Original Article

Detection of Tetrahydrocannabinol in Commercial Consumables: A Survey-Based Study with Real Time Samples

Nupoor Gopal Neole[1], Anil Harishchandre[2]

[1]Research Scholar, Center for Nano and Material Science, JAIN (Deemed-to-be University)Jain Global Campus, Kanakapura, Ramanagaram, Bangalore, India. [2]Assistant Professor, Institute of Forensic Science, RashtrasantTukadoji Maharaj Nagpur University, Nagpur, India.

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Abstract

The study aimed to detect the presence of tetrahydrocannabinol (THC) in commercial consumables, specifically focusing on real-time samples. The study highlights the widespread use and cultivation of cannabis, with various compounds such as THC and Cannabidiol (CBD) present in the plant. THC is responsible for the psychoactive effects, while CBD is non-intoxicating and may have therapeutic benefits. The study also discusses the legal and social acceptance of cannabis across countries and regions. The study emphasizes the need for analysing street samples of pan-masala and other tobacco-containing orally consumable products for the presence of cannabis alkaloids, as they are often used as a concealment step for selling and purchasing cannabis. The experimental details include using chemicals and solvents from commercial sources, thin-layer chromatography (TLC) plates and an Ultraviolet-Visible spectrophotometer for detecting THC. The study used Dragendroff (DD) and Fast Blue B (FBB) reagents to identify THC. The THC quantities detected were 15.32 µM, 29.2 µM, 7.15 µM, and 5.3 µM respectively in the collected sample.

Keywords: Tetrahydrocannabinol, Cannabis, Cannabinoids, Dragendroff reagent, Fast blue B reagent, Oral consumable products.

Introduction

Cannabis plants produce many compounds of possible medical importance. Cannabis is among the very oldest of economic plants providing humans with fiber for spinning, weaving cloth, and making paper; seed for human foods and animal feeds; and aromatic resin containing compounds of recreational and medicinal value[1,2]. Human selection for varying uses and natural selection pressures imposed by diverse introduced climates have resulted in a wide variety of growth forms and chemical compositions[3,4] Innovative classical breeding techniques have been used to improve recreational drug forms of Cannabis, resulting in many cannabinloid-rich cultivars suitable for medical use. The biosynthesis of cannabinloid compounds is unique to Cannabis, and cultivars with specific chemical profiles are being developed for diverse industrial and pharmaceutical uses[5,6]. The active principle is not an alkaloid but a fat-soluble oleoresin, cannabinol. It is absorbed both from the digestive and respiratorytract.

Corresponding Author: Nupoor Gopal Neole. Research Scholar, Center for Nano and Material Science, JAIN (Deemed-to-be University), Jain Global Campus, Kanakapura, Ramanagaram, Bangalore, India.

E-mail: n.nupoor@jainuniversity.ac.in

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Cannabiscontains numerous chemical compounds called cannabinoids, with delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) being the most well-known[7,8]. THC is responsible for the psychoactive effects associated with cannabis use, while CBD is non-intoxicating and may have potential therapeutic benefits[9]. Cannabis has been utilized for medicinal, recreational, and spiritual purposes in different cultures worldwide. Its legality and social acceptance vary across countries and regions.

Commonly, there is tingling and numbness of the extremities or in sleep for about six hours after the consumption of cannabis in its different forms. Rarely, drowsiness may be followed by coma and collapse, and death may occur from respiratory paralysis[10–12]. The harmful effects of THC can include impaired memory and cognition, decreased coordination and motor skills, increased heart rate, anxiety and paranoia, respiratory issues, and potential addiction or dependency[13,14] the biogenetic precursor in the biosynthesis of the psychoactive Δ9-tetrahydrocannabinol (THC Prolonged or heavy use of THC can also impact mental health, leading to an increased risk of psychosis or exacerbation of existing mental health conditions[15,16]. It's important to note that the specific effects can vary depending on individual factors such as dosage, frequency of use, and personal susceptibility. Insanity in India is often attributed to the chronic use (addiction) of this drug in any form and is known as hashish insanity[17]. In some cases, delusions of grandeur or persecution develop. Following the continued use of cannabis or rarely after its consumption for the first time, he may run amok. The minimum lethal dose of chars is about 2g, of ganja is about 8g, and for bhang about 10g per kilo body weight. Death may occur in about 12 hours in acute poisoning[18–20] Cannabis products are by far the most abused drugs on the illicit drug market. Cannabis can be grown in virtually any country. Production of herbal cannabis (marihuana) is widely dispersed, existing in almost every country in the world[21]. Cannabis in different forms is under the umbrella of NDPS act, but from past 2 decades its being observed that their infusion with street tobacco products and pan masala has started as an concealment step for there selling and purchasing one of the infamous case is of "Bhola Manucca" that occurred in Mumbai, it has now become a serious consent to have a prominent overview on the street market of local orally consumable products and in last few years the ratio of drug addiction has increased by 24% in India and in Maharashtra by 8% due to this reasons the steer samples of "pan-masala" and other tobacco containing, orally consumable products are needed to be analyzed for the presence of cannabis alkaloids for the assisting the legal system.

Experimental details:

Chemicals and Instruments:

The experiments were conducted in aerobic condition, using chemicals and solvents from commercial sources without further purification or recrystallisation. The thin-layer chromatography (TLC) plates used are 0.25 mm Merck TLC silica gel plates.

To remove volatile solvents, an IKA rotary evaporator with a dry diaphragm pump (10-15 mm Hg) was utilized, and the process continued until a constant weight was achieved using an oil pump (<300 mTorr). The UV-VIS spectrophotometer used was the Labindia UV-3200 model, capable of scanning speeds ranging from 2-3000 nm/min. This instrument incorporated a tungsten and deuterium lamp, which allowed for detection within the wavelength range of 190-1100 nm using a photomultiplier detector.

Reagent Preparation:

Dragendroff (DD) reagent was prepared by adding glacial acetic acid to bismuth subnitrate and potassium iodide in distilled water[22]. Fast Blue B (FBB) reagent was prepared by mixing fast blue B salt in anhydrous sodium sulphate[22]. All the prepared reagents were stored separately in amber-coloured reagent air-tight bottles.

Sample collection and preparation:

Street samples of various chewing tobacco products were collected to test the hypothesis using random selection or chance sampling, wherein each item in the population had an equal chance of being included in the sample. The collected samples consisted of loose (handmade) and packed samples, totalling 39, with 16 belonging to the loose category and the remaining 23 being filled materials. The weight of the open samples ranged from 5 to 8 g, while the packed samples ranged from 2 to 8 g.

Figure 1: Collected steer samples of "pan-masala" and other tobacco containing, orally consumable products.

Sample preparation:

For each collected sample same procedure was carried out. A 5mg sample was weighed and dissolved in deionized distilled water. A small amount of petroleum ether was added to the solution, and the entire mixture was subjected to a sonicator bath at room temperature for 15-20 minutes. Following this, the mixture underwent filtration using Whatman filter paper no. 45. The resulting filtrate was then transferred to a separating funnel for liquid-liquid extraction. In this process, 5 ml of petroleum ether was added, and the funnel was shaken to ensure thorough mixing. After allowing the aqueous layer and petroleum layer to separate, the petroleum layer was carefully removed. The original aqueous layer was subsequently washed with petroleum ether, repeating this step 3-4 times. All of the collected petroleum layers were combined and concentrated using a Rotar-evaporator. The resulting concentrated extracted samples were then subjected to further examination specifically targeting THC.

Results and discussion

Colour test and TLC:

The 39 extracted samples were initially tested with DD reagent to assess the success of the extraction process. The DD reagent test is commonly used for detecting alkaloids, resulting in a bright yellow colour when any alkaloid is present.

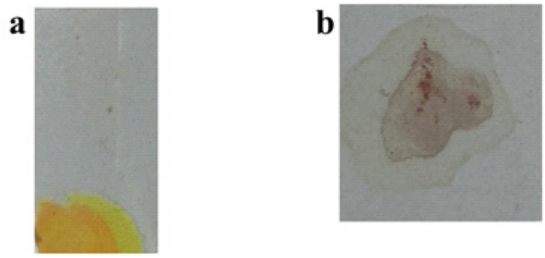

Figure 2: Positive colour test with DD reagent (a) and with FFB reagent (b).

Out of the 39 samples, 29 showed a positive result for the DD reagent test, indicating the presence of alkaloids. These 29 samples were then subjected to the FBB test, which targets explicitly THC. Among these samples, samples **5, 15, 27** and **33** displayed a purple-red colour during the FBB test, confirming THC's presence[23–25]. In both cases, false-positive and false-negative standards were used to verify the exact colour change.

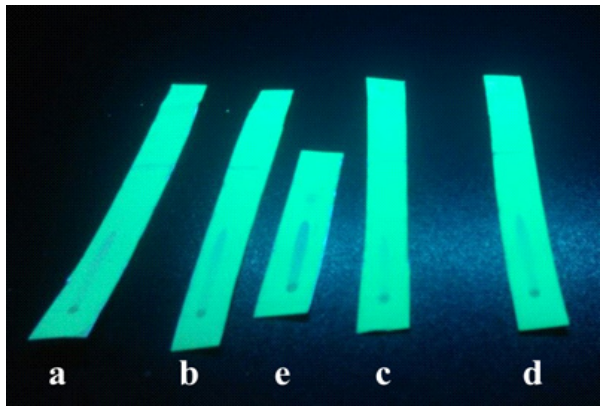

Figure 3: THC illustrating controlled THC sample (e), collected sample 5 (a), 15 (b), 27 (c) and 33 (d).

The samples that gave positive tests for both the Dargendroff reagent test and the Fast Blue B test were further tested by thin-layer chromatography (THC) and UV spectrometry, and the results were compared with the standard sample.

Table 1: TLC result for the collected samples 5, 15, 27 and 33.

Sample	hRf
Standard THC	37
05	36.66
15	36.54
27	37.11
33	37.02

Ultraviolet-Visible spectroscopic analysis:

A stock solution of 5 mM for the THC standard was prepared, and with serial dilution, the required concentration was prepared. The series of serially diluted concentrations of standard were analysed with the UV-VIS spectrometer[26,27]. The THC peak was observed around 278 nm[28]Δ9-tetrahydrocannabinol (or Δ9-THC. As the concentration increased, the peak height and area under the peck is also increased Figure 4a. Using this data, the calibration curve was plotted against a linear concentration range of 1-200 µM versus the area under the curve.

To further validate the presence of THC, UV-VIS spectroscopy was employed. This analytical technique provides a reliable means of identifying specific compounds based on their characteristic absorption patterns in the ultraviolet-visible range. The UV-vis spectroscopy confirmed the presence of THC in the previously identified samples **5, 15, 27** and **33.**

Using this calibration curve, Figure 4b, the THC concentration in the positive resulting samples **5, 15, 27** and **33** were calculated to be 15.32 µM, 29.2 µM,7.15 µM and 5.3 µM respectively.

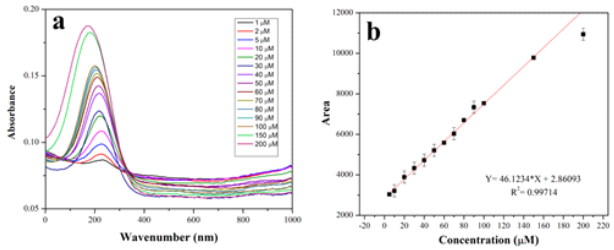

Figure 4: UV spectra (a) and calibration curve (b) for THC controlled sample in the concentration range of 1-200 µM.

Conclusion

The study describes the detection of THC in commercial consumables using real-time samples. Cannabis is a versatile plant with various uses and legality varies across countries. Cannabis products are widely abused drugs, and their infusion with street tobacco products and pan masala has become a concern. The study conducted experiments using chemicals and instruments and prepared reagents for detecting THC in analysing street samples of pan-masala and other tobacco-containing orally consumable products**5, 15, 27** and **33** were calculated to be 15.32 µM, 29.2 µM,7.15 µM and 5.3 µM respectively, with the help of calibration curve of standard THC sample with R^2 value 0.99714.

Ethical Clearance: Taken form dissertation committee, Institute of Forensic Science with informed consent from the vendors.

Conflict of Interest: Nil

Source of Funding: Self

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