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## RESEARCH ARTICLE

### IDENTIFICATION OF A NOVEL COMPOUND IN POST-MORTEM SPECIMEN OF A SEXUALLY ASSAULTED VICTIM BY GC-MS ANALYSIS

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#### ABSTRACT

Modern society is witnessing a considerable ascend in sexual assault against women in India by using several illicitly abused psychoactive substances. Isopulegol is a terpene-based alkaloid found in cannabis plants and can also be synthetically manufactured as a designer drug. It is used as a precursor chemical for menthol manufacturing which is used as a flavoring agent in food products. This paper presents identified novel drug Isopulegol (a terpene-based plant alkaloid) in the post-mortem samples of rape victims. Thin Layer Chromatography (TLC) and GC-MS analysis were conducted on the extracts of biological samples. This case highlighted the role of forensic toxicologists and forensic chemists or drug analysts to explore drugs with abuse potential. Forensic examination of a reported case unearthed that this plant alkaloid has the potential to depress the central nervous system of an individual, enabling it to be used for offense expeditions.

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## INTRODUCTION

Drug-facilitated crimes (DFCs) include the commencement of an offense under the influence of a chemical substance that is capable to impede the senses, motor activity, cognition, and memory of the individual with the outcome of distorted perceptions, altered response to the stimuli, decision-making ability and amnesia. Usually, an incapacitating chemical substance is covertly given to the victim in combination with an alcoholic beverage or water to take advantage or harm the person in a non-resistible or unconscious state where the victim is not aware of this adulteration. Though, the victim ingests the substance willingly offense is commenced without his/her consent under the influence of administered substance under the category of DFCs.<sup>1</sup> Based on the nature of the act, such offenses under the influence of drugs can be classified into DFCs that include acts like robbery, blasphemy, extortion, etc. whereas acts that involve sexual indulgence are termed drug-facilitated sexual assault (DFSA). In a document issued by the United Nations Office on Drugs and Crime (UNODC), it was mentioned that there exists a wide range of substances like alcohol, over-the-counter medicines (OTCs), illicit psychoactive drugs, and prescription medicines. But, Ketamine, Rohypnol, and gamma hydroxybutyrate (GHB) are the drugs that gained attention from social media. Despite these drugs' eminence in society, illicit prescription medications and OTCs serve as good choices of substance for fulfilling malicious intentions because of their inexpensiveness, easy accessibility, and intensified synergetic effects when used in conjunction with alcohol.<sup>2</sup>

Substances used to facilitate such offense act on the central nervous system (CNS) as a depressant to slow down the body's metabolism and produce effects similar to effects exhibited during alcohol or anesthetic intoxication. The outcome of the pharmacology of such substances on the human body include euphoria, non-resistance, amnesia, loss of voluntary actions, impeded perception and motor activity, blurry vision, slurred speech, drowsiness, and high doses may cause mortality. Sexual assault like rape cases predominantly occurs in both rural and urban areas. Nowadays literate and upper-class strata of the community promptly youngsters are indulged in such offenses attributable to grounds like moral values shortfall, transformed lifestyle, and more exposure to illicitly abused substances or alcohol. However, little scientific record of this fact is available but a notable trend can be ascertained from the instances reported in the media. One of the most important reasons for this scientific loophole is that sexual assault cases are reported comprehensively as rape offenses rather than in a more peculiar manner as DFSA. National control record bureau (NCRB) reported that for the year 2019, there were 7.9% rape cases, 17.9% kidnapping & abduction of women, 21.8% assault on women with intent to outrage her modesty, and 30.9% cruelty by husband or his relatives but no data on DFSA was accessible.<sup>3</sup> In addition to this lack of awareness in the police system about new chemical substances, the amnesic effect of drugs on victims, indistinguishable overlapping symptoms of drug and alcohol, deprivation of research or data collection in forensic science laboratories to determine the frequency of these cases adds fuel to worsen the condition. It was reported that the actual number of similar offenses may surpass the number of recognized cases since offenses are not reported.<sup>4</sup> Subsequently, crucial information like how and what kind (conventional/new form) of the drug was utilized, and its source cannot be extracted henceforth the true picture may not get

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depicted. It was mentioned in the literature that in India, 70% of drug-facilitated sexual assaults were embarked beneath the intoxication impact of illicit drugs or alcohol.<sup>5</sup> Forensic analysis of this case lead to the recovery of a novel plant alkaloid "Isopulegol" more scientifically a terpene conferred in the cannabis plant that was utilized to commit a sexual offense that rendered the victim in a resistless state. Its alternative source can be generated through synthetic manufacture from the precursor monoterpene *Citronellal* predominantly procured by the secondary metabolism of plants. Its transformation of citronellal into isopulegol involves acid-catalyzed cyclization followed by hydrogenation of isopulegol to procure menthol.<sup>6</sup> This transformation of isopulegol from *Citronellal* is an intermediate pathway required in menthol fabrication in various commercial industries. The cyclization reaction during isopulegol synthesis yields four stereo centers namely (-)-isopulegol, (+)-neoisopulegol, isopulegol, and neo-isopulegol.<sup>6,7</sup> At present times, it is well known for its distinctive properties like mint, pleasant odor, and instant cooling effect hence serving as an effective precursor substance for manufacturing menthol. Multiple benefits of isopulegol are reported in the literature including a potent source of anti-inflammatory and antioxidant properties,<sup>8</sup> capable to cure tumors,<sup>9</sup> facilitating pain-relieving actions (analgesic effects) and antihyperlipidemic,<sup>10</sup> and can also be employed in manufacturing compounds with anti-influenza and anti-viral properties.<sup>11,12</sup> Toxicological data gathered through experimental studies on mice discerned that isopulegol LD<sub>50</sub> administered orally for a rat is 1.03 ml  $\pm$  0.10 ml per kg with observed symptoms like ataxia, salivation, respiratory congestion, and central nervous system depression during initial hours after administration. In the same study effects on the human dermal surface using a variety of isopulegol-containing commodities were conducted but it doesn't provide any significant data on this drug lethality over the human population.<sup>13</sup>

**Case history:** A 17-year-old female patient was brought to casualty in an unconscious state by her father and the Doctor declared the patient dead. The Investigation Officer called the crime team for the inspection of the place of the incident or crime scene. On the spot, police recover a handwritten confession letter written by the victim in which she admitted that the two persons gave her some liquid by mixing something in it, then raped her. The postmortem of the deceased was conducted at GTB hospital and viscera exhibits/specimens of the deceased were sent to the Chemistry Division, Forensic Science Laboratory for the expert opinion.

## MATERIALS AND METHODS

**Materials:** Toxicological viscera samples containing the stomach, pieces of the small intestine, liver, kidney, and blood sample were received from the hospital after the postmortem examination of the deceased and were sent to the Chemistry Division, Forensic Science Laboratory for the examination. All the chemicals and reagents were used of analytical grade for the analysis.

**Digestion of viscera:** To recover the suspected drug analyte in a biological specimen, the prime step is to digest the viscera and extract the analyte using a suitable extraction technique. Here, in this work, the Liquid-liquid extraction method was employed for extraction purposes. Digestion of viscera was performed following the methodology given by Jaiswal *et al.*, (2017).<sup>14</sup> A content of 50 gm stomach was chopped into small pieces and macerated in 100 ml of a glass beaker. The macerated material was then treated with 10 ml of glacial acetic acid and 10 gm of anhydrous sodium sulphate. The mixture was then incubated in a water bath at 60°C for 3-4 hours. The content was then allowed to cool before being filtered with filter paper. The extracted filtrate was used to extract suspected drugs. The extraction was divided into two fractions: acidic and basic.

### Extraction of suspected analyte

**Acidic Extraction:** The procured filtrate and 50 ml of diethyl ether were added to the separating funnel and shaken vigorously for 12-15 minutes.

The organic layer or the ether layer was isolated and the same process was repeated two more times to recover the maximum amount of analyte. All three ether layers were combined and allowed to pass through a funnel of anhydrous sodium sulphate prior to being evaporated to dryness. The evaporated residue was used for TLC and GC-MS analysis.<sup>14</sup>

**Basic Extraction:** The aqueous acidic layer procured after acidic extraction was first made alkaline by the addition of ammonium hydroxide that shifted the pH towards 9-10. Afterward, the basic layer was extracted with 50 ml of ether:chloroform (3:1) mixture and then shaken for 10-15 minutes in a separating funnel. The first ether layer obtained after filtration was labeled as BE1. Further, extraction from the aqueous layer was performed twice. The ether layer after separation was labeled as BE2 and BE3. All three layers were passed together through anhydrous sodium sulphate using a funnel. Residue collected after evaporation was used for TLC and GC-MS instrumental analysis.<sup>14</sup>

**Neutral Extraction:** Accordingly, the aqueous basic layer procured above was made neutral by the addition of glacial acetic acid that shifted the pH toward 7. Then, it was extracted using 50 ml chloroform and shaken vigorously for 10-15 minutes. After this was done, the layer of chloroform was separated and labeled as NE1. Further, two times extraction was done repeatedly and organic layers were separated and labeled as NE2 and NE3. All three organic layers procured were added together and allowed to pass through anhydrous sodium sulphate. After evaporation, the dried residue was utilized for TLC and GC-MS analysis.<sup>14</sup>

**Preliminary analysis by Thin Layer Chromatography (TLC):** All the filtrate was employed for the screening purpose by using the TLC method. The protocol for the preliminary examination of the sample was followed by the method of Manual of Forensic Toxicology (2005).<sup>15</sup>

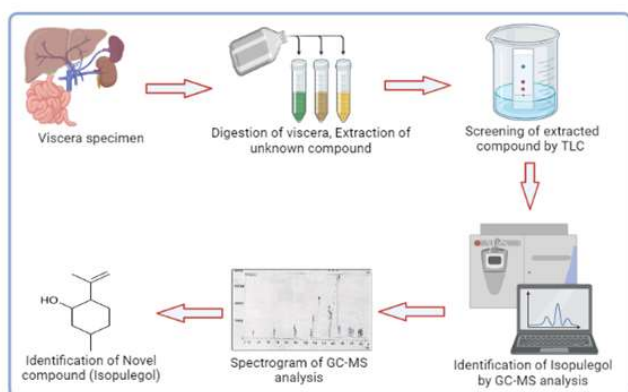
**Test for Acidic compound:** The acidic extract obtained is examined to detect the presence of barbiturates, benzodiazepams, salicylic acid, aspirin, lysergides etc. Ethylacetate: Hexane (18:6) was employed as a running solvent to elute the sample components. Ferric perchloric nitric (FPN) was used as a spraying reagent.

**Test for Basic compound:** The basic extract obtained is examined to detect the presence of mainly plant alkaloids. Two solvent systems namely chloroform: methanol (9:1) and methanol: ammonia (100:1.5) were employed as mobile phase to elute the sample components. Dragendorff's reagent was used as a spraying agent.

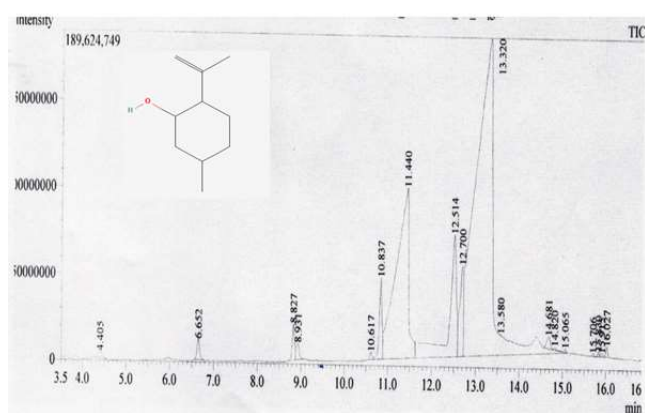
**GC-MS analysis:** The extracted sample produced positive results in early TLC testing or screening and was employed for the GC-MS analysis (Figure 1). Analyses were performed on GC-MS-QP2020NX (SHIMADZU), JAPAN coupled with a mass spectrometer (SHIMADZU Technologies) was used for compound analysis. A DV-5MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) was used to separate compounds.

High-purity helium was applied as the carrier gas. The following conditions were used: column flow rate: 1.0 mL/min; split injection, split ratio: 100:1; injection volume: 1  $\mu$ L; and injection port temperature: 280°C. The temperature procedure was as follows: 0–3 min, 150–150°C; 3–16 min, 150–280°C and 16–26 min, 280–280°C. The MS working conditions were as follows: the electron ionization energy was 70 eV, the full-scan acquisition was used in the range of 45–550 m/z, the ion source temperature was 230°C, the transmission ion temperature was 280°C, and the four-stage pole temperature was 150°C. The identification of each peak in the total ion flow chromatogram was automatically retrieved from the National Institute of Standards and Technology (NIST) MS search 2.4 as the standard mass spectrometry database and verified with standard mass

spectrometry. Some components were confirmed with the retention value of a standard sample.



**Fig. 1. Schematic diagram of extraction, screening, and identification of Isopulegol from post-mortem specimen**



**Fig. 2. GC-MS chromatogram and Isopulegol structure for the basic viscera specimen fraction Peak Report TIC**

## RESULTS AND DISCUSSION

The TLC for the suspected basic drug gave positive results in comparison to the working standard while TLC for the suspected acidic drug gave negative results in comparison to the working standard. The spectral quality of each chromatographic peak in the TIC's mass spectrum was examined. Each TIC peak's mass spectra were properly examined and searched using electronic commercial or approved in-house spectral libraries. Ten (10) compounds were identified in the analyzed viscera samples by using GC-MS analysis shown in Table 1.

**Table 1. Compounds detected in the basic fraction of viscera sample using GC-MS Technique.**

Peak#	Name	R. Time	Area
1	7-Tetradecene, (Z)-	4.405	22170080
2	Cyclotetradecane	6.652	48851782
3	Cetene	8.827	78520503
4	Tetradecanoic acid	8.931	46398635
5	Ethyl 9-hexadecenoate	10.617	15278265
6	Hexadecanoic acid, ethyl ester	10.837	200294218
7	n-Hexadecanoic acid	11.440	2005425758
8	9-Tetradecanol, (Z)-	12.514	868504206
9	1-Hexadecanol	12.700	314216713
10	Isopulegol	13.320	5231634921

The GC-MS examination of the exhibits in the Forensic Laboratory concluded that the exhibits results revealed the highest specific surface area for the compound Isopulegol at the retention time (TR) of

13.320 min (Figure 2). Identification of components was achieved based on their retention indices and interpretation of the mass spectrum was conducted using the database. Isopulegol is a monoterpene alcohol intermediary in the production of menthol that can be found in essential oils from a variety of plants. Silva *et al.* (2007) revealed that a single intraperitoneal dose of Isopulegol at 25 and 50 mg/kg had depressant- and anxiolytic-like effects in male mice.<sup>16</sup> A literature study revealed that contrary to widely abused drugs like GHB, ketamine, ethanol, or Rohypnol, a scarce amount of data is available on Isopulegol abuse potential and its pharmacological impact on the human body. As of today, this is the first case of sexual assault under the influence of the drug Isopulegol that was received in a Forensic Laboratory, Rohini, New Delhi. Forensic examination of viscera elucidated the presence of Isopulegol in visceral samples and it might be possible that it is capable to produce pharmacological effects where the intended victim failed to perceive the environment. Isopulegol may likely have an impact indistinguishable from other cannabis alkaloids and its mint-like properties, cooling effect makes the substance an effective choice to be used by perpetrators without getting identified. The perpetrator takes advantage of available literature on conventional drugs of abuse and its byproducts manufactured for commercial purposes that are readily available in the market. Analysis of such substances possesses challenges for the forensic analyst during case examination. In this context, deficiency of cognition of the offense by police about the fact that these substances produce symptoms analogous to alcohol intoxication may mislead the case, procrastination in reporting the case due to the amnesic impact of the substance on the victim, the high metabolic rate of substance that may infer toxicological finding with detrimental results, paucity of scientific research on unidentified substances and their metabolites with abuse capacity, the inexistence of optimized methodology for their examination may hamper the scientific inputs that is requisite for the justice system to make judgments. Unawareness about designer drugs and their presence in commercial consumable products can lead to their misuse because such compounds are not easily detectable in routine testing and also a series of new compounds can be manufactured with minute changes in the parent drug structure with intensified potency. Recognition of such setbacks and communicating them to the scientific community may generate awareness that is considerably required to counter such offenses by directing victorious research. Multidisciplinary research must be conducted including disciplines like chemistry, pharmacology, or toxicology, and knowledge can be utilized for forensic applications and to generate enough scientific data to scrutinize such chemicals and their synthetic drugs under regulation.

## CONCLUSION

This study highlights the importance of toxicological testing in identifying acute strychnine adverse effects. The compound was first identified in the sexually assaulted victim, it is quite evident from forensic examination of viscera that Isopulegol has abuse potential and its utilization as a flavoring agent for manufacturing commercial edible products can allure the perpetrator to misuse the substance. The substance abuse trend has shifted from conventional drugs to designer drugs and is a matter of concern as synthetic analogs can be prepared clandestinely in different forms with slight modifications in the parent drug structure. Researchers must conduct studies in this direction to illuminate the pharmacological impact of this alkaloid-based designer drug. India, being an Asian country confronts the downside in terms of case reporting because the female victim may feel shamed, lack strength and support from family, held responsible for defaming the reputation of the family in society therefore victims fail to report the case. The impact of social pressure and Asian shame is so immense that the victim is inclined and finds it easy going with committing suicide rather than facing the post-offense embarrassment and societal abandonment. To depict the true picture of DFSA offenses at the ground level, the criminal justice system must categorize the rape offense; as under the influence of a drug or without the use of such a tool, provision related to this offense must be enshrined exclusively,

training must be imparted to police personnel to identify the offense in either of the two mentioned categories and regulatory body must be enacted to maintain the record of such offense so that its trend (incline/decline in the number of case, type of novel substance misused) can be monitored. The major responsibility relies on the researchers of the scientific community, to explore or carry out studies to identify the substance of abuse potential and report the finding to the scientific community and thereafter to the public community preferably girls to generate awareness. Also, this prevalence of Asian shame must be diminished through interaction with the masses, induction of counseling, and awareness program.

**Conflicts of Interest:** The authors declare that they have no conflicts of interest.

**Ethical Approval:** As per Journal Policies, ethical approval was not required for this manuscript.

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