JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

TODDY AS ILLICIT BEVERAGE IN FORENSIC ANALYSIS

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ABSTRACT:

The term "toddy" generally refers to an alcoholic beverage made by fermenting sap, which is often derived from various palm trees. This traditional drink is well-known in some areas for its distinct flavour and cultural significance. some unscrupulous producers may utilize chemicals to increase or speed up the fermentation process. Synthetic yeast, preservatives, and accelerators are common compounds used in adulteration for huge production. It is crucial to highlight, however, that the use of such compounds is unlawful and presents health concerns. In order to identify different compounds, this investigative study uses sophisticated chromatographic techniques such as GC-MS and HPLC to lay a foundation in the chemical composition of toddy. These chemical fingerprints are crucial indicators in forensic analyses and are essential in differentiating between legal and illicit sources. The study tackles the widespread problem of adulteration in the manufacture of illicit toddy, improving forensic techniques to identify typical manipulations and protect against health hazards. Toddy is a valuable tool in forensic investigations, particularly when it comes to cases of poisoning or illicit alcohol production. Toxic additives, like methanol, can be identified through sample analysis, and the chemical and microbiological composition of the samples aids in tracing the sources and production processes and provides vital evidence in the fight against the production and distribution of illicit alcohol.

Keywords: Toddy, illicit, Beverage, TLC, Analysis, Adulterants &HPLC

1. INTRODUCTION

In order to promote optimal health, food quality and safety are very important. However, tainted or contaminated food is a main cause of human disease and results insignificant nutritional loss (R. Nageswara Rao et.al., 2004).

Based on the wine's current stage of fermentation palm toddy, or fermented sap, has an alcohol concentration ranging from 3.3% to 4.0% and a pale colour. Its pH is approximately 3.6 (O. Lasekan & K.A. Abbas 2010).

A traditional alcoholic beverage known as toddy is created by fermenting sap, also known as exudate, which is obtained by cutting the tips of unopened coconut or palm tree flowers. Given that toddy is a naturally occurring beverage, we proposed that the native bacterial populations in palm saps generate a number of metabolites that could alter the fresh sap and transform it into a beverage with potential uses (Souvik Das & Jyothi Prakash Tamang 2023).

It's a locally made, sociable beverage that's popular throughout Asia, especially It is a regional, social beverage that originated in Asia and is mostly enjoyed in Bangladesh, Sri Lanka, and India. (P. Fellows, IT Publs, 1997; Traditional foods, processing for business).

Beer, domestic liquor, illicit liquor, and foreign liquor manufactured in India (IMFL). Indian-made foreign liquor is distilled liquor with a western flavour that is produced in India with official government licences. This category includes alcoholic beverages with a maximum alcohol level of 42.8%, such as brandy, gin, rum, vodka, whisky, etc. Distilled alcoholic beverages known as "country liquors" are produced with inexpensive raw materials such sugarcane, palm, rice, coconut, or coarse grains. Country spirits is made with permission from the government and sold only in that district (Tanu Priya & Amandeep Kaur 2019).

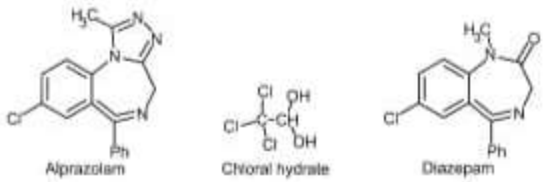
Due to an increase in consumer demand and a scarcity of palm or coconut trees, these products have frequently been adulterated and diluted with chemicals like benzodiazepines, diazepam, or chloral hydrate, which are dangerous when combined with alcohol and have been shown to cause fatal poisonings in forensic toxicological investigations. It has been found that female drinkers are more likely than men to abuse benzodiazepines (A.T. Pandhare et.al., 2015). A little girl was also reported to have alprazolam poisoning, which resulted in coma and respiratory depression. These psychoactive drugs have sedative properties and are illegally mixed into toddy to improve its potency (K. Fujiwara, Sber et.al., 1956).

Under excise rules, adulterant toddy is illegal for consumption, necessitating the development of acceptable analytical procedures for determining psychotropic chemicals in fermented food or alcoholic beverage (A.T. Pandhare et.al., 2015).

The common illicit substances include chloralhydrate, phenobarbitone, saccharin, and diazepam which could be used to induce addiction. (Bhagwat D. Mali et.al., 2005). Toddy contains a variety of adulterants, including chloral hydrate [CHL], alprazolam [ALP], and diazepam [DIA].

Numerous techniques, such as gas chromatography, thin-layer chromatography, capillary electrophoresis, enzymatic techniques, liquid chromatography, phthalocyanine-based voltametric screen-printed electrodes, and stochastic sensors, have been reported for the analysis of biogenic amines. (Lee, Yoo, and Shin 2015, Cioates Negut et al. 2020).

Because of its excellent sensitivity and selectivity, high-performance liquid chromatography with ultraviolet or fluorometric (PL) detection has become more and more prominent among analytical techniques. (Tahmouzi, Khaksar, and Ghasemlou 2011).



(R. Nageswara Rao et.al., 2004)

2. THE ADULTERANTS OF TODDY

2.1. CHLORAL HYDRATE

Chloralhydrate is a dangerous compound that is illegally blended with toddy. CHL has been designated as a priority chemical for further toxicological evaluation since it is genotoxic (M.S. Prasad et.al., 1976) and hepatocarcinogenic in male mice (M.M.D. Santos et.al., 2002) The presence of chloral hydrate is indicated by a strong red colour in the Fujiwara test, (A.T. Pandhare et.al., 2015) Moreover, chromatographic methods based on anion exchange and reversed-phase processes exist. to detect the presence of chloral hydrate (S. Haworth et.al., 1983).

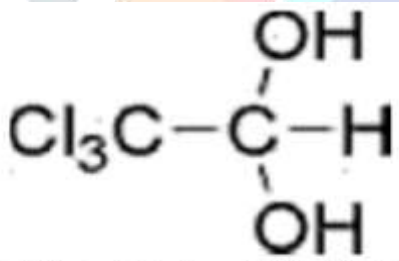


Fig.1 Chemical structure of Chloral hydrate.

(A.T. Pandhare et.al., 2015).

2.2. DIAZEPAM

Several allegations have surfaced of diazepam and alprazolam being illicitly put into toddy to improve its intoxication potential. While the media continues to report on rising violence, health issues, and deaths caused by the drinking of tainted toddy. It is said that a number of instrumental methods, such as spectrophotometry, HPLC, and polarography, are available for determining diazepam and other compounds such as phenobarbitone and saccharin in various matrices (Bhagwat D. Mali et.al., 2005).

2.3. ALPRAZOLAM

Alprazolam, along with diazepam, is a highly poisonous drug. This psychotropic chemical is illegally mixed into toddy to improve its potency. A case of alprazolam poisoning in a young child with coma and respiratory depression has also been recorded.

All of these psychoactive substances [CHL, DIA, ALP] can be detected utilizing various methods and procedures.

There are various methods for detecting adulterants. A review of the literature reveals that there are numerous experimental methods available, including thin layer chromatography, head space gas chromatography, reversed-phase HPLC, and so on. this article reviews various methods and suggests which method should be used for the greatest outcomes.

This emphasizes the need for robust forensic techniques to detect adulteration and ensure public safety. Few studies have specifically focused on toddy, making it an understudied area. Forensic analyses commonly involve examining alcohol content, microbial contaminants, and chemical additives. Comprehensive research is essential to establish reliable mdifferentiating between legal and illicit toddy, safeguarding public health and regulatory frameworks.

3. INSTRUMENTAL ANALYSIS OF TODDY SAMPLES CONSTITUTING ADULTERANTS

3.1. THIN LAYER CHROMATOGRAPHY

The introduction emphasizes the significance of ensuring the safety of toddy, which is prone to adulteration with medicinal products and additives. The common illicit substances include chloralhydrate, phenobarbitone, saccharin, and diazepam, which could be used to induce addiction. The literature review mentions several instrumental approaches for determining these chemicals, but emphasizes the limits that make them inappropriate for typical forensic work. As a result of its ease of use and speed, TLC is a popular approach for assessing many samples (Bhagwat D. Mali et.al., 2005).

The experimental section describes the chemicals and reagents employed, which include standard diazepam, phenobarbitone, saccharin, and chloral hydrate solutions. The TLC plates were covered with silica gel, and the plates were developed using three mobile phases. For the concurrent identification of diazepam, phenobarbitone, and saccharin, a novel chromogenic reagent employing chlorination followed by the application of o-tolidine was developed. The recovery trials showed that the TLC approach recovered almost 90% of the diazepam, phenobarbitone, and saccharin from toddy samples. According to the findings section, the compounds emerged as bright blue spots on the TLC plates following detection.

The mechanism of the reaction involves the chlorination of compounds with certain functional groups, which results in the synthesis of chloramines, which then oxidize o-tolidine to produce a blue quinonoid compound.

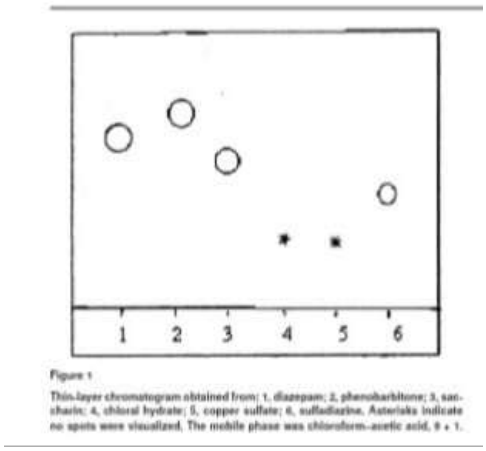


Figure 1

Thin-layer chromatogram obtained from: 1, diazepam; 2, phenobarbitone; 3, saccharin; 4, chloral hydrate; 5, copper sulfate; 6, sulfadiazine. Asterisks indicate no spots were visualized. The mobile phase was chloroform–acetic acid, 9 + 1.

(Bhagwat D. Mali et.al., 2005).

The research finds that the established TLC method, which employs a single sensitive chromogenic reagent, is appropriate for identifying diazepam, phenobarbitone, and saccharin in toddy samples. The approach is distinguished by its ease of use, speed, and specificity, making it ideal for regular forensic analysis of toddy to assure human well-being and regulatory compliance.

TLC Determination of Diazepam, Phenobarbitone, and Saccharin in Toddy Samples by (Bhagwat D. Mali et.al., 2004)

Research on the forensic examination of Teddy bears great importance, especially in light of the potential presence of pharmaceutical compounds such diazepam, alprazolam, and chloral hydrate. Although natural fermentation has always been linked to toddy, reports of adulteration using psychotropic chemicals have surfaced, raising grave concerns for public health.

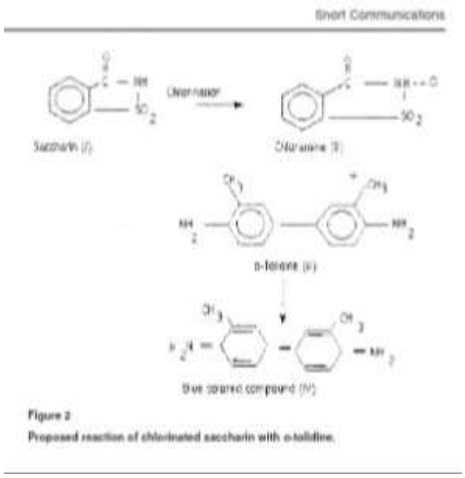
The importance of TLC as a potent analytical technique for identifying then measuring pharmaceuticals in intricate matrices like toddy is highlighted in the literature. The separation and identification of diazepam, alprazolam, and chloral hydrate in alcoholic beverages have been studied using TLC, which lays the groundwork for its use in toddy analysis.

The study's literature analysis highlights the limitations of existing experimental methods for determining diazepam, phenobarbitone, and saccharin in diverse matrices, such as spectrophotometry, HPLC, (J.B.F. Parry Loyd et.al., (1988) 179–181) (V. Shankar et.al., (1989) (M.L. Puttermans et.al., 1984) and polarography. (C.G. Kontoyannis et.al., 1999), (M. Yano et.al., 1992) .

These procedures are judged inadequate for typical forensic work requiring the study of numerous samples.

Because of its sensitivity and specificity, TLC can be used to test several substances at once. However, because of their distinct composition, toddy matrices provide difficulties when attempting to adapt TLC methods.

Additionally, a number of reagents for the detection of diazepam, phenobarbitone, and saccharin have been reported. Mercurous nitrate reagent is one such reagent.



(Bhagwat D. Mali et.al., 2005).

3.2. HEAD SPACE GAS CHROMATORAPHY

The main objective of the study is to advance the use of HSGC in a quick and accurate way to detect chloral hydrate in toddy samples. It is noted that the procedure is suitable for forensic analysis. The article Analytical reagents were employed. ELGA Pure Lab Altra deionised glass-distilled water, chloral hydrate. A PerkinElmer Clarus 500 Headspace Gas Chromatograph was used, which was outfitted with an Auto sampler, FID, and a Wax column. (A.T. Pandhare et.al., 2015).

Chromatographic Conditions:

1. Toddy samples are thermostated at 80°C in the Headspace, on the auto-sampler for 20 minutes.
2. The GC conditions used an Elite-Wax columns with an isothermal column temperature of 50°C kept for 9 minutes.
3. Carrier gas: 50 kg/cm2 nitrogen, FID temperature 230°C.
4. For data collecting and processing, Complete Chrom software was utilized.

Toddy specimens were filtered, and 1 cc of this supernatant is thermostated at 80°C for a period of twenty minutes within the auto-sampler. The chromatograph was filled with sample vapour. Under the same settings, conventional chloral hydrate sample were tested Rotation timings were compared to standards to determine the amount of chloral hydrate, and peak areas were used to estimate its quantity. After ethyl alcohol was eliminated at 4.31 minutes and chloral hydrate was eluted at 7.31 minutes, the chromatogram indicates that the ideal conditions for separation were met. (A.T. Pandhare et.al., 2015).

Calibration Curve: A curve was plotted after injecting various amounts of chloral hydrate in ethanol. The chemical eluted at 50°C, which was deemed appropriate for the investigation.

Accuracy and Precision: The method's accuracy was evaluated using the usual addition procedure, with reliability reflected in peak areas. Five repeat assays of toddy contained chloral hydrate were used to assess precision.

The SGC method is reliable and convenient for forensic analysis, proving to be an important instrument for detecting adulteration in traditional alcoholic beverages.

under the framework of this initiative. Chloral hydrate was determined with head space gas chromatography equipped with a flame ionization detector. The literature also included reports of liquid chromatographic techniques based on union exchange and reverse phase mechanisms. (S. Haworth et.al., 1983)

The given study tackles a significant issue of toddy adulteration, a traditional alcoholic beverage brewed from the sap of coconut or palm trees. The adulteration of the toddy with chloral hydrate, a non-barbiturate and benzodiazepine chemical, has aroused concerns due to its potential risks, particularly when combined with alcohol. According to the report, the inclusion of chloral hydrate is frequently meant to improve the strength of toddy, creating major health hazards and, in rare cases, lethal poisoning.

The work emphasizes the importance of developing effective analytical methods for detecting and quantifying chlorallhydrate in brewed fermented beverages such as toddy, especially given the potential for broad exposure to this dangerous toxin**.**

The literature review offers an overview of prior approaches for detecting chloral hydrate in toddy. The Fujiwara test, which produces a strong red hue is an indication for a base, is widely utilized to diagnose chloralhydrate. However, the study highlights the limits of present approaches. (A.T. Pandhare et.al., 2015).

These assays, which employ UV and reduced conductivity detectors, may yield colours that are similar for numerous organic substances containing halogens, lowering their selectivity. The paper then presents the application of headspace gas chromatography (HSGC) using a flame ionization detector for chloral hydrate determination, presenting it as a simple, quick, and reliable approach for both qualitative and quantitative measurement for chlorallhydrate contamination in toddy.

For the examination of volatile substances, HS-GC is a good choice. However, for the successful identification of less volatile or non-volatile chemicals, liquid chromatography or other methods may be needed. The type of substances and the particular analytical needs determine which technique is best. The use of HS-GC to identify and quantify volatile chemicals in alcoholic beverages, including toddy, has been demonstrated in the literature.

Headspace Gas Chromatography (HS-GC) emerges as a valuable tool in this context, offering a non-invasive method for identifying and quantifying volatile compounds.

Research in the literature underscores the successful application of HS-GC for detecting chloral hydrate in various matrices, including alcoholic beverages. This technique allows the separation and analysis of volatile compounds without direct sample injection, minimizing potential matrix interferences.

Studies have shown that chloral hydrate, being a relatively volatile compound, is amenable to HS-GC analysis. The method provides sensitivity and specificity, enabling forensic investigators to distinguish between adulterated and non-adulterated samples effectively.

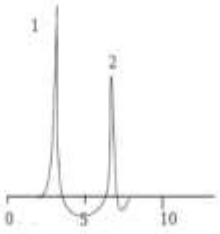


Fig-2

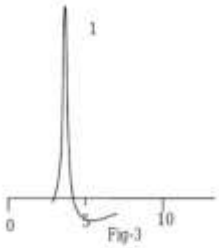


Fig-3

Fig.2 Typical HS-GC chromatogram of Toddy, with adulteration of chloral hydrate (1- ethanol & 2 -chloral hydrate).

Fig.3 Typical HS-GC chromatogram of Toddy, without adulteration of chloral hydrate (1- ethanol).

(A.T. Pandhare et.al., 2015).

However, limitations exist in the application of HS-GC to toddy analysis

3.3. REVERSED PHASE HIGH PERFORFANCE LIQUID CHROMATOGRAPHY

The paper RP-HPLC chromatography was used to separate and determine the presence of alprazolam, chloralhydrate, and diazepam in traditional alcoholic beverages in order to detect adulteration(. RAO, R. Nageswara et.al., 2004). focuses on using Reversed-Phase High-Performance Liquid.

The study's major goal is to develop and deploy an RP-HPLC technique for identifying and isolating certain adulterants from traditional alcoholic beverages, such as alprazolam, chloralhydrate, and diazepam (. RAO, R. Nageswara et.al., 2004).

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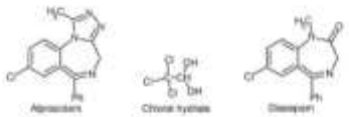

Fig. 1 Chemical structures of the adulterants studied in the present investigation.

(R.Nageswara Rao et.al., 2004)

The authors discuss their findings from a study that used RP-HPLC to detect particular contaminants in traditional alcoholic beverages. The study stresses the method's efficiency in isolating and identifying Alprazolam, Chloralhydrate, and Diazepam. The conclusion emphasizes how well the procedure works for identifying adulteration in these beverages.

Utilizing reversed-phase high performance liquid chromatography, the adulteration of conventional alcoholic beverages can be identified by separating and measuring alprazolam, chloral hydrate, and diazepam. R. Nageswara Rao et.al., 2004).

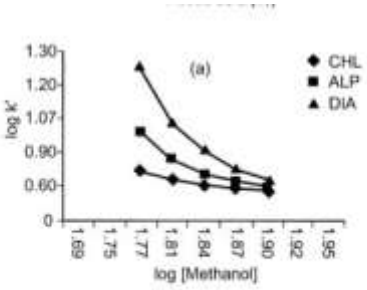

Fig. 2 Effect of concentration of (a) methanol and (b) acetic acid on the retention of CHL, ALP and DIA.

(R. Nageswara Rao et.al., 2004)

RP-HPLC is the method used in this study to identify adulteration in traditional alcoholic beverages by separating and identifying drugs such as diazepam, chloral hydrate, and alprazolam. The review of the literature in the article likely offers the context on the prevalent problem of alteration in conventional beverages containing alcohol, highlighting the necessity for robust analytical methods to identify and quantify specificity.

The review may highlight past approaches used to detect alprazolam, chloral hydrate, and diazepam in similar matrices. Techniques like RP-HPLC have most likely been investigated in the literature for their usefulness in separating and measuring these medications. The limits of present approaches, as well as the requirement for preciseness, sensitivity, and accuracy in identifying these illicit substances in traditional alcoholic beverages, are likely to be stressed.

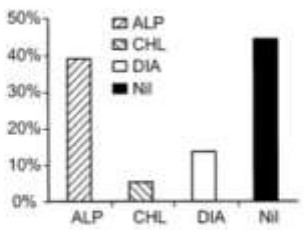

Fig. 5 Bar diagram depicting the percentage of adulteration of toddy with different substances.

(R. Nageswara Rao et.al., 2004)

RP-HPLC has been successfully used in the separation and quantification of pharmaceutical compounds such as alprazolam, chloral hydrate, and diazepam in a variety of matrices. This approach has a high sensitivity and specificity, allowing for precise identification and quantification of these chemicals in complicated mixtures such as toddy.

Potential interference from toddy components, as well as the requirement for method tuning to improve separation efficiency, may be challenges in RP-HPLC analysis for toddy matrices.

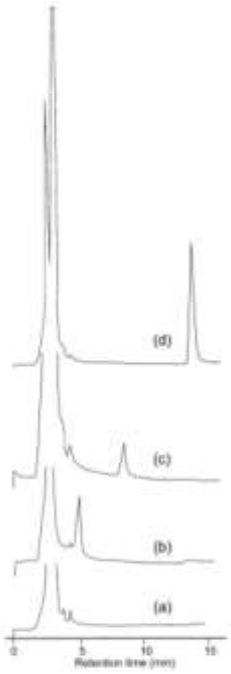

Fig. 4 Typical HPLC chromatograms of toddy, (a) without adulteration and containing, (b) CHL, (c) ALP and (d) DIA.

(R. Nageswara Rao et.al., 2004)

4. ADVANTAGES

1. Versatility: RP-HPLC is suited for a wide range of chemicals, including polar and non-polar analytes. Its ability to handle a broad range of samples makes it applicable to numerous industries, including medicines, environmental analysis, and food testing.

2.High Sensitivity: It has a high sensitivity, allowing it to detect trace levels of analytes, which is extremely useful in applications where low concentrations of target substances must be evaluated.

3.Resolution: RP-HPLC has exceptional resolution, allowing the partition of complicated mixtures into separate components. This is critical for precise documentation of chemicals in a sample.

4.Speed and Efficiency: The approach enables speedy analysis, which contributes to high laboratory throughput. The use of shorter columns and smaller particle sizes improves separation efficiency.

5.Ease of Use: RP-HPLC is a reasonably simple procedure that requires little sample preparation. The method is well-established, and there is a large range of stationary and mobile phases accessible, allowing for method development flexibility

6.Applicability of Non -Volatile compounds: Contrasting gas chromatography, RP-HPLC can analyse non-volatile and thermally unstable chemicals, broadening its use to a broader spectrum of substances.

These benefits contribute to the popularity and effectiveness of RP-HPLC in analytical laboratories across a wide range of scientific disciplines

5. CONCLUSION:

In conclusion, this highlights the significance of employing various analytical methods for detecting adulteration in toddy, a traditional beverage. TLC emerges as a potent technique for identifying pharmaceutical compounds like diazepam, alprazolam, and chloral hydrate. However, adapting TLC to toddy matrices presents challenges due to their distinct composition.

Headspace Gas Chromatography (HS-GC) is effective for volatile compound analysis, demonstrated in the finding of chloral hydrate in fermented beverages. Despite its success, limitations exist, necessitating consideration of alternative techniques for non-volatile mixtures.

A primary focus is on (RP-HPLC), particularly for the identification of diazepam, chloral hydrate, and alprazolam. Although RP-HPLC has a high degree of specificity and sensitivity, it has drawbacks such as the requirement for method optimization and possible interference with toddy components.

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