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Analysis of Caffeine in Kola Nut Extracts by High-Performance Liquid Chromatography

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ABSTRACT

Kola nut is a perennial tree of the Sterculiaceae family, native to Africa's rainforests. Their caffeine-rich seeds are used as a flavoring in soft drinks. Caffeine belongs to the Xanthines family. It is found in numerous beverages, including coffee and tea. This study used High-Performance Liquid Chromatography (HPLC-DAD) to compare methanol and ethanol extracts, estimate the concentration of caffeine extracted at the same conditions and compare it to standard caffeine. Results show that the concentration of caffeine extracted in 10 microliters of methanol is 393.04 ppm, while its concentration in the same amount of ethanol is 331.16 ppm. The concentration of caffeine in methanol is higher than its concentration in ethanol. Thus, the use of methanol to extract caffeine from kola nut seeds is better than ethanol. We recommend further studies for the determination of other compounds found in kola nut seeds.

Keywords: Kola nut; Caffeine; High-performance liquid chromatography

INTRODUCTION

Natural products are diverse compounds with various biological activities and potential applications [1]. The extraction of natural products involves the separation of desired compounds from their natural sources using various methods [2]. Solvent extraction is the most common method for extracting natural products. It involves using a solvent, such as ethanol or methanol, to dissolve the desired compounds from the source material. The solvent is then evaporated to obtain the extract [3].

Kola nut is an important seed tree that belongs to *Sterculiaceae* familly. It is commonly grown in India, Brazil and Africa. The literature review indicated the efficacy of kola nut seed in producing stimulants, wines and soft drink (Coca-Cola) [4]. Medically, kola nuts were found to have a markedly stimulating effect. It is used in nervous debility, states of weakness and depression because of its caffeine content [5].

Caffeine belongs to the Xanthines family. It is the oldest natural component, known as a stimulant, found in coffee, tea and kola nuts [5, 6]. High Performance Liquid Chromatography (HPLC) is a sophisticated analytical technique widely employed to identify and quantify different chemicals in complicated mixtures [7].

The extraction of caffeine from kola nut seeds and its analysis by HPLC was conducted in several studies. The validation and development of a sensitive, simple and economical HPLC method for quantifying caffeine in different beverages were described by Alkhamaisah, et al. [8]. The moisture, ash, crude protein and caffeine contents of different varieties of kola nuts were determined using conventional methods by Olaoye [9]. Yalwa and Bello investigated the moisture, fat and caffeine contents of different kola nuts and found variations based on freshness and growing soil [10].

This study aimed to identify and quantify caffeine found in kola nut seeds' methanol and ethanol extracts using a High Performance Liquid Chromatography Diode Array Detector (HPLC-DAD).

MATERIALS AND METHODS

Plant material and Chemicals

Metronidazole and Miconazole nitrate gel formulation dosage form and drug substance were procured from Encube Ethical Pvt. Ltd., Isopropyl Alcohol (IPA, 99.9%), Sodium Hydroxide (NaOH, 99.9%), Hydrochloric Acid (HCl, 36.5%) and Hydrogen Peroxide (H_2O_2, 30%) had been purchased from Merck India Ltd.

Preparing methanolic and ethanolic extracts

After crushing two amounts of 0.2505 g of kola nuts, one was dissolved in ten milliliters of methanol and the other in ten milliliters of ethanol for 72 hours and shaken at 40°C. The extracts were filtered and then directly injected into the HPLC-DAD.
The methanolic and ethanolic extracts were concentrated using a rotary evaporator and weighed to calculate the extraction yield percentage. The extraction yield percentage (% E.Y) was calculated as follows:

$$\%E.Y = \frac{weight\ of\ exract\ yield}{initial\ weight\ of\ sample} \times 100 \qquad (1)$$

Preparation of standard solutions

A suitable amount of caffeine was weighed and then dissolved in water. The stock solution contained caffeine at 100 ppm. Standard solutions of 1, 3, 5, 7, 9 and 10 ppm were prepared by diluting the stock solution. After being filtered, the standard solutions were injected into the HPLC-DAD directly.

Conditions of HPLC chromatographic

A Shimadzu system was used for the HPLC analysis. The mobile phase's flow rate was maintained at 0.8 mL/min. The separation was carried out on a column (SUPELCOTM LC-18, 250 mm × 4.6 mm). A Diode Array Detector (DAD) was equipped with a deuterium lamp at a wavelength of 254 nm. The mobile phase consisted of (0.1%) titrafluoroacitic acid in water in HPLC-grade as solvent A and acetonitrile as solvent B.

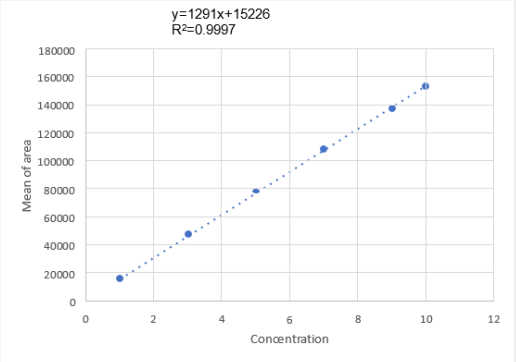

Figure 1: The calibration curve of caffeine.

RESULTS AND DISCUSSION

The area of the peak values in six solutions of the caffeine standard was measured. Each standard solution was injected in triplicate. The standard linear calibration curve obtained is shown in Figure [1]. A linear relationship between the peak's area and concentration of standard solutions (R^2=0.9997) shows that the regression line approximates the actual data points. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated according to the formulas:

$$LOQ = 10\ \sigma/a \qquad (2)$$

$$LOD = 3.3\ \sigma/a \qquad (3)$$

Where σ is the blank's standard deviation and a is the calibration curve's slope. The values of LOQ and LOD were equal to 0.683 and 0.225 ppm, respectively.
To compare the concentration of caffeine extracted using two polar solvents (methanol and ethanol) from two samples with the same weight and extraction time (72 hours), ten microliters of the solution were injected into the HPLC-DAD. The caffeine concentrations in methanol and ethanol solutions were 394.26 and 332.19 ppm, respectively. Table 1 shows the results of caffeine content from methanol and ethanol extracts. The results show that methanol is better than ethanol for caffeine extraction. Figure 2 shows the chromatograms of methanol, ethanol extracts and caffeine standard.

Table 1: The HPLC's results of caffeine content in methanolic and ethanolic extracts.

Readings	EtOH's extract			MeOH's extract		
	Retention time (min)	Area of the peak	Concentration (ppm)	Retention time (min)	Area of the peak	Concentration (ppm)
1	10.786	5193462	341.01	10.804	6041872	396.72
2	10.776	5003798	328.55	10.812	6034406	396.24
3	10.775	4980290	327	10.808	5936716	389.82
Mean	10.779	5059183	332.19	10.808	6004331	394.26
Standard deviation	0.0061	116881.3	7.676	0.004	58675.46	3.85

After evaporating the solvents, it was found that the extraction yield percentage of the methanol extract was 39.92%, whereas the extraction yield percentage of the ethanol extract was 23.95%. Thus, the percentage of the extract produced by utilizing methanol as a solvent is greater than that produced using ethanol (Figure 2).

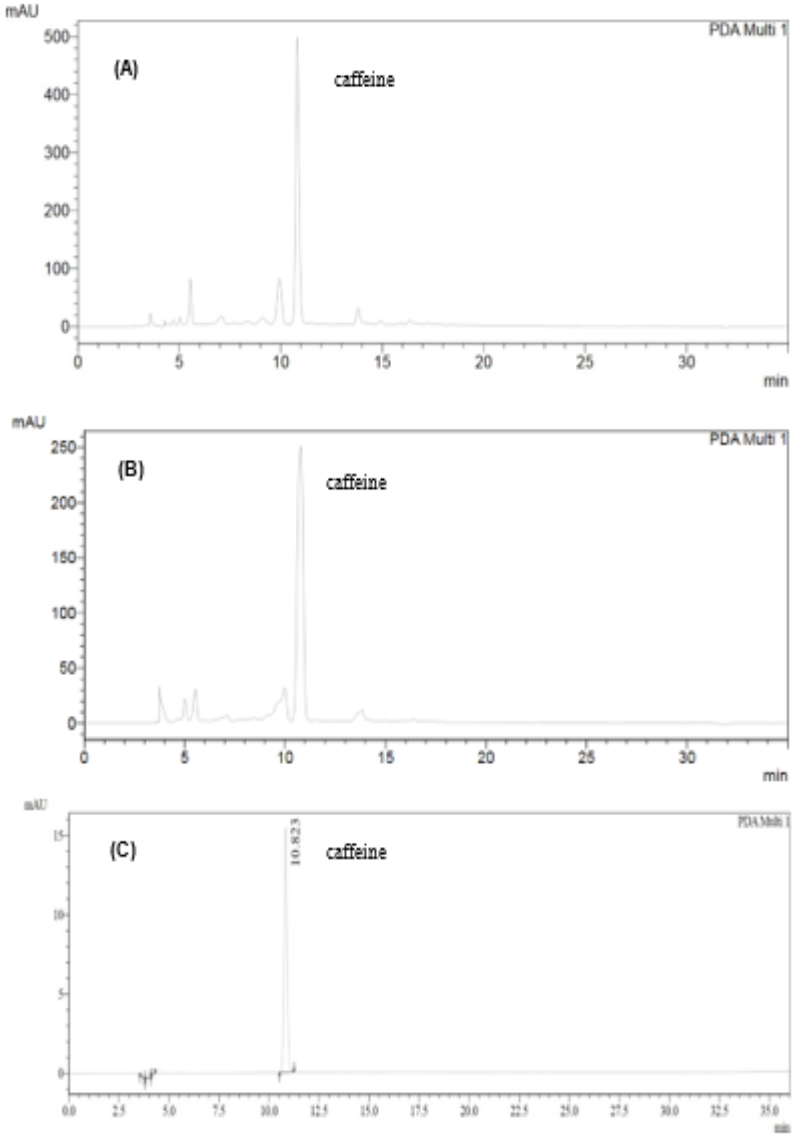

Figure 2: The chromatograms of methanol. Note: A: Ethanol; B: extracts; C: Caffeine standard.

CONCLUSION

Choosing the appropriate solvent is the first step in the solvent extraction process. In our study, we compared the use of ethanol and methanol to extract caffeine from kola nut seeds by the HPLC-DAD. Results show that the caffeine extracted in methanol is better than its concentration in the same amount of ethanol. Ultimately, we recommend further studies of other ingredients in kola nut seeds. We also recommend using green solvents in the extraction process.

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