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Case Report

Critical chemical study of 'wearing apparels' extract of a decomposed dead body by GC-MS method providing evidences for conviction of murder case

Suman Kr. Chakrabarti*, Pranab Chowdhury[2], Juthika Debbarma**, Pradipta Narayan Chakrabarty**, Utpal Ch De*****

*Chemical Science Division, Tripura State Forensic Science Laboratory, Agartala, India.
**Forensic Medicine & Toxicology department, Agartala Govt Medical College & GBP Hospital, Agartala, India
***Dept. of Chemistry, Tripura University, Agartala, India

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Corresponding author : S. K. Chakrabarti, Chemical Science Division, Tripura State Forensic Science Laboratory, Agartala, India. E-mail: csuman1973@gmail.com

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Abstract

This study describes the critical analysis of extracts from the wearing apparels of a decomposed dead body by chemical and GC-MS techniques to provide irrefutable evidence for conviction of accused. Following the brutal murder of a child, the body was concealed in the bamboo ceiling of the perpetrator's living room. Crime spot was left with no substantial evidence as it was fired by a group of aggrieved neighbours, posing a significant challenge to convict the accused. However, chemical and GC-MS analysis of trace amounts of room cleaning disinfectant collected from the crime scene indicated the presence of compounds namely α-pinene, eucalyptol, d-limonene, γ-terpinene, camphor, fenchol, citronellal, terpinen-4-ol, α-terpineol, citronella, geraniol, and citral which were very similar to the extracts from the wearing apparels of the dead body. This finding along with other physicochemical analyses led to established the crime and conviction of the accused. Therefore, this study showcasing unique significance of GC-MS techniques in the field of forensic analysis as a led support for conviction.

Key words: Disinfectants; forensic science; wearing apparels; GC-Mass.

Introduction

The identification and conviction of perpetrators in murder cases often rely heavily on forensic evidence. Homicide, in its various forms, has always been a crucial focus for forensic investigators, who must provide convincing results to the judiciary based on forensic analysis. Modus operandi studies reveal that perpetrators often attempt to evade responsibility by employing new methods to conceal their crimes.[1] Common practices include burying, burning, or dumping the corpse in remote areas. When such methods are not feasible, offenders may conceal the body in other ways, such as staging the scene to resemble a suicide.[1] Instances have been documented where bodies were hidden in freezers, under ice, cut into pieces and packed in airtight containers, or preserved with formaldehyde.[2] In cases where freezing or cooling is not an option and the decomposing body emits disagreeable odor, it prompts the offender to find ways to mask the smell. Present case involves an offender from a low-income family who, after brutally murdering a child as revenge, was unable to dispose of the body and concealed in the bamboo made ceiling of his own

living room. To suppress the decomposition odor, the offender repeatedly applied a lemon-scented room disinfectant available at a nearby market. Despite these efforts, the degradation smell gradually intensified over time which eventually lead the recovery of the child's body. Following the recovery, an emotionally agitated mob set fire to the crime scene, degraded prima facie evidences complicating the investigation. Nonetheless, trace chemical analysis of the crime scene liquid detected at the time of careful crime scene investigation [1,3] and extracts from the clothing of the decomposed body played a crucial role in establishing the crime and presenting evidence in court.

Brief of the incidence

In a semi-urban area on the outskirts of an Indian city, there were a few families living in clusters with more or less normal relationships. A fifteen-year-old boy was living with his family, while a five-year-old child lived with his mother and grandparents in the neighboring house. The little child often visited the elder boy's house and used to play there with him. One day, the child had some issues with the elder and was beaten by the older boy. The family members of the child raised this issue and argued with the elder boy's parents. On that occasion, the elder boy's family threatened the child's family with severe consequences in the future. After some days, the child again started visiting the elder boy's house again and one day, after his morning meal, he went to the older boy's house, but reportedly had gone missing. Naturally, a rampant and frantic search began for the missing child by his family members, neighbors, and interestingly, the older boy's family too. According to the child's family members, he was last seen entering the older boy's house but was not seen coming out. This compelled the grandparents of the child and locals to have a strong suspicion of the elder boy's house. The locals also kept a strong vigilance over that house. The family members of the elder boy had actually murdered the child and tried to conceal the crime by keeping the dead body in the ceiling of a room. They were waiting for scopes to remove and dump the dead body. They attempted to suppress the possible decomposition smell of the dead body. It was winter (January), and decomposition was a little slow but not stopped. Despite all efforts, the body started emitting a decomposition smell. When the decomposition smell became intense, the accused family members used locally available lemon scented disinfectants but could not suppress it. The local inhabitants finally traced the body in the ceiling of the older boy's house eleven days after the murder.

The child's body was found clothed, with both the upper and lower limbs tied at the wrists and ankles by a single green colour nylon rope with multiple folds and knots. White adhesive tape in two layers was found sticking to the lower part of the face, extending from the right mastoid and overlapping the lower part of the helix of the right ear, covering the entire lower part of the face, including the mouth and nostrils, up to the lower part of the helix of the left ear (Fig. 1A-D).

Fig. 1 **A** Body of the child**.**
B close up of the face tied with adhesive tape
C Close up view of tied hands**.**
D knot position of hands and leg.

Materials and Methods

Viscera samples *i.e.,* stomach and small intestine with contents, a section of the liver, the full spleen, and half of each kidney from the dead body and blood samples were collected during the post mortem examination and sent to the forensic laboratory. In addition, various items of the child's clothing were collected *viz.* a half-sleeve T-shirt, a full-sleeve black shirt with indigo stripes, blue jeans with graphics on one side, the curtain in which the body was wrapped, and a green underwear soiled with faecal matter (Fig. 1E-I). A plastic bottle containing approx 2.0 ml of white coloured and perfumed (lemon scented) fluid

labelled as "Doctor's Phenyl" (crime spot liquid) was collected from the crime spot (Fig. 1J).

Fig. 2 Wearing apparels of deceased -
E T shirt.
F full sleeves black shirt.
G jeans pant with some graphics.
H close up view of the pant**,**
I curtain used for wrapping the dead body in ceiling.
J plastic bottle containing approx 2.0 ml of white coloured & perfumed fluid collected from crime spot.

Pre-coated TLC plates (Merck, cat no. 1.5554.0007), CAMAG twin trough TLC chamber, commercially available room cleaning disinfectant (similar brand as recovered from crime place), marketed formulations of pesticides, sodium nitrate, sulphuric acid for phenol identification (Liebermann test), iodine crystals, anisaldehyde, diphenyl amine (DPA), sodium hydroxide, silver nitrate, palladium chloride, mercuric chloride, diphenyl carbazone, sodium hydroxide, aluminium oxide, charcoal, solvents *viz*. ethyl acetate, toluene, n-hexane, acetone, methanol, chloroform, were purchased from E. Merck and used without further purification. Thermo Scientific Trace 1300 Gas Chromatograph, ISQ7000, with column: TG-5MS, 30m×0.25mm×0.25 µm integrated with single Quadrupole Mass Spectrophotometer was used for requisite GC-MS analysis.

Specific colour Tests

Liebermann's colour test for phenol (as suspected to be used for suppression of dead body degradation) was done for the collected crime spot liquid as per standard protocol.[4] In brief, to a small amount of the sample in test tube was added few crystals of solid sodium nitrite and few drops of conc. sulphuric acid. The resultant solution was poured into aqueous alkali wherein no colouration was observedindicating the absence of phenolic compounds.[4,5]

TLC analysis

The viscera samples were subjected to thin-layer chromatography (TLC) to investigate the presence of common poisons, such as organophosphorus (OP), organochloro (OC), and carbamate group pesticides[6]. For this analysis, the samples were extracted with n-hexane for 24 hours. The extract was then percolated through neutral alumina, concentrated, and spotted onto several TLC plates along with corresponding standard references.The plates were developed in a twin trough TLC chamber with saturation pads with a solvent system of n-hexane-acetone (4:1) and visualized under UV light (254 nm). Different spraying reagents were used to visualize the spots in the TLC plates: DPA in ethanol for OC, palladium chloride in water for OP, and Tollen's reagent for carbamates. To investigate the presence of sedatives like benzodiazepines, the viscera extract (for drug analysis) was also analyzed by TLC, (developed in chloroform-methanol (8:2), and visualized under UV light using pure benzodiazepine (alprazolam) as a reference standard. Further UV visualization was done after spraying with Dragendorff's reagent. Besides, all the wearing apparel and covering cloths of the dead body (Fig. 2 E -I) were extracted with ethanol, filtered, and passed through neutral alumina and activated charcoal to remove undesired biological materials and dyes. The extract was then concentrated, subjected to TLC analysis using silica gel pre-coated aluminum plates, developing in ethyl acetate-toluene (9:1), and visualized in an iodine chamber as well as under UV light (254 nm). Additional visualization methods included spraying with anisaldehyde solution [7,8] followed by heating at 100-105°C for 10 minutes and subsequent UV exposure (365 nm) of the TLC plate for further validation of the observations. A commercially available room-cleaning fluid with a lemon-like smell that visually matched the crime scene liquid was used as a standard reference.

GC-MS Analysis

GC-MS (Thermo Scientific Trace 1300 Gas Chromatograph, ISQ7000) analysis of both the samples *viz.* crime spot liquid and the jeans pant(waist portion) extract were carried out for anticipated similarity of these two samples in terms of major chemical compositions.

Method development

Before injecting the samples (the extract and crime spot liquid) into the GC-column they were successively passed through activated neutral alumina, charcoal to remove the biological residues, dyes from the jeans pant and traces of faecal matters. Samples were then filtered through a 0.45µm filter discs (Milipore) for removal of fine particles, if any. In the context of method standardization for effective separation of chemical components in GC-analysis various methods have been developed and tested. At the initial, a portion of 2 µl extract was subjected to GC analysis employing gradient method within temperature range of 50°C to 280°C.[6] Various temperature settings, holding time, temperature gradient were applied under different programmable options (**Table 1**). Helium was used as carrier gas passing through an oxy trap and a moisture trap successively. Flow rate of 1.0 ml/minute of carrier gas was maintained and TG-5MS, 30m×0.25mm×0.25 µm column was used for effective separations. In built EIMS mass detector was used enabling detection within mass range of 25-300 amu.

Results

Post mortem observations

Post mortem examination of the viscera samples i.e., stomach and small intestine, liver portion, spleen (full) and kidney, blood (found to be putrefied) revealed that the stomach contained about 300 gm of yellow coloured semi digested food with recognisable rice. Mucosa was congested. Post mortem examinations also reported that the whole body was blotted and there was development of greenish black colouration. Both the eye balls protruding, emission of strong foul smell. Nose was depressed and tongue protruded between the teeth's. Penis and scrotum swollen, maggots of different stages had developed and found crawling in the different orifices. Loosening of scalp hair and partial skeletonization of the finger tips were observed.

Colour Test

Liebermann's colour test for phenolic type compounds was performed for the crime spot liquid as well as for the commercial product (bought from nearby market) with a prior validation of the test against standard phenol. Negative results ruled out the presence of any phenolic compounds in either of the liquids. Similar test done for the extracts of wearing apparels of the dead body and the negative results which indicated the absence of phenolics in the extract too. The absence of phenol was also supported by the post mortem examination which reported no sign of corrosive effects on the dead body except it's advanced state of natural putrefaction.[2,9]

TLC Studies

Specific TLC for citronellol like compound (a common constituents of lemon scented fluids)[13-18] for exhibit J and the commercially available liquid suggested the presence of citronellol like compounds in both the fluids. This observation prima facie supported the fact that the liquid collected from the site of incident (crime spot liquid) and commercially available (bought from nearby market) liquid disinfectants were same. Moreover, TLC analysis for the extracts of jeans pants (waist portion), crime spot liquid and the commercial product collected from the local market revealed that they were similar in composition as spots with same R_f valueswere observed. This finding was further strengthened by the testimony of the local shopkeeper from whom several bottles of disinfectants liquids were purchased by the accused family members.

GC-MS Analysis

With the aforesaid information in hand, all the liquid samples, viz., wearing apparel extracts and crime spot samples, were subjected to GC-MS analysis. Different GC-MS programs with various controlled temperature gradients were applied for optimized results (maximum peak) from the analytes (Table 2). The program with a temperature range of 50-120°C was found to be

effective for the elution of possible components. Similar chromatograms (Fig. 3 & Fig. 4) were obtained for both the liquids and the reference standard (commercial sample). Analysis results of these chromatograms in terms of m/z values revealed that all the samples had similar chemical compositions. Searching the preinstalled NIST library for mass-specific compounds indicated the presence of compounds namely α-pinene, eucalyptol, d-limonene, γ-terpinene, camphor, fenchol, citronellal, terpinen-4-ol, α-terpineol, citronella, geraniol, and citral (Fig. 5). All these compounds, along with their m/z values and intensities, are summarized in Table 2, and their fragmentation patterns are also depicted.

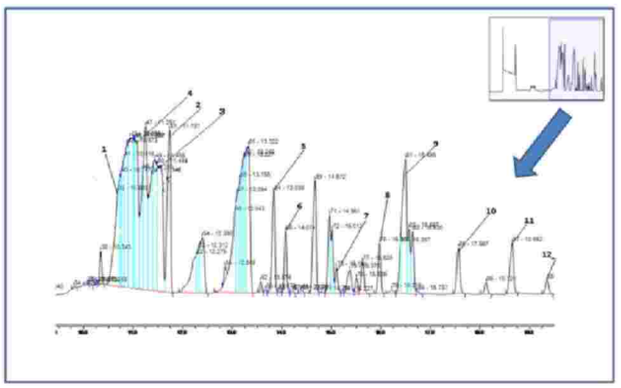

Fig. 3 Chromatogram of extracted liquid

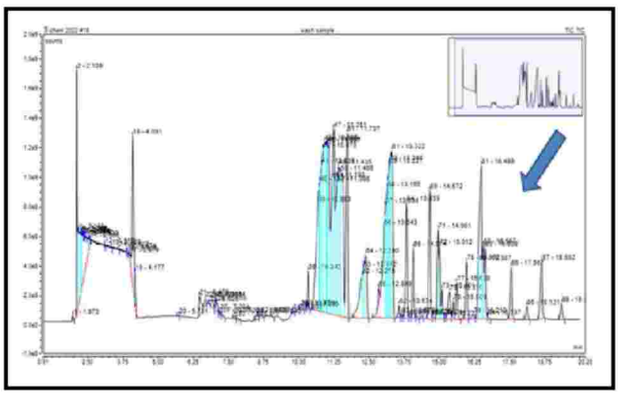

Fig. 4 Chromatogram of reference standard

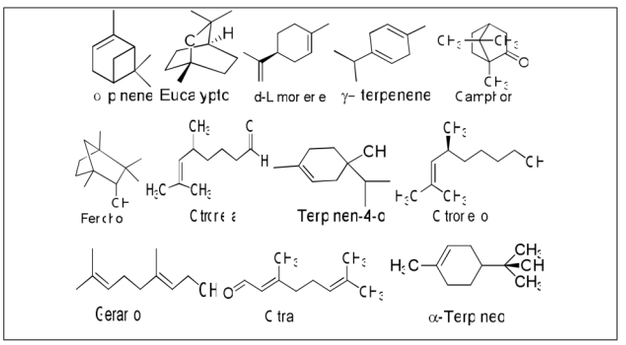

Fig. 5 Structural formulae of the compounds detected

Discussion

Post mortem investigations suggested that the deceased was killed on the same day he had gone missing. During the course of time the rigor mortis had passed away. The autopsy report also opined that the death was due to smothering. No intoxicants, poison or any sedative drugs were present in the stomach as revealed in the chemical examination of the viscera. The absence of any intoxicants, poisons or sedative drugs ruled out the other means of homicide. The house where the body was concealed was gutted by fire by the angry mob. So there were no direct evidences left with the investigating agency to establish the link of accused family with the murder case by law. It was left with only few (~2.0) ml of lemon scented white coloured fluid (later on identified as a common room cleaning disinfectant) collected from crime spot and the wearing apparels of the dead body. The jeans pant of the dead child had an about one-inch-thick elastic band at the back side. Being at the backside of the body and disinfectant liquid being applied repeatedly to suppress the degradation smell of the dead body, it had soaked quite a good quantity of disinfectant liquid and also had little opportunity to evaporate. Moreover, being winter season, the evaporation was slow. The body was found in advanced stage of putrefaction, with maggots and skeletonisation of finger tips. Thus, detection of disinfectant residues in the wearing clothes was the only way to support the prosecution. Hence, a similar brand (Doctor's phenyl) of disinfectant was collected from the market. As there was no specific *a priori* knowledge about the compositions of commercially available local made disinfectants, except the probable presence of anionic surfactants, pine oil/citronella oil, camphor oil, binary anionic surfactants;[19] TLC studies were undertaken for qualitative chemical profiling of all the analytes *viz.* residues of wearing apparels of the dead body, crime spot liquid as well as the disinfectant purchased from the local market. To establish connectivity of the murder case with the accused person with irrefutable evidences, the matching of the collected white coloured fluid with the residues of disinfectant in the wearing apparels of the dead body was the only way. The body was recovered from the ceiling of the accused house after eleven days, To suppress the

degradation smell, the accused and his family members went on pouring the liquid disinfectant with strong lemon smell. Due to the strong vigilance by the family of deceased and local people, the accused persons could not find any scope to remove the dead body and so they were compelled to use cheap commercial liquid disinfectants. This fact was in favour of finding the disinfectant residues extracted from the waist portion of the jeans pant of the body. Thus, eventual matching of the extracted residue of wearing apparels of the body and the residual liquid collected from crime spot with the commercial product of same brand purchased from the local market was a strong evidence to establish the link of accused family with the murder case. Accordingly, the 2.0 ml liquid collected from the incident site having lemon like smell was subjected to TLC to find the possible matching of visually similar and commercially available product in the local market.

Focussing on the analysis of disinfectants fluids revealed that there were no specific formulations as that of the Chlumsky disinfectant [17,18] for such cheap room cleaning fluids manufactured locally. However, this kind of disinfectants usually consisted of pine oil/citronella oil (10-40% by wt), camphor oil (2-5% by wt), binary anionic surfactants (10-25% by wt (Material Safety Data Sheet, 2009).[19] This fact also has been experimentally supported by the GC-MS analysis (Table 1) and corresponding literature data.[20-30]

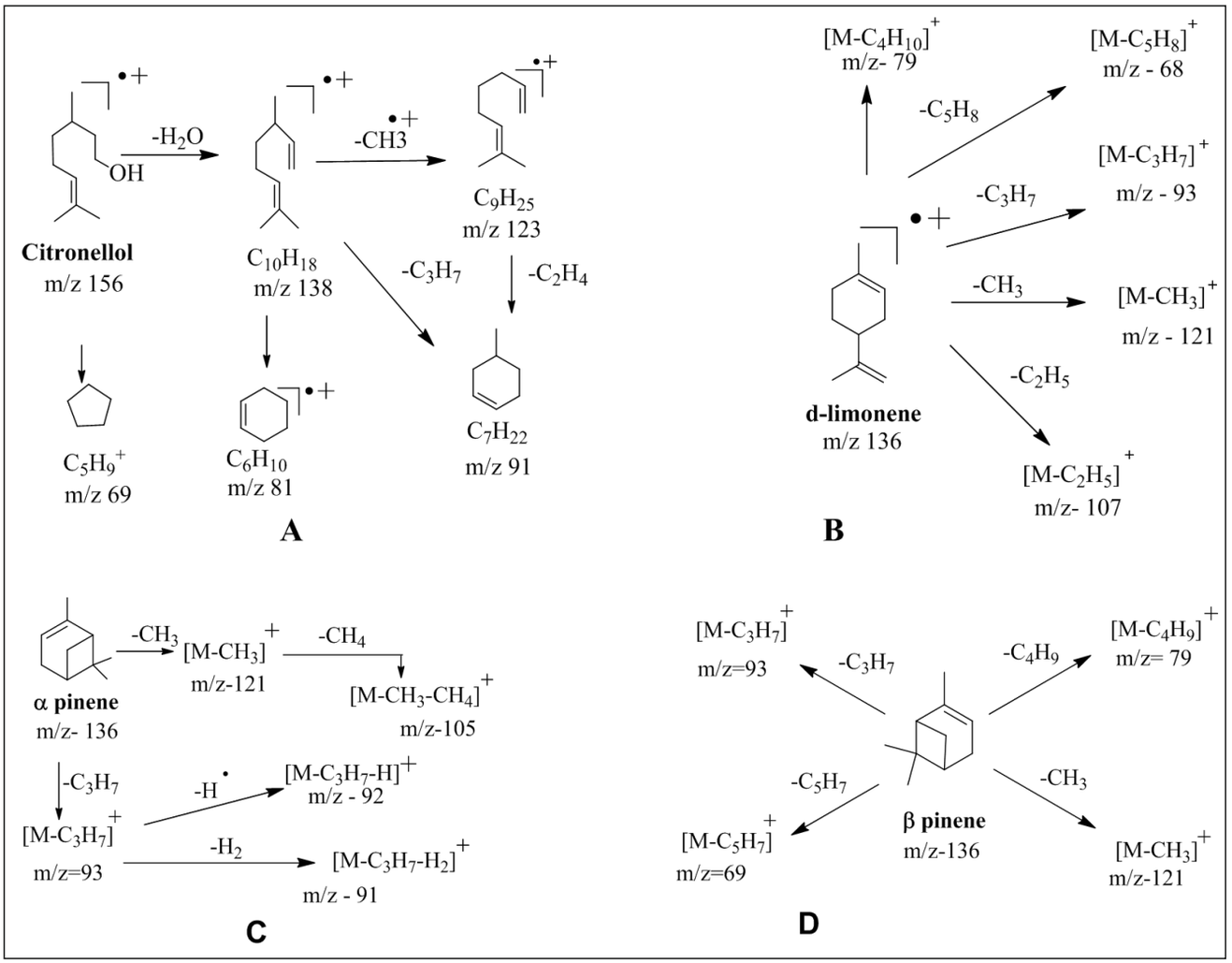

Fig. 6 Mass fragmentation study of four compounds

A-Citronellol, **B**-d-Limonene, **C** -α Pinene, **D**-β Pinene

The probable mass fragmentations of four major components *viz.* citronellol, d-limonene, α–pinene, β–pinene found in the GC-MS analytes are presented in Fig. 6. The study of the mass fragmentation supports the mass data obtained from the GC-MS analysis. Literature study [31-34] reveals that the principal components detected in lemon peel oil are γ-terpinene, d-limonene, and citral. Although citral (3,7-dimethyl-2,6-octadienal) is the major component but other two isomers geranial and neral are also present in the lemon scented herbal plants. It is likely that the tertiary terpene alcohols are formed to different extent by hydration of terpenes during contact with acidic juice and water during extraction.[10,15, 35] Citronellol is found in citronella oil which is used in normal room cleaning and disinfectant fluids due to its strong odour and the insect repellent property. Because of these, the fluid with the lemon smell was used to suppress the decomposition smell of the dead body.

Citronellal, a mono terpenoid aldehyde is the main component that gives the citronella oil a distinctive lemon scent.[11,13,16,21,36-38] The chemical profiling of all the samples were finally ascertained by their GC-MS analysis [39] in a low temperature programme (Entry 3 in Table -1) The program optimization enabled detection of maximum compounds. The volatile compounds remained undetectable at high temperature programs as observed during the study. In this study, the low temperature program carried out for GC-MS analysis proved to be successful for detection of the targeted molecules. The similar pattern of chromatograms confirmed similar chemical compositions. Such analysis can also be carried out in similar cases where volatile compounds with strong odour has been used to suppress the smell of death. These irrefutable evidences establish the crime and link of the accused family. The parents of the accused were sentenced to rigorous imprisonment and the elder boy who was a minor at the time of commission of offence was sent to juvenile correctional home.

Conclusions:

The identification and conviction of perpetrators in criminal cases often rely heavily on forensic evidence. In this study, analysis of trace samples extracted from wearing apparels of the deceased linked the convict with the murder case. Conviction of the accused has established the necessity of logical amalgamation of chemical analysis techniques and scientific thoughts in forensic field. Due to lack of substantial evidences to link the accused by law, it was a challenge to the authors with a very trace amount of ordinary room cleaning disinfectant collected from crime spot. However, systematic study of trace chemicals and identification supported the judiciary with irrefutable evidence to convict the accused by law.

Limitations of the study:

It was not possible to quantify the amount of the different ingredients detected from the extracts as the individual items could not be obtained in their pure forms.

Declaration:

This is an original piece of work and it has not been published anywhere or sent anywhere for consideration for publication. All the photos and relevant materials used in this paper are collected by the authors.

Conflict of interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the paper.

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Table -1: Various GC-methods employed in GC-MS analysis of the samples

Temp. range(°C)		Temp. gradient (°C)	Hold temp (°C)	Hold time min	Flow rate (ml/min)
Initial temp.	Final temp.				
50	280	--	50	01	01 ml/min
		20°/min to 150°	150	05 min	
		10°/min to 280°	280	15 min	
75	250	--	75	2 min.	01 ml/min
		10°/min to 200°	200	10 min	
		5°/min to 250°	250	2 min.	
50	120	--	50	01 min	01 ml/min
		5°/min to 120°	120	05 min	
100	250	--	100	01 min	01 ml/min
		5°/min to 150°	150	05 min	
		2°/min to 250	250	05 min	

Table-2: Mass fragmentation of major peaks in GC-MS

Sl No	$R_{t\ (min)}$	m/z values (% intensity)	Name of the compound
1	10.67	67(8.4), 77(28.5), 83((0.1), 93(99.9), 105(10.2), 121((13.6), 136(7.2)	α-Pinene
2	11.72	43(99.9), 81(65.4), 71(49.2), 93(38.0), 96(24.6), 108(56.0), 139(35.5), 154(36.2)	Eucalyptol
3	11.35	68(99.9), 93(78.3), 79(39.1), 107(25.2), 121(28.6), 136(27.6)	D-limonene
4	11.02	93(99.9), 77(31.8), 105(8.2), 121(26.9), 136(29.0)	γ-Terpinene
5	13.83	41(50.), 55(31.1), 69(36), 81(73.3), 95(99.9), 108(48.1), 152(33.2)	Camphor
6	14.06	55(17.9), 69(28.4), 81(99.9), 84(21.5), 93(14.9), 111(16.5), 121((14.9), 154((1.5)	Fenchol
7	15.09	41(84.7), 55(44.2), 69(99.9), 81(20.1),95(61.8), 111(23.7), 121(29.1), 136((11.6), 154((14.5)	Citronellal
8	15.96	71(99.9), 43(44.7), 55((22.3), 81(11.7), 86(26.7), 93($43.2), 111(52.6), 125(1.9), 136(7.3), 154(17.6)	Terpinen-4-ol
9	16.49	59(99.9), 67((23.5), 81((39.4), 93((69.4), 107((8.0), 121((58.7), 136((49.0)	α-Terpineol
10	17.55	41(94.2), 55((48.0), 69((99.9), 81(51.5), 95((44.8), 109(18.0), 123((24.8), 138(0.2), 156(5.3)	Citronellol
11	18.64	41(60.0), 53(11.2), 69(99.9), 93(14.3), 111(7.8), 123(13.8), 139(3.1), 154(1.2)	Geraniol
12	19.34	69(99.9), 84((28.6), 94(16.6), 109(8.8), 123(8.6), 137(9.8), 152(4.3)	Citral

Statements and declarations

Author contribution statement:

Suman Kr. Chakrabarti: Conceptualization, Material preparation, photography, data collection and analysis. **Pranab Chowdhury, Juthika Debbarma, Pradipta Narayan Chakrabarty**: Post mortem examination and viscera sample collections. **Suman Kr. Chakrabarti & Utpal Ch De:** Data compilations & manuscript preparations, Methodology, Validation, Supervision. All authors contributed to the final text and approved it.

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