that the observed strong band at 1636 cm^{-1} may also be attributed to the bending vibrations of O-H bonds of water molecules as residual amounts of water is expected, even with the careful preparation of samples from aqueous solutions.[18,19]

In the finger print region, weak absorption bands at 1362 cm^{-1} (Fig. 2) and 1360 cm^{-1} (single band) (Fig. 3) are probably due to C–N stretching probably arising from an aromatic compound that overlays C-H deformation (bending and out-of-plane) vibrational bands which is hardly seen as very weak shoulder (see Fig. 2) that would be normally observed at ~ 1430 cm^{-1}.[20,21] Thus, the observed vibrational frequencies are indicative of the presence of aromatic compound(s) containing nitrogen in tuibur which corroborates with the preliminary LC-MS study.[9] In the fingerprint region, the area of the

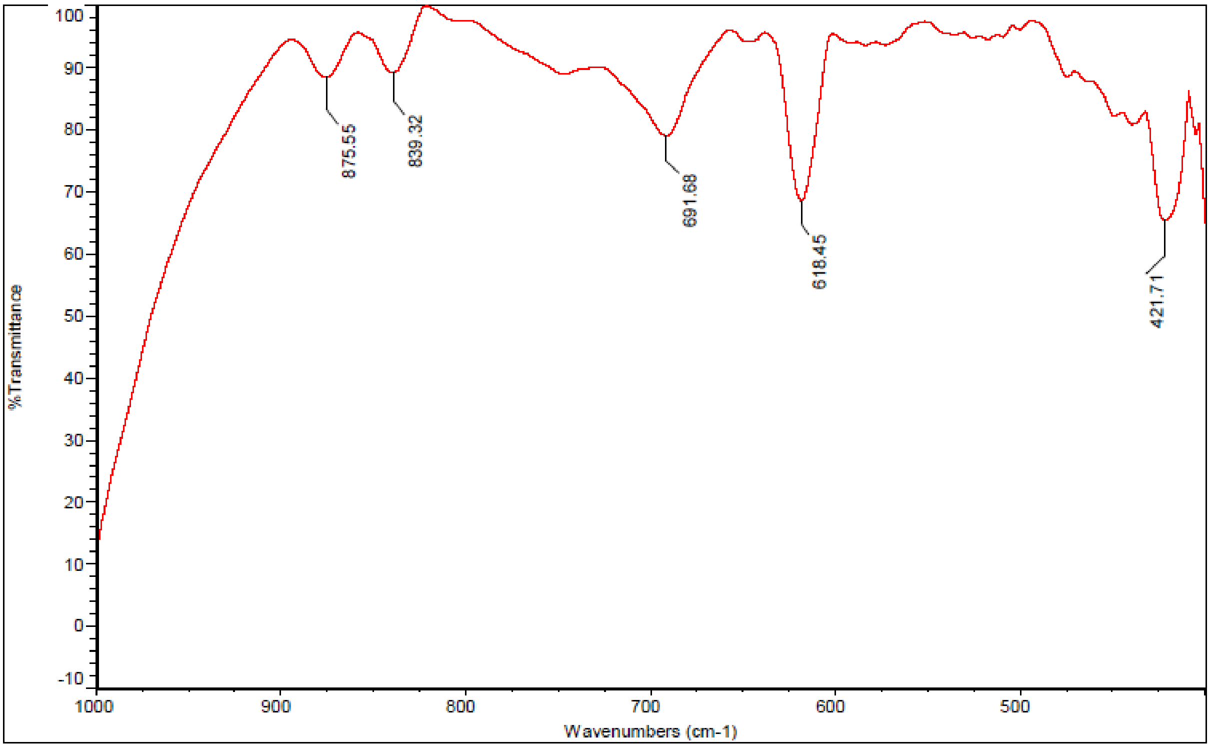

Figure 1. FTIR spectrum of tuibur.

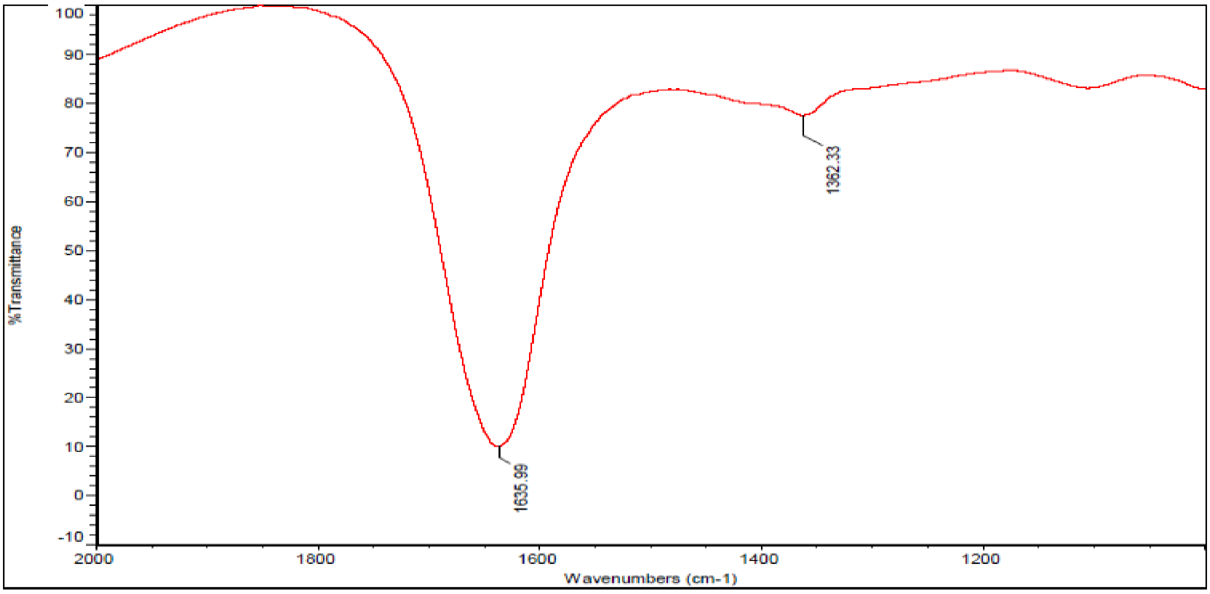

Figure 2. FTIR spectrum of tuibur solution in 2000-1000 cm^{-1} region.

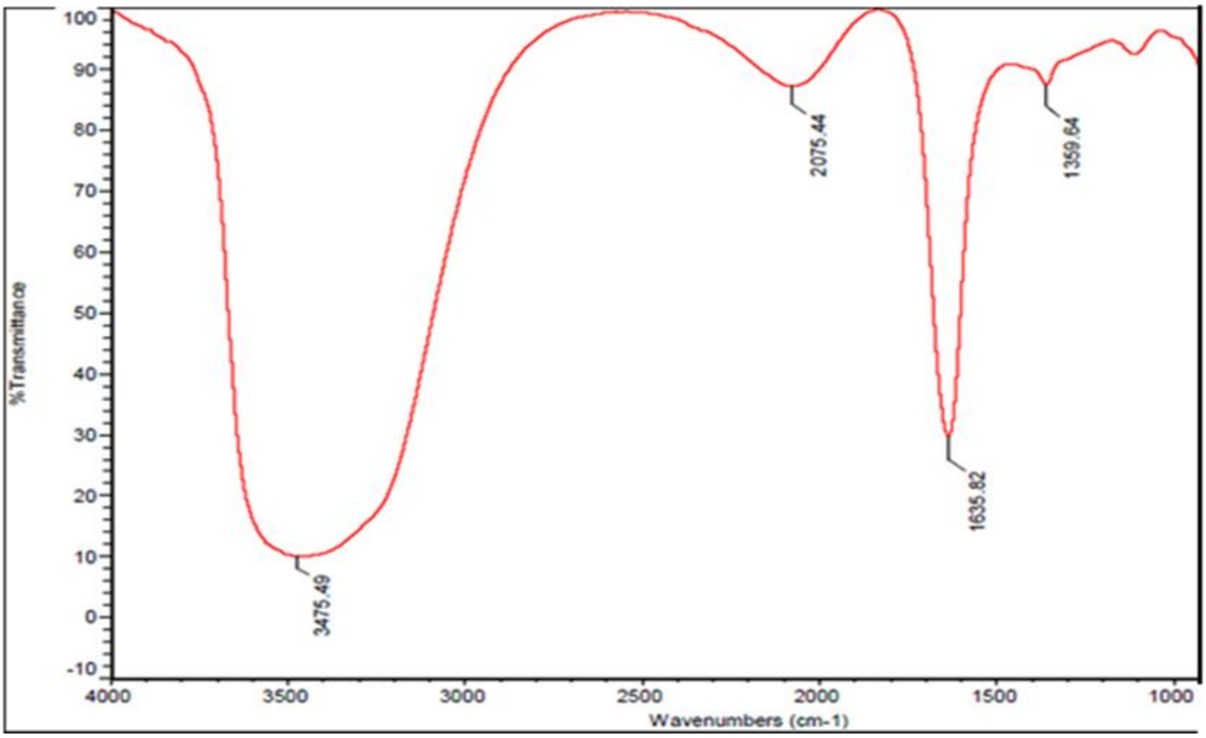

Figure 3. FTIR spectrum of tuibur in the range of 4000-1000 cm^{-1}.

C–O single bond vibration from 1200 to 1000 cm^{-1} is occupied by a small band at 1100 cm^{-1} (a single weak band) along with a weak band at 1000 cm^{-1} (Fig. 2), which may be assigned to the phenyl ring breathing mode. In addition, often overlooked in similar cases, but very important in this spectrum, is the distinctly small and broad signal at 2075 cm^{-1} indicating the possible presence of cyano group[8] as free inorganic cyanide ion exhibits stretching vibrations at 2080 cm^{-1}. Moreover, cigarette smoke is known to contain cyano group as HCN.[12,15] Alternatively, the distinctly small intensity band at 2075 cm^{-1} may also be attributed to C=N bond present in nitrogen containing heteroaromatics such as pyridine. Furthermore, due to the observation of vibrational bands corresponding to cyano/ring C=N group, the characteristic overtone vibrations of aromatic moiety between 2000 – 1800 cm^{-1} can be hardly detected in the present study.

The absorption band at 3476 cm^{-1} (Fig. 3) is rather unstructured and shows a very broad band that dominate the IR spectrum of tuibur, probably indicating the presence of hydrogen bonded labile proton attached with electronegative oxygen (O–H) or nitrogen (N–H). Moreover, the breadth of the unstructured band (~ 800 cm^{-1}) in the region belonging to O–H/ N–H bonds, in general, depends on the degree of hydrogen bonding and the polarity of the solvent. It is also important to note that it would be a challenge to extract meaningful bonding vibrations from FT-IR measurements alone in aqueous solutions due to the strong interference of water in the mid-IR region as dominant valence vibration of O-H bonds in water molecules and any further attempt at explanation would be purely speculative and premature. Yet, the challenges imposed by aqueous environments, in recent years, may be partially mitigated by employing careful preparation methods for samples and the subsequent spectral measurements in transmission measurement mode.

CONCLUSION

In summary, FT-IR spectroscopy was successfully applied for the identification of different functional groups of a mixture of chemical components present in tuibur solution. A short article such as this can present only a brief outline of the presence of various chemical moieties within the chemical constituents of tuibur. It is also important to mention here that the detailed characterization of tuibur solution by combining FT-IR with Raman spectroscopy along with liquid chromatography-mass spectrometry (LC-MS) will provide a unique opportunity to explore the composition of tuibur besides the structural and functional aspects of various chemical components present in it, which is currently underway.

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