



EXTRACTION, CHARACTERIZATION AND COMPARATIVE ANTIOXIDANT ACTIVITY OF CAFFEINE FROM KOLANUT, TEA LEAVES, AND COFFEE BEANS

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ABSTRACT: Caffeine is a naturally occurring stimulant that affects the central nervous system. It helps increase alertness, reduce fatigue, and improve concentration by blocking the action of a brain chemical called adenosine, which normally promotes sleep and relaxation. That means, it is a drug that stimulate (increases the activity of) your brain and nervous system. This study investigates the caffeine content and antioxidant properties of kolanut (*Cola nitida*), bitter kola (*Garcinia kola*), garlic (*Allium sativum*), and ginger (*Zingiber officinale*) using High-Performance Liquid Chromatography (HPLC) and free radical scavenging assays. The samples were collected, processed, and analyzed for moisture content, caffeine concentration, and antioxidant activity using the DPPH and FRAP assays. HPLC results confirmed the presence of significant caffeine levels in kolanut and bitter kola, whereas garlic and ginger contained negligible amounts. The antioxidant activity varied among the samples, with garlic exhibiting the highest free radical scavenging ability, followed by ginger, kolanut, and bitter kola. These findings provide scientific validation for the traditional use of these plants, emphasizing their potential applications in food, pharmaceutical, and nutraceutical industries. Further studies are recommended to explore additional bioactive compounds and optimize extraction methods.

Keywords: Bitter Kola, DPPH, FRAP, Garlic, HPLC, Kolanut

1.0 INTRODUCTION

Caffeine is a natural stimulant belonging to the methylxanthine class, found in coffee, tea, kola nuts and certain plants, known for its ability to enhance alertness, reduce fatigue and stimulate the central nervous system. Natural products have long been a cornerstone of medicine, nutrition, and cosmetics due to their bioactive compounds. In recent years, attention has turned to the antioxidant properties of various plant-based sources as potential remedies for oxidative stress-related diseases. Antioxidants play a critical role in neutralizing free radicals, which are implicated in conditions like cancer, cardiovascular diseases, and aging (Lobo *et al.*, 2015). Kolanut, bitter kola, garlic, and ginger are widely consumed and recognized in traditional medicine for their health benefits, yet their antioxidant potential deserves further exploration and characterization (Lobo *et al.*, 2015).

Kolanut (*Cola nitida*) is a caffeine-rich nut indigenous to West Africa and is widely used as a stimulant. Apart from its psychoactive effects, studies have shown that kolanut contains polyphenols, flavonoids, and other phytochemicals that exhibit antioxidant properties (Ejele & Ogu, 2017). These compounds are believed to scavenge free radicals, thereby reducing oxidative damage in cells (Ajayi *et al.*, 2019).

Bitter kola (*Garcinia kola*), another staple in African traditional medicine, is celebrated for its therapeutic properties, including anti-inflammatory, antimicrobial, and antioxidant activities. Research indicates that its high flavonoid and tannin content contributes significantly to its ability to combat oxidative stress (Farombi *et al.*, 2015). However, detailed characterization of its antioxidant mechanisms remains underexplored (Ajayi *et al.*, 2019).

Garlic (*Allium sativum*) is a globally renowned spice and medicinal plant with a well-documented history of use in combating cardiovascular and immune-related disorders. Its antioxidant activity is attributed to sulfur-containing compounds like allicin and organosulfur derivatives, which play a crucial role in reducing lipid peroxidation and scavenging reactive oxygen species (ROS) (Banerjee *et al.*, 2013). This underscores its potential for therapeutic applications (Singh *et al.*, 2022).

Ginger (*Zingiber officinale*) is another versatile spice known for its anti-inflammatory and antioxidant properties. Bioactive compounds such as gingerols, shogaols, and paradols are responsible for its free radical scavenging activity (Rahmani *et al.*, 2014). These properties make ginger a promising candidate for combating oxidative damage in biological systems.

The characterization of bioactive compounds in these plants is essential to understand their antioxidant mechanisms. Techniques such as spectrophotometry, high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) are pivotal for identifying and quantifying these compounds (Cheng *et al.*, 2016). Such analyses provide a scientific basis for their use in nutraceuticals, pharmaceuticals, and functional foods (Rahman *et al.* 2021).

Given the widespread consumption of these plants, their antioxidant properties could offer accessible and cost-effective solutions to health challenges associated with oxidative stress. This study seeks to bridge the gap in knowledge by characterizing the antioxidant compounds in kolanut, bitter kola, garlic, and ginger, thereby providing scientific validation for their traditional uses (Rahman *et al.* 2021).

Oxidative stress, linked to numerous chronic diseases, underscores the need for safe and effective antioxidants, as concerns over synthetic antioxidants persist. Despite their widespread traditional use, the antioxidant properties of kolanut, bitter kola, garlic, and ginger remain underexplored, with limited scientific characterization of their bioactive compounds. Existing research often lacks comprehensive methodologies, hindering the full utilization of these plants in health interventions and nutraceuticals. This study addresses these gaps by evaluating and characterizing the antioxidant properties of these plants, providing scientific validation for their traditional use and promoting their potential as natural alternatives to synthetic antioxidants.

The increasing prevalence of oxidative stress-related diseases and the rising demand for safer and more sustainable healthcare solutions necessitate the exploration of natural antioxidants. Kolanut, bitter kola, garlic, and ginger are widely used in traditional medicine, yet there is insufficient scientific evidence to fully validate their antioxidant potential and bioactive compounds. Addressing this gap is crucial for bridging traditional knowledge with modern science.

Given concerns over the safety and side effects of synthetic antioxidants, this study is justified in its aim to identify and characterize natural alternatives that are both effective and accessible. Additionally, the comparative evaluation of these plants will provide valuable insights into their relative effectiveness, guiding their use in therapeutic, nutraceutical, and industrial applications. The study also supports the global shift toward sustainable and affordable health interventions, particularly in resource-limited regions where these plants are abundant and culturally significant.

The aim of this study is to characterize and evaluate the antioxidant properties of caffeine produced from kolanut, bitter kola, garlic, and ginger in providing scientific validation as a potential natural alternative to synthetic antioxidants.

2.0 METHODOLOGY

2.1 Materials

2.1.1 Sample

Bitter kola, kolanut, garlic and ginger, were the samples material used.

2.1.2 Reagents

While the reagents used are: Distilled water, methanol, acetonitrile

2.1.3 Apparatus

Among the apparatus used are: Conical flask, volumetric flask, beaker, water bath, pipette, sample bottles, foil paper, analytical weighing balance.

2.1.2 Sample Collection

Samples were obtained at Oja-Timi in Ede, Osun State by market survey and were brought into Chemistry laboratory of Federal Polytechnic Ede.

2.1.3 Sample Preparation

Bitter kola, ginger, kolanut and garlic were peeled and diced into smaller part and then each sample was placed in a foil paper, then it was left to air-dry for 72hours(3days), after air drying, each sample was blended in an electronic machine. After that each grinded sample was poured inside container and the container was labeled.

2.1.4 Extraction of Caffeine

0.3g of Bitter kola, kolanut, garlic, ginger samples were weighed in 250ml conical flask. Then 200ml of distilled water was added and placed over water bath (100degreec). Extraction was done for half hour(30min). Then the solution was cooled, volume maintained to 250ml and filtered through whatmann number 1 filter paper. 1 ml of the filtrate was pipetted into clean 10ml volumetric flasks and made to the mark with distilled water. Thus, prepared sample was then filtered through microfilter (0.2micrometer) and filled with HPLC vials for analysis.



PICS 1 & 2: Caffeine Power Products Samples

2.2 METHODOLOGY

The following parametric analysis was carried out using HPLC Instrumentation: DPPH Radical Scavenging Assay (to evaluate the antioxidant activity of the caffeine extracts); Ferric Reducing Antioxidant Power (FRAP) Assay (to assess the reducing power of the caffeine extracts); and Moisture Content Determination

3.0 RESULT AND DISCUSSION

3.1 RESULTS

The following parameters were observed;

3.1.1 Moisture Content

The results of the moisture content determination are presented in Table 3.1.

Table 3.1: Moisture Content of the Sample

Sample	W ₁	W ₂	Moisture Content (%)
Kolanut	10.00	8.50	15.00
Bitter kola	10.00	8.60	14.00
Garlic	10.00	7.80	22.00
Ginger	10.00	7.90	21.00

3.1.2 Antioxidant Activity Test

3.1.2.1 Results for DPPH Assay

The results of the DPPH free radical scavenging activity for each sample are presented in Table 3.2.

Table 3.2: Results for DPPH Assay

Sample	Inhibition
Kolanut	45.0
Bitter kola	38.5
Garlic	60.2
Ginger	52.8

3.1.2.2 ABTS Free Radical Scavenging Activity

The results of the ABTS free radical scavenging activity for each sample are presented in Table 3.3.

Table 3.3: ABTS Free Radical Scavenging Activity

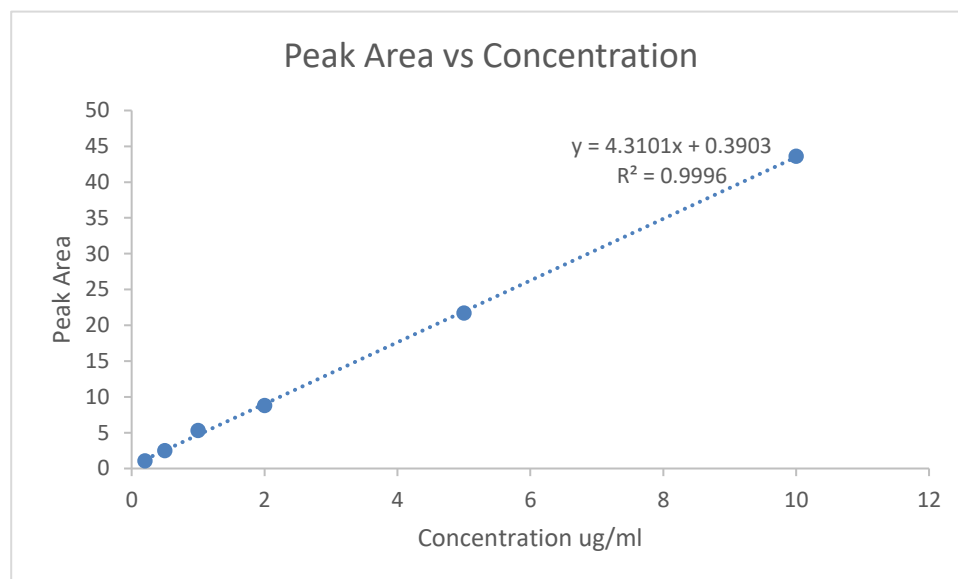
Sample	Inhibition
Kolanut	55.0
Bitter kola	47.5
Garlic	72.4
Ginger	64.0

3.1.3 Calibration Data and Chromatographic Conditions

The calibration curve for caffeine was constructed by plotting the peak area (PA) against the concentration of the caffeine standard. The concentrations of the caffeine standard solutions were prepared in varying concentrations and injected into the gas chromatograph under optimized conditions. The resulting peak areas were recorded and used to generate a linear calibration curve. The calibration data for caffeine are presented in Table 4.4, while Figure 4.1 shows the graph of Peak Area vs. Concentration of Caffeine.

Table 3.4: Calibration data

Concn ug/ml	PA1	PA2	Concn ug/ml	Average PA
0.2	1.03	1.05	0.2	1.04
0.5	2.4	2.6	0.5	2.5
1.0	5.2	5.4	1.0	5.3
2.0	8.4	9.2	2.0	8.8
5.0	21.4	22.0	5.0	21.7
10.0	43.9	43.3	10.0	43.6

**Fig 3.1:**

3.1.4 Calibration Curve for Caffeine Standard

A graph representing the relationship between the caffeine standard concentration and peak area is shown in Figure 3.1. The x-axis represents the concentration of caffeine ($\mu\text{g/mL}$), and the y-axis represents the corresponding peak area (PA1 or PA2) measured by the gas chromatograph. The calibration curve shows a strong linear correlation, which is indicated by the high correlation coefficient ($R^2 = 0.9996$).

3.1.5 Caffeine Content in Plant Extracts

The caffeine content in the kolanut, bitter kola, garlic, and ginger extracts was determined using the calibration curve established in Section 3.3. The resulting caffeine concentrations for each plant extract were calculated and presented in Table 3.5. The corresponding graph is shown in Figure 3.15.

Table 3.5: Amount of Caffeine in the extract

Extract	PA1	PA2	Average PA	Concentration (µg/ml)
Bitter cola	0.86	0.97	0.915	0.121737315
Garlic	1	0.8	0.9	0.118257117
Ginger	1.3	1.3	1.3	0.211062388
Kola nut	1.4	1.6	1.5	0.257465024

***PA is peak area**

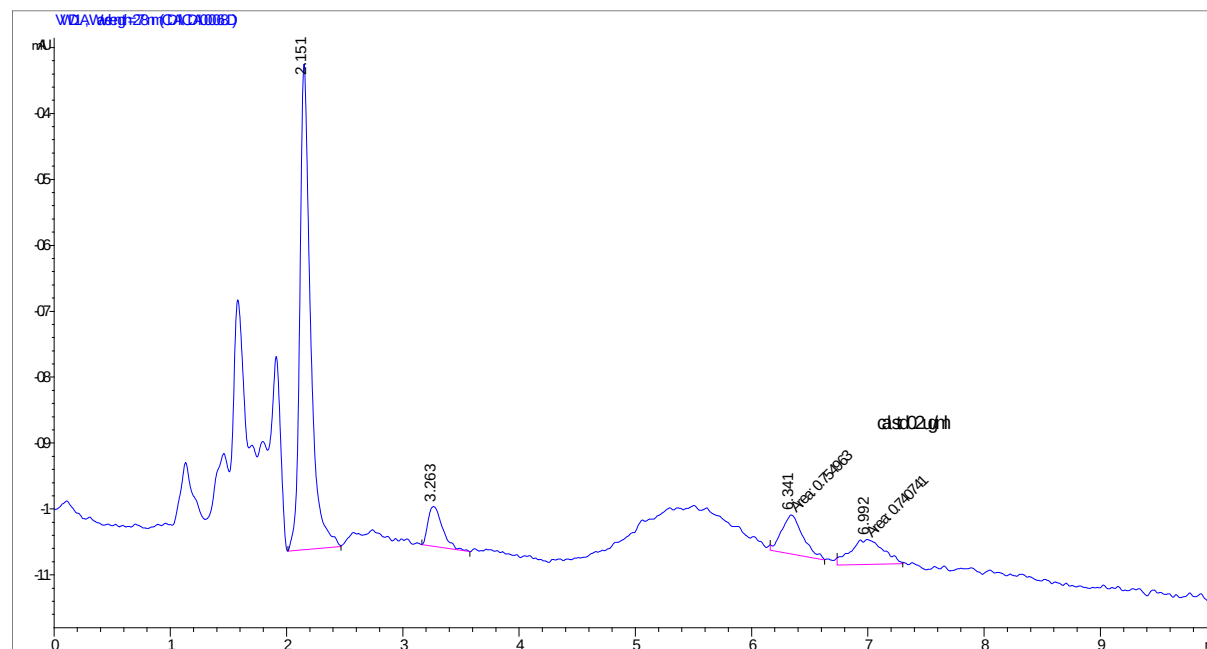


Fig 3.2: Chromatogram for 0.2 ug/ml

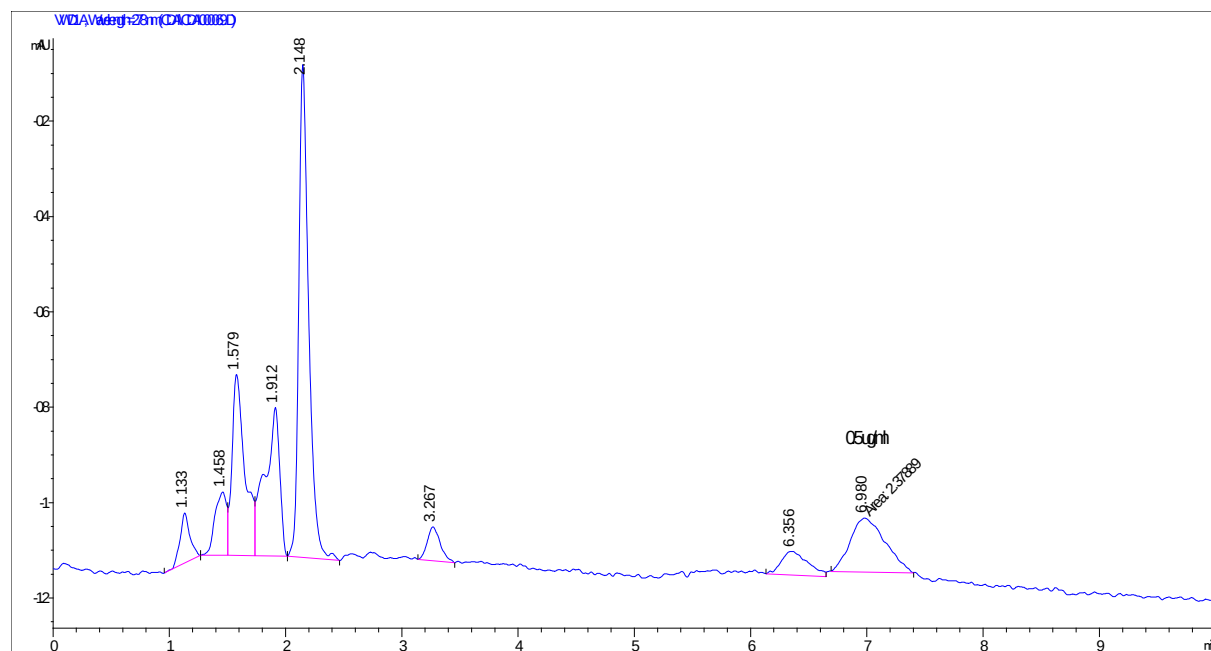


Fig 3.3: Chromatogram for 0.5 ug/ml

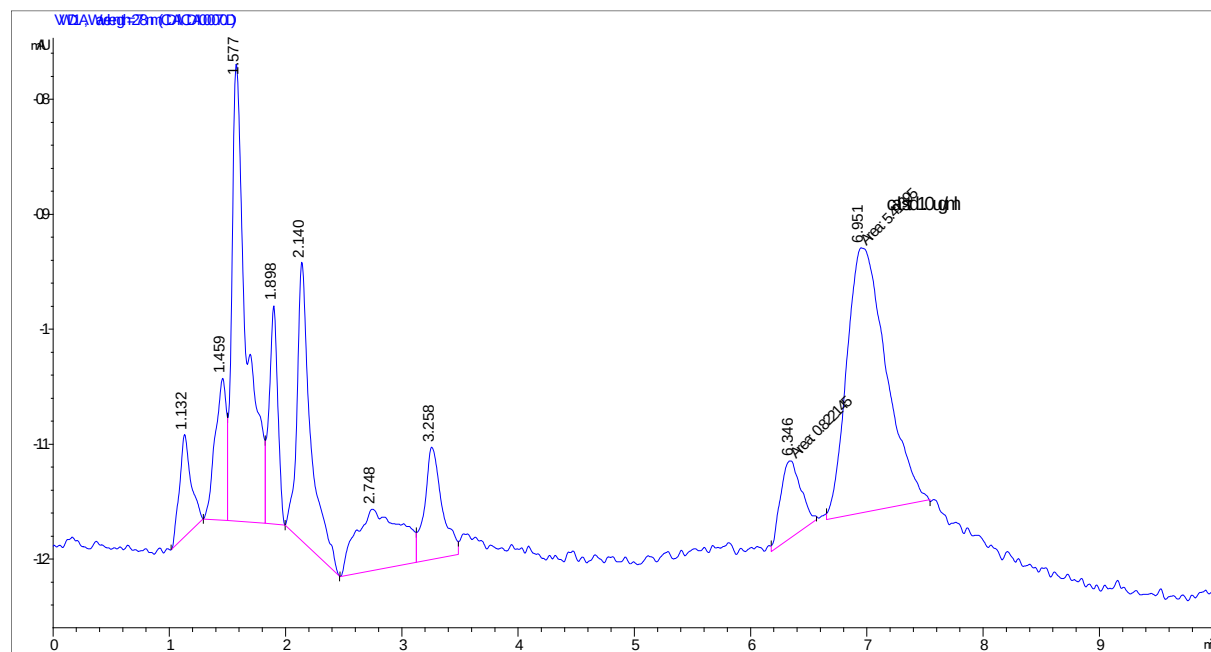


Fig 3.4: Chromatogram for 1.0 ug/ml

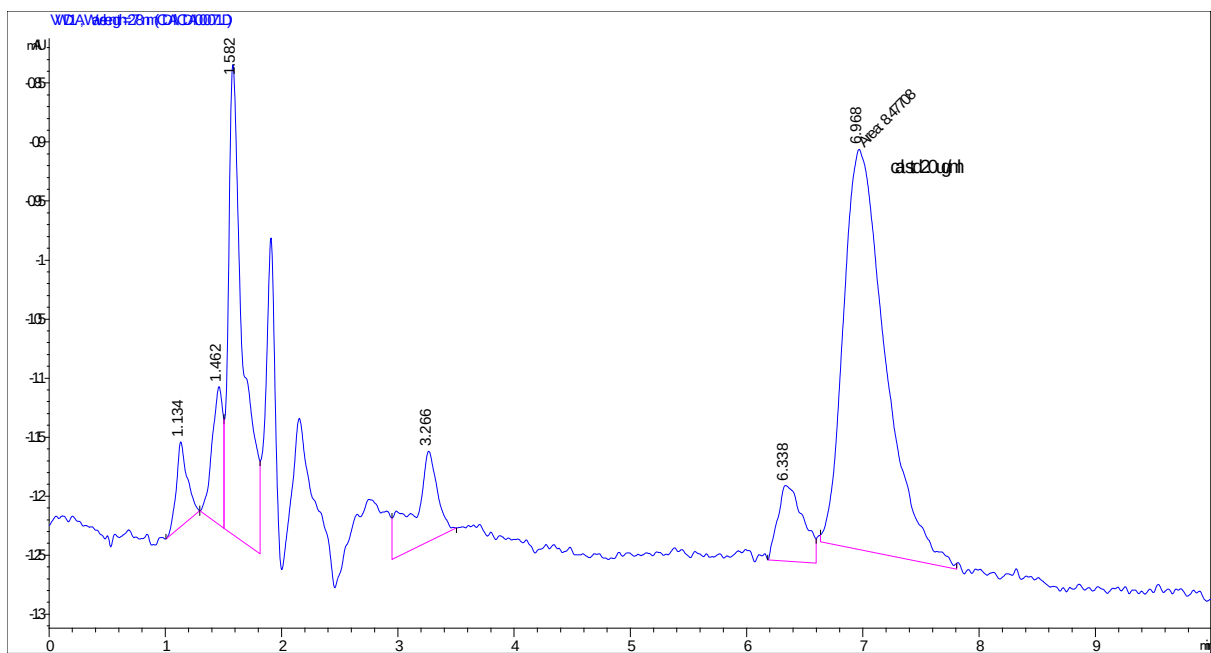


Fig 3.5: Chromatogram for 2.0 ug/ml

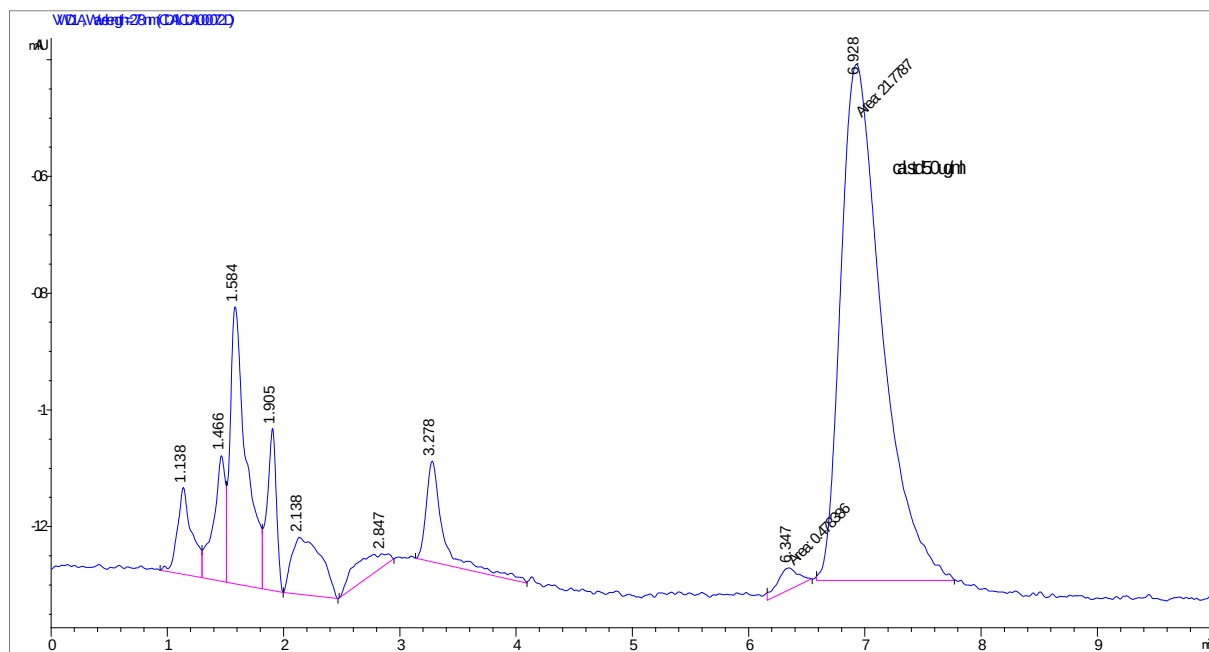


Fig 3.6: Chromatogram for 5.0 ug/ml

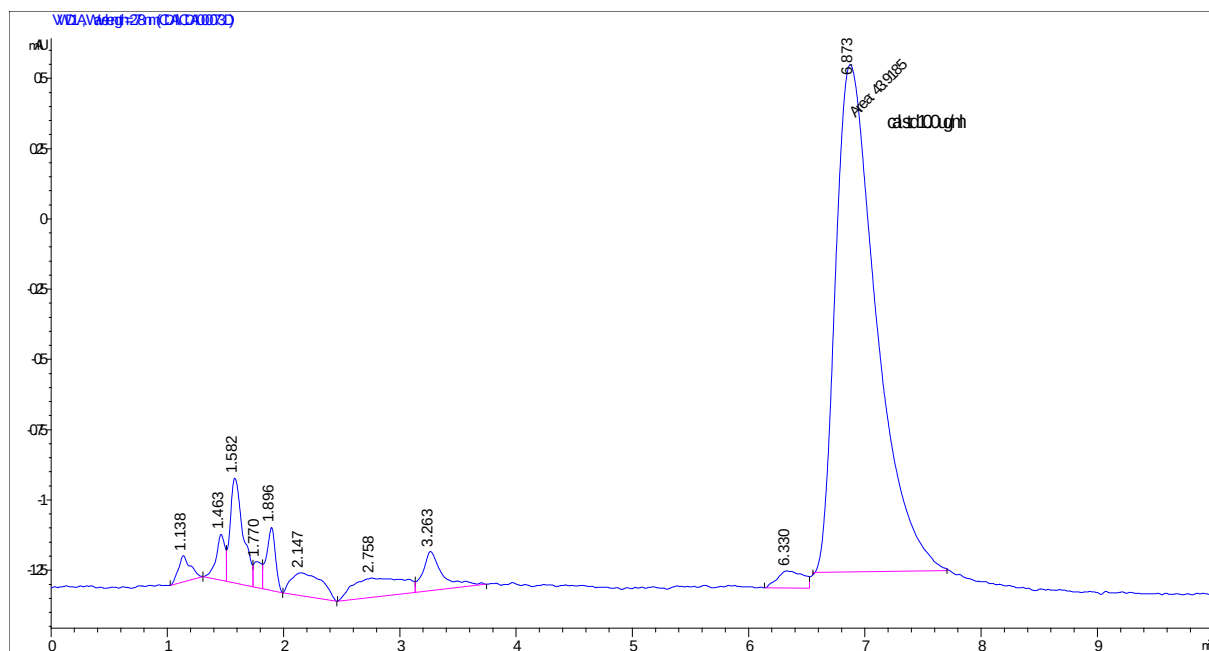


Fig 3.7: Chromatogram for 10.0 ug/ml

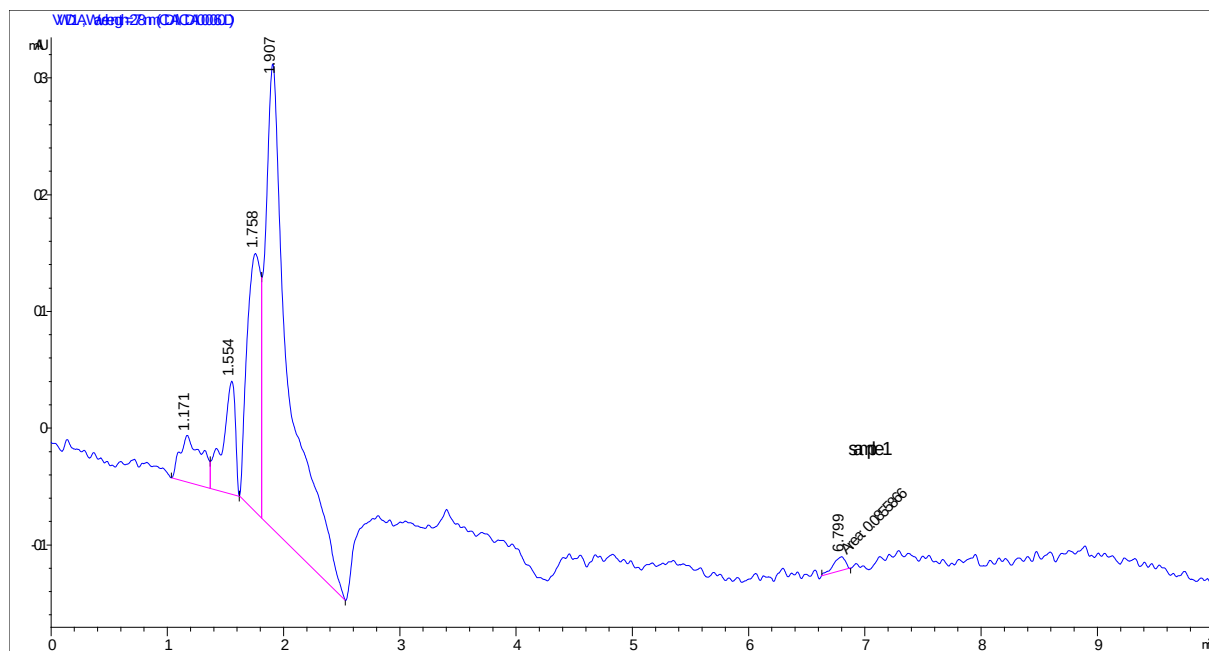


Fig 3.8: Chromatogram for Bitter kola

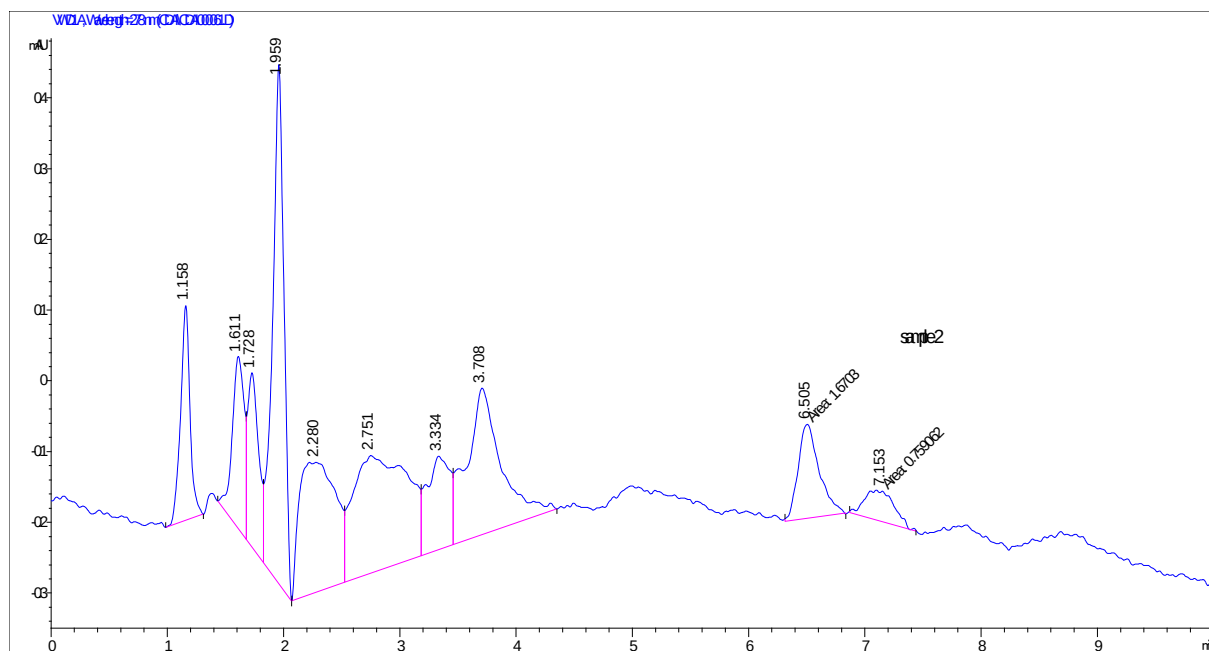


Fig 3.9: Chromatogram for Bitter kola

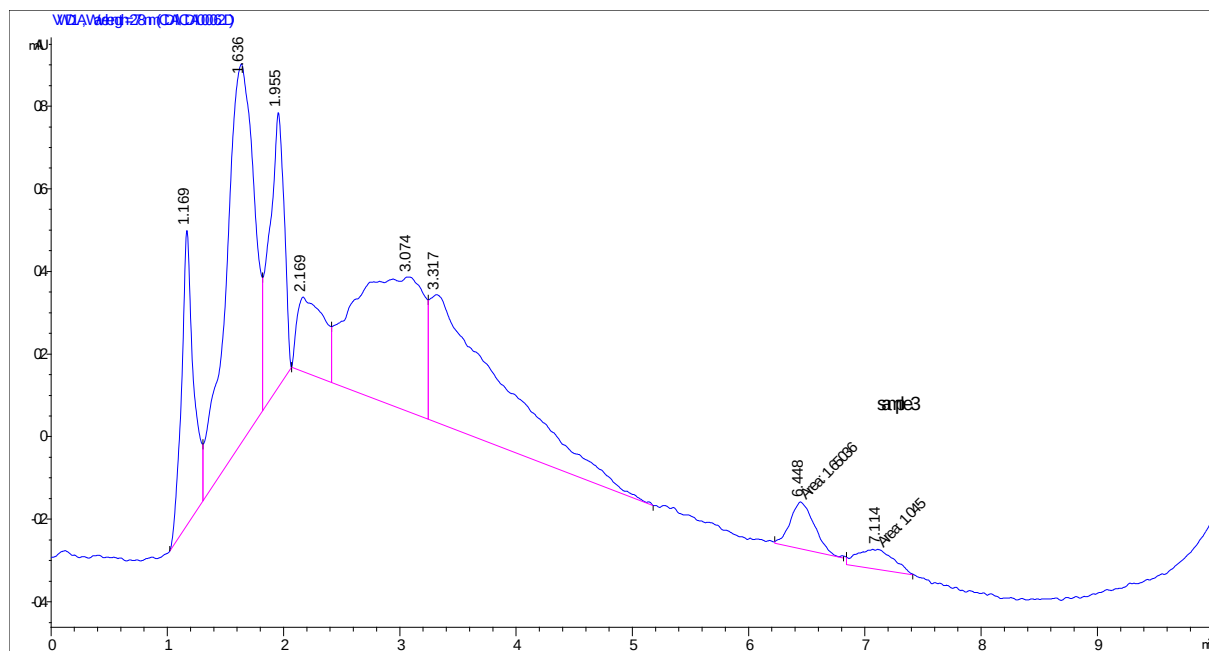


Fig 3.10: Chromatogram for garlic

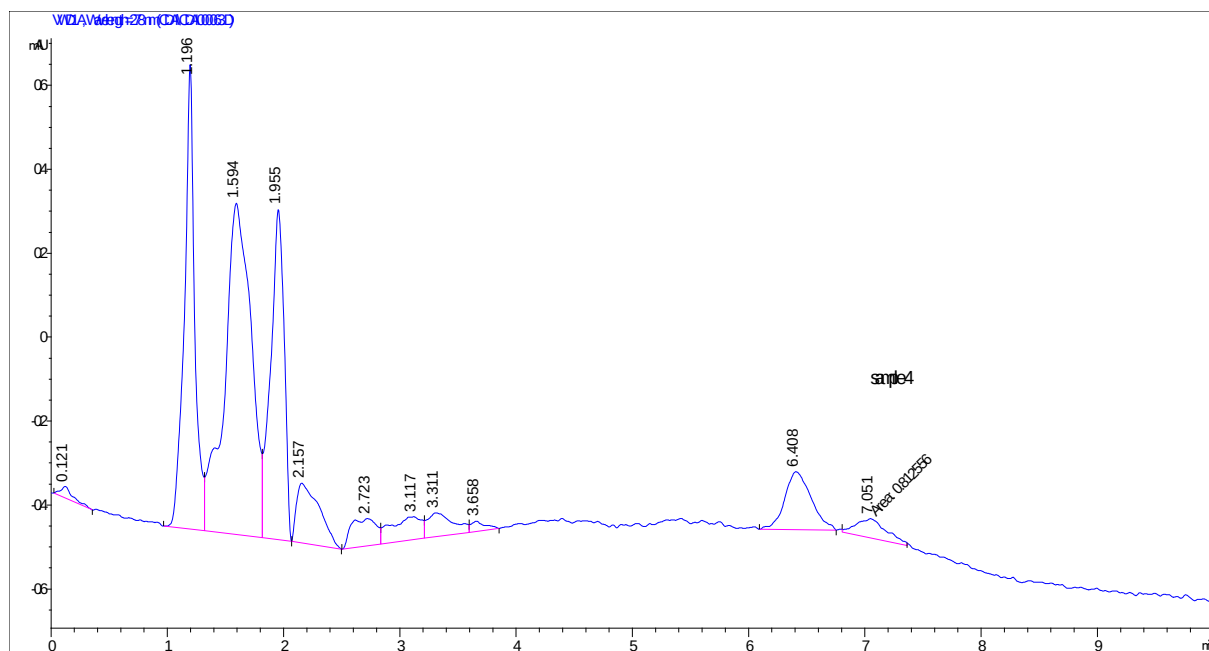


Fig 3.11: Chromatogram for Garlic

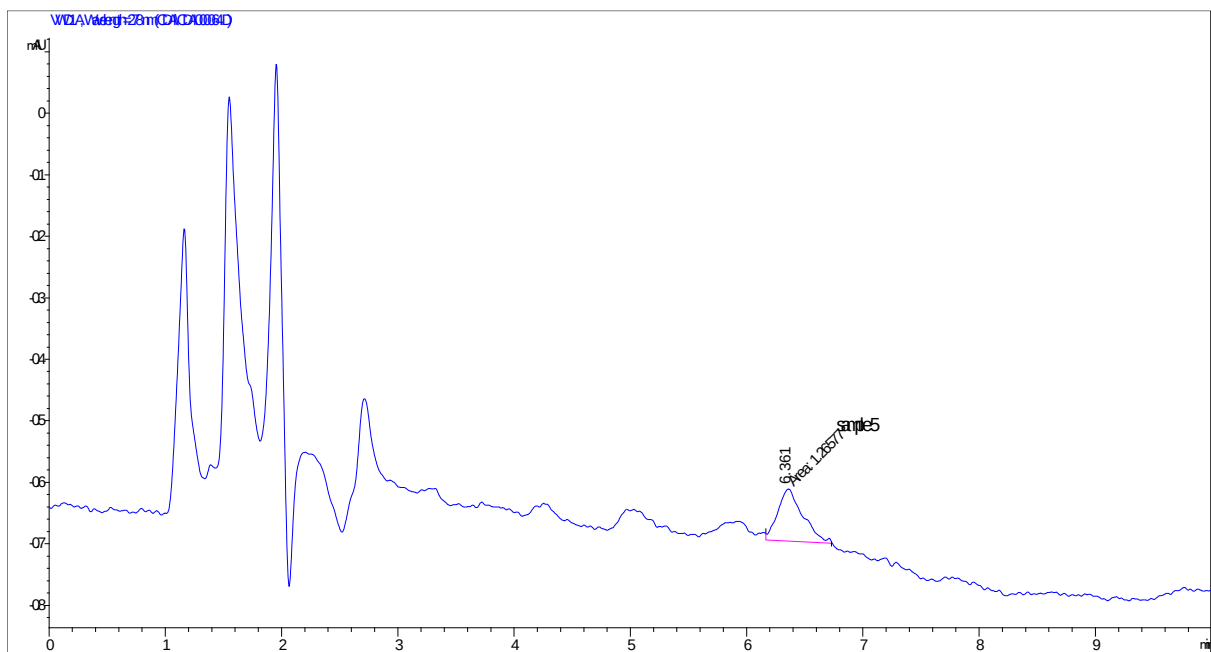


Fig 3.12: Chromatogram for ginger

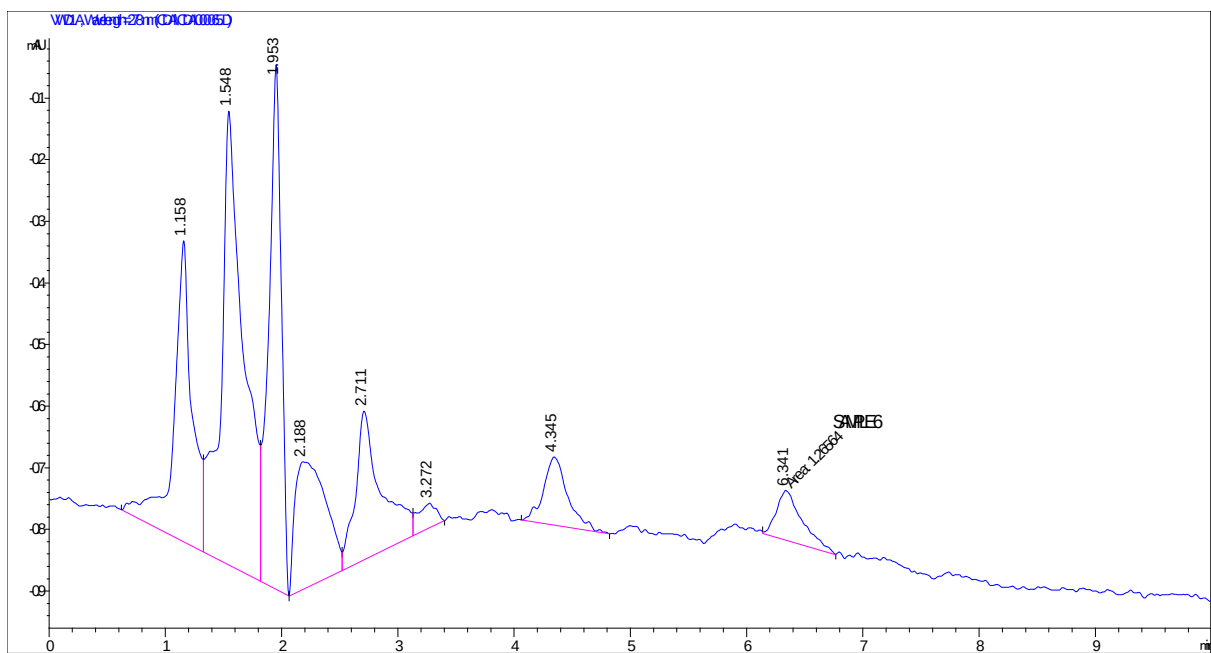


Fig 3.13: Chromatogram for ginger

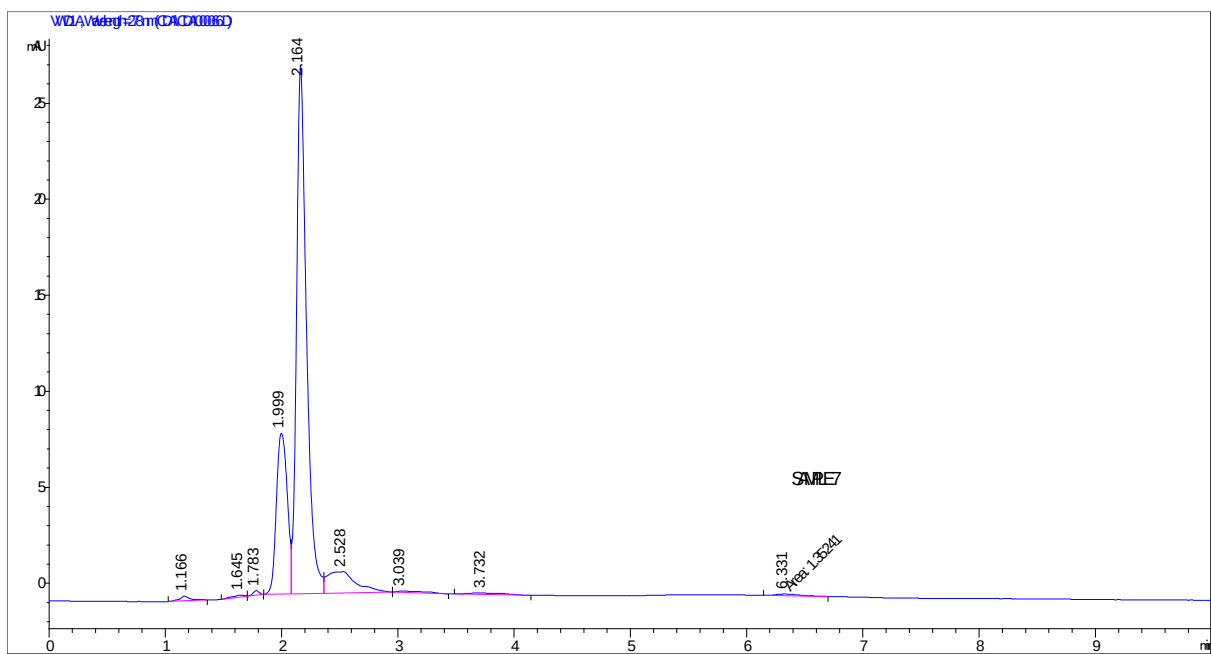


Fig 3.14: Chromatogram for kolanut

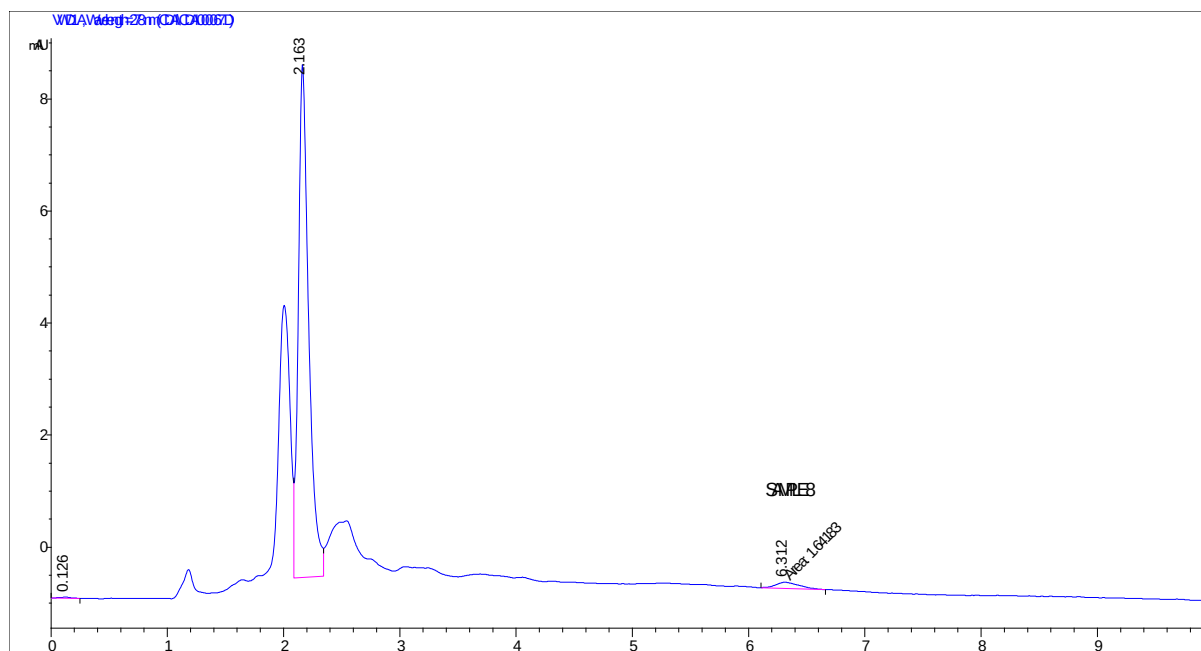


Fig 3.15: Chromatogram for SAMPLE 8

3.1.6 Interpretation of Chromatograms

The chromatograms obtained from the HPLC analysis were analyzed to determine the presence and concentration of caffeine in both standard solutions and natural product samples. The interpretation was based on retention time, peak intensity, and peak area, comparing the samples to standard caffeine chromatograms at different concentrations.

3.1.7 Standard Caffeine Chromatograms (A1 Series)

The chromatograms labeled A1 represent the standard caffeine solutions at varying concentrations, ranging from 0.2 $\mu\text{g/mL}$ to 10.0 $\mu\text{g/mL}$. Each chromatogram displayed a peak at a consistent retention time, characteristic of caffeine. As the concentration increased, the peak intensity and area also increased proportionally. These chromatograms served as reference points for identifying caffeine in the sample chromatograms.

3.2 Discussion

3.2.1 Moisture Content

The moisture content of the plant samples varied significantly, with garlic and ginger showing higher moisture contents (22.00% and 21.00%, respectively) compared to kolanut and bitter kola (15.00% and 14.00%, respectively). This suggests that garlic and ginger may have a higher water content, which could influence their shelf life, stability, and possibly their antioxidant properties. The lower moisture content in kolanut and bitter kola may indicate better preservation for storage. Garlic exhibited the highest DPPH scavenging activity (60.2%), followed by ginger (52.8%). Kolanut (45.0%) and bitter kola (38.5%) demonstrated moderate antioxidant activity. These results suggest that garlic has a potent ability to scavenge free radicals, which may be attributed to its bioactive compounds such as allicin. Ginger, with its bioactive gingerols, also showed significant antioxidant potential.

3.2.2 Antioxidant Activities

Garlic exhibited the highest ABTS radical scavenging activity (72.4%), followed by ginger (64.0%), kolanut (55.0%), and bitter kola (47.5%). These results correlate with the DPPH assay, confirming that garlic has strong antioxidant properties. The ABTS assay results further validate the significant antioxidant potential of ginger and kolanut, suggesting their potential use in medicinal applications.

3.2.3 Chromatograms of Caffeine Composition of the Samples

➤ Bitter Kola (Samples B1 & B2)

The chromatograms labeled B1 and B2 corresponded to bitter kola. These samples exhibited significant peaks at the same retention time as the caffeine standards. The intensity and peak area were relatively high, indicating a notable concentration of caffeine in bitter kola. The results confirmed that bitter kola is a rich source of caffeine, consistent with existing phytochemical studies.

➤ Garlic (Samples B3 & B4)

The chromatograms labeled B3 and B4, corresponding to garlic, did not show peaks at the caffeine retention time. The absence of such peaks suggested that caffeine was either not present or existed in negligible amounts within the samples. However, additional peaks at different retention times were observed, which may be attributed to bioactive compounds naturally present in garlic.

➤ Ginger (Samples B5 & B6)

The chromatograms labeled B5 and B6, representing ginger, also did not exhibit any distinct peaks at the retention time characteristic of caffeine compare to that of the standard. This finding suggested that ginger does not contain caffeine in detectable amounts. Instead, other peaks appeared at different retention times, likely corresponding to compounds such as gingerol, which is a major bioactive component of ginger.

➤ Kola Nut (Samples B7 & B8)

The chromatograms labeled B7 and B8, corresponding to kola nut, closely resembled the standard caffeine chromatograms. Well-defined peaks were observed at the caffeine retention time, confirming the presence of caffeine. The peak areas and intensities were relatively high, further supporting that kola nut is a natural source of caffeine, similar to bitter kola.

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

The study successfully analyzed the caffeine content in selected natural products using High-Performance Liquid Chromatography (HPLC), along with complementary tests such as moisture content analysis, Ferric Reducing Antioxidant Power (FRAP) assay, caffeine extraction efficiency, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity. The HPLC chromatograms revealed that bitter kola and kola nut contained significant caffeine concentrations, as indicated by their distinct peaks matching those of standard caffeine solutions. Conversely, garlic and ginger did not show caffeine-related peaks, confirming the absence of caffeine in detectable amounts. The moisture content analysis provided insight into the storage stability of the samples, with variations observed across the different natural products. The FRAP and DPPH assays

demonstrated antioxidant properties, with certain samples exhibiting notable free radical scavenging abilities. The caffeine extraction process was optimized to ensure maximum yield, allowing for a more precise determination of caffeine levels.

Overall, the results confirmed that bitter kola and kola nut serve as natural caffeine sources, while garlic and ginger contain alternative bioactive compounds with potential health benefits. The findings contribute to the broader understanding of natural product composition and their potential applications in food, pharmaceuticals, and nutraceuticals.

4.2 Recommendations

Below are necessary recommendations:

➤ **Further Quantitative Analysis of Bioactive Compounds**

Beyond caffeine, additional chromatographic techniques such as HPLC-MS or GC-MS should be used to identify and quantify other bioactive compounds present in garlic and ginger.

➤ **Optimization of Caffeine Extraction Methods**

Different solvents and extraction techniques should be explored to enhance the efficiency of caffeine extraction from natural sources, ensuring maximum yield and purity.

➤ **Comparative Antioxidant Studies**

A broader study comparing the antioxidant activities of caffeine-containing and non-caffeine-containing samples should be conducted to assess their potential health benefits.

➤ **Application in Functional Food and Pharmaceutical Industries**

The findings suggest that bitter kola and kola nut could be further explored for use in energy-boosting products, while garlic and ginger may be beneficial for antioxidant and therapeutic applications.

➤ **Storage and Processing Effects on Caffeine Content**

Future studies should investigate how different processing techniques (drying, roasting, and fermentation) affect caffeine content in natural sources, as well as their overall stability over time.

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