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Determination of Alcohol in Botanical Extracts Using a Simple and Cost-Effective Oven-Based Assay

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ABSTRACT

A simple assay for the estimation of ethanol concentration is essential during the extraction of bioactive compounds in botanical research. This study presents an assay developed to measure ethanol levels in different extracted samples. Utilizing the boiling point properties of ethanol in conjunction with a hot air oven, the method is capable of determining a minimum standard alcohol content of 2% by volume, with a precision of ± 0.5%. This technique proves particularly advantageous for identifying ethanol that may have been inadvertently collected alongside the target compounds during the extraction process. Furthermore, this assay has been demonstrated to be a cost-effective, simple and accurate means of determining ethanol concentration in unknown solutions.

Keywords: Bioactive Compounds, Boiling Point, Ethanol, Plant Extract, Simple Method.

INTRODUCTION

Plant-derived bioactive compounds have attracted substantial interest in recent decades due to their promising applications in pharmaceuticals, nutraceuticals, and cosmetics (Sasidharan *et al,* 2011; Kinghorn and Falk, 2002; Harborne, 1998; Dai and Mumper, 2010; Rostagno *et al,* 2003). The extraction of these compounds typically involves the use of solvents, with ethanol being a widely preferred option due to its low toxicity, environmental compatibility, and efficiency in extracting a broad range of polar and semi-polar compounds (Harris, 2015). However, residual ethanol in the final extract can present several challenges. Accurate quantification of ethanol content is essential for ensuring the safety and consistency of plant extracts across different applications, monitoring the efficiency of extraction and purification processes, and complying with regulatory limits on residual solvents in food and pharmaceutical products. Moreover, the presence of ethanol may interfere with bioactivity assays, making its precise measurement even more critical. Ethanol's effectiveness as a solvent is largely attributed to its capacity to dissolve a wide spectrum of phytochemicals (Dai and Mumper, 2010). Additionally, the concentration of ethanol used during extraction can significantly affect the yield and composition of the extract (Rostagno *et al.* 2003), further emphasizing the need for accurate determination of any residual ethanol. Several factors can complicate the accurate determination of ethanol in plant extracts, including: The complex matrix of plant extracts, which may contain interfering compounds (Dai and Mumper, 2010; Rostagno *et al,* 2003). The volatility of ethanol, which can lead to losses during sample preparation (Dean,1998; Miller and Miller, 2018). The wide range of ethanol concentrations that may be encountered.

Various analytical techniques are employed to quantify ethanol in plant extracts, including: Gas Chromatography (GC) (Caputi *et al,* 1968; Williams and Rosser, 1981; Holmberg and Sievänen, 1980; Skoog *et al*, 2007). This is a widely used method due to its high sensitivity and selectivity. GC allows for the separation and quantification of ethanol from other volatile compounds in the extract. High-Performance Liquid Chromatography (HPLC) (Skoog *et al*, 2007; Harris, 2015). HPLC offers an alternative method, particularly for non-volatile or thermally labile compounds. This method is also very useful for the determination of many of the bioactive compounds themselves, therefore can be used in conjunction with the bioactive compound analysis. Enzymatic Methods (Marazuela, 1996). These methods utilize enzymes that specifically react with ethanol, providing a rapid and accurate determination. Spectroscopic Methods (Vidal *et al,* 2003). Methods like Near-infrared spectroscopy can be used for rapid determination of ethanol. This

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research article aimed to provide an overview of the importance and methodologies employed for the determination of ethanol in bioactive compound extracts derived from plant samples.

MATERIALS AND METHODS

This study developed a simple assay for the estimation of ethanol concentration in botanical extracts, leveraging the boiling point properties of ethanol (Lide, 2004).

Sample preparation

Standard ethanol solutions at concentrations of 2%, 4%, 6%, 8%, and 10% (v/v) were prepared by diluting absolute ethanol (99.9% purity) with sunflower oil, following established methods (Dean, 1998; Miller and Miller, 2018). Botanical extract samples, potentially containing residual ethanol, were collected for analysis. All samples were stored in tightly sealed vials at room temperature (25 ± 2°C) until further testing.

Assay procedure

Accurately measured 1 ml aliquots of each standard ethanol solution and unknown sample were transferred into pre-weighed, heat-resistant glass vials (Harris, 2015; Skoog *et al*, 2013; Dean, 1998). The initial weight of each vial containing the sample was recorded. The vials were then placed in a preheated hot air oven, maintained at 80°C. This temperature was selected to ensure the evaporation of ethanol (boiling point ~78°C) (Lide, 2004). The vials were incubated in the oven for 24 hrs. This duration was empirically determined to ensure complete evaporation of ethanol from solutions with concentrations up to 100% w/v. Following incubation, the vials were removed from the oven and allowed to cool to room temperature. The final weight of each vial was recorded. The difference between the initial and final weights was calculated, representing the weight of evaporated ethanol.

Calculation of alcohol concentration

The percentage of Alcohol (w/v) in each sample was calculated using the following formula: Alcohol Concentration (w/v %) = (Weight of Evaporated Alcohol / Initial Sample Weight of Alcohol with Oil) x 100

Validation and accuracy assessment

The accuracy of the assay was assessed by comparing the measured alcohol concentrations of the standard solutions with their known concentrations (Harris, 2015; Skoog, 2013; Miller and Miller, 2018). The precision of the assay was evaluated by performing replicate measurements (n=3) of each standard and unknown sample (Dean, 1998). The minimum detectable ethanol concentration was determined by analyzing low-concentration standard solutions.

Statistical analysis

The mean and standard deviation of replicate measurements were calculated (Dean, 1998). Linear regression analysis was performed to assess the correlation between known and measured ethanol concentrations (Dean, 1998).

RESULTS AND DISCUSSION

The developed assay demonstrated its ability to accurately estimate ethanol concentrations in both standard solutions and botanical extracts. The weight loss observed after heating the samples in the hot air oven correlated directly with the initial ethanol concentration. The measured ethanol concentrations of the standard solutions closely matched their known concentrations. Linear regression analysis revealed a strong positive correlation ($R^2 > 0.99$) between the known and measured values, indicating high accuracy (Table 1 and Fig 1). Replicate measurements of all samples exhibited a precision of ± 0.5% v/v, confirming the reliability and reproducibility of the assay. The assay successfully detected ethanol concentrations as low as 2% v/v, demonstrating its sensitivity for low-level ethanol determination. The assay effectively quantified residual ethanol in various botanical extracts, highlighting its applicability in real-world scenarios.

The utilization of the boiling point differential between ethanol and sunflower oil, facilitated by a hot air oven, provided a simple and effective method for ethanol quantification (Lide, 2004). The 80°C temperature was crucial, ensuring the evaporation of ethanol while minimizing oil loss, thereby maintaining the accuracy of the assay. The assay is simple and required only 24 hours of heating, enabling simple determination of ethanol concentration. The method utilized readily available laboratory equipment and did not require expensive reagents or specialized instrumentation (Harris, 2015; Skoog *et al*, 2013; Smith, 2003). The procedure was straightforward, requiring minimal technical expertise. The assay demonstrated high accuracy and precision, making it a reliable tool for ethanol quantification (Miller and Miller, 2018). Due to the simple nature of the

Table 1: Standard Calibration Data

Sr. No.	Percent of standard alcohol solution (w/v %)	Loss of alcohol (w/v %)
1	0	0
2	2	0.092
3	4	0.255
4	6	0.402
5	8	0.524
6	10	0.649

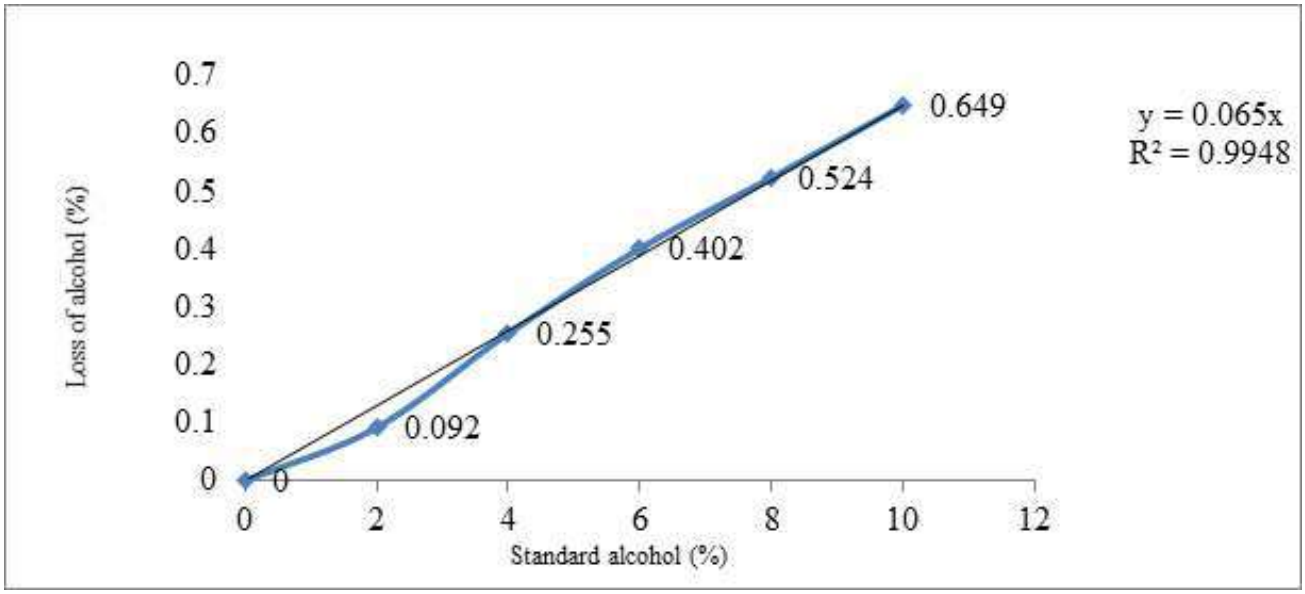

Fig 1: Calibration graph of spiked alcohol

equipment; this method could be very useful. Limitations of this method is the method relies on the assumption that ethanol is the primary volatile component lost during heating. The presence of other volatile compounds with similar boiling points could potentially interfere with the assay. The method is limited to the determination of ethanol in liquid samples. While the method can detect 2% standard ethanol, very low residual amounts of ethanol can be estimated using standard curve. This simple assay offers a valuable tool for researchers and practitioners involved in the extraction and purification of bioactive compounds from plant materials (Holmberg and Sievänen, 1980; Skoog, 2007; Sasidharan *et al,* 2011; Kinghorn and Falk, 2002; Harborne, 1998). It enables efficient monitoring of residual ethanol levels, ensuring product quality and compliance with regulatory standards. The method can be readily implemented in laboratories with limited resources, facilitating ethanol quantification in diverse settings.

CONCLUSION

The developed assay provides a cost-effective, simple, and accurate method for the determination of ethanol concentration in botanical extracts. Its simplicity and reliability make it a valuable tool for quality control and process optimization in botanical research and related industries. Additionally, the method's ability to detect ethanol as low as 2% with ± 0.5% precision ensures dependable monitoring during extraction processes. Its adaptability to various extract types and ease of implementation make it particularly useful for both academic and industrial laboratories involved in phytochemical studies.

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