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Original Research Article

Textile Dye Potential of *Cola acuminata* Extract: Phytochemicals, Antioxidant and Bioactive Performance Investigation

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Abstract: This study undertakes a comprehensive investigation into the phytochemical profile and potential dye properties of *Cola acuminata* extracts obtained using a range of solvents, including methanol (MeOH), ethyl acetate (EtOAc), and hexane (n-hexane). Phytochemical screening revealed a diverse array of bioactive compounds, with the MeOH extract exhibiting high levels of phenolic compounds, flavonoids, and tannins, underscoring its efficacy in extracting polar phytochemicals. The MeOH extract demonstrated superior antioxidant activity across multiple in vitro assays, including the hydroxyl radical scavenging assay (HRSA), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, with inhibition percentages of 57.43%, 65.59%, and 62.88%, respectively. The estimated IC50 values for the HRSA and DPPH assays were approximately 300 µg/mL, indicating that higher concentrations are required to achieve significant antioxidant activity relative to the positive control, butylated hydroxytoluene (BHT), which exhibited a maximum inhibition of 75.25%. The EtOAc extract was found to be rich in terpenoids and quinones, suggesting its potential for extracting semi-polar compounds with putative antimicrobial properties. The n-hexane extract contained notable amounts of phenolic compounds and tannins, albeit with a lower overall phytochemical diversity. The presence of alkaloids in all extracts highlights their biological significance, including potential anti-inflammatory and antimicrobial activities. The findings of this study underscore the potential of *Cola acuminata* as a valuable source of natural dye and bioactive compounds with applications in the pharmaceutical and leather tanning industries. Future research directions should focus on the application and characterization of specific phytochemicals responsible for coloring activities, as well as elucidating their action on leather during tanning process.

Keywords: *Cola Acuminata*, Phytochemical Profile, Dye Potential, Bioactive Compounds, Natural Dye.

1. INTRODUCTION

Dye is a colored substance used to impart a variety of colors to the substrate. Since 1856, synthetic dyes have been widely used for coloring leather and textiles. But since synthetic dyes are produced from non-renewable petroleum (Manicketh *et al.*, 2021), they not only destroy the environment during synthesis but also release effluents into water bodies during application which affect aquatic life (Kumar *et al.*, 2013); (Carmen Zaharia, 2012). The leather industry consumes a large amount of azo dyes (C Zaharia & Suteu, 2014) a class of harmful synthetic dyes. Aspect of leather product production with minimal impact on the ecological balance, affecting both human and environmental health. Since the late 20th century, researchers have focused on the use of natural dyes in leather dyeing. Research has been reported on the extraction of dyes from plant sources such as Rubia tinctorum roots, henna leaves (Ajekwene & Oguzie, 2024), eucalyptus bark, tea leaves, turmeric rhizomes, and beetroot (Egbujor *et al.*, 2019). In accordance with the above efforts, this study was carried out to color vegetable tanned sheepskin crust leather using a dye extracted from kola acuminata Schott nuts (Sterculiaceae)(Ugwuowo *et al.*, 2021). The use of this plant, although known to local dyers as a traditional leather dye, has not been optimized in terms of extraction

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and dyeing parameters. However, available literature cites the use of the plant in northern Nigeria as an antimicrobial agent and panacea for acute malaria. The aim of this research is to highlight the use of Schott acuminata kola nuts (Sterculiaceae) as a dye source and to optimize the subsequent extraction and dyeing processes. The efforts are expected not only to help local dyers but also to introduce a sustainable dye for the leather processing industry.

2. MATERIAL AND METHODS

2.1 Cola Acuminata Sample Collection and Location

Fresh *Cola Acuminata* nut sample was collected in élig-mfomo town from lékié division in center region of Cameroon. The geographical zones coordinates are: 4°37'43'' North latitude and 11°42'.58'' East longitude. The obtained sample was identified and authenticated by Cameroon Herbarium National center in comparison with the reference species N°18605 SFR/Cam as *Cola Acuminata* Schott (Sterculiaceae). The samples were dried and ground to a powder using a ceramic mortar and were then stored in a sealed container for further use. Figure 1 shows the caption of the selected samples as presented below.

Figure 1: *Cola Acuminata* Schott (Sterculiaceae) sample

2.2 Chemical Reagents

The reagents used in this work were of analytical quality and used without prior purification. Mayer's reagent was prepared by mixing 1.35g of $HgCl_2$ and 3.6g of KI in 100mL of distilled water to detect the presence of alkaloids. Ammonia solution (NH_4OH) (20%) was used to highlight free anthraquinones. Folin-Ciocalteu's reagent is a mixture of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and phosphotungstic acid ($H_3PW_{12}O_{40}$) of yellow color, which by reduction, during the oxidation of phenols, gives a mixture of blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}). The presence of flavonoids was detected using concentrated hydrochloric acid (HCl) in the presence of some magnesium (Mg) chips. The tannin content of the sample was evaluated using vanillin and that of gallic tannins by 1% FeCl3.

2.3 Preparation and Extraction

Kola nuts were harvested in Elig-Mfomo (Cameroon). Kola nut powder was obtained after drying and grinding the samples. The extraction process was carried out by making a triple maceration in methanol, hexane, and/or ethyl acetate of the powder for 72 hours. Approximately 1 kg of mass with a particle size between 2 and 3.2 mm was introduced into one Liter of solvent. The extracts were then filtered and concentrated using a rotary evaporator. These extracts are called, respectively, methanolic extract; hexane and ethyl acetate extract. These extracts contain secondary metabolites that will be highlighted by quantitative and qualitative phytochemical analyses.

2.4 Proximate Analyses

The proximate analyses were performed according the Association of Official Analytical Chemists AOAC. The Ash content was examined according to AOAC method (AOAC, 1990). Briefly 5g of the sample was balanced in to a pre-heated and cooled ceramic crucible and calcinated in Nabertherm muffle furnace at 550°C four 6 hours. The experience was done in triplicate to avoid statistical errors. The resulted material was the cooled Ash which has been balanced again. The Ash (%) percentage was calculated using the following expression as presented in equation 1:

$$Ash\ (\%) = \frac{M2-M0}{M1-M0}\ x\ 100 \quad (1)$$

With M0 = mass of the empty crucible; M1= mass of the crucible containing sample; M2= mass of the crucible containing Ash.

Regarding the Moisture, which is a process to eliminate water in a sample until a constant mass, exactly 5g of the sample was weighted in an empty crucible and placed in a Dry air oven at 105°C for 12 hours. After cooling the sample was weighted once more until a constant mass. The moisture content was calculated using equation 2:

$$Moisture(\%) = \frac{M2-M0}{M1-M0}\ x\ 100 \quad (2)$$

With M0 = mass of the empty crucible; M1= mass of the crucible containing sample; M2= mass of the crucible containing dried sample.

2.5 Phytochemical Screening of Extracts

Phytochemical screening is a qualitative or quantitative analysis based on precipitation and/or coloring reactions. These allow the detection of the presence or absence of secondary metabolites such as alkaloids, quinones, flavonoids, saponins, tannins, sterols and reducing sugars in Kola.

2.5.1 Alkaloids, Terpenoids, Glycosides, Quinone Test

2 mg of plant material was added to a 50 mL beaker previously containing 10 mL of sulfuric acid (H_2SO_4) (10%), and the mixture was left stirring for 3 hours. After filtering the mixture, 1 mL of the filtrate was added to a test tube, then 5 drops of Mayer's reagent were added, and the mixture was homogenized. The appearance of a yellowish-white precipitate indicates the presence of alkaloids (Hamadou *et al*., 2020).

For this test, 5 mL of methanolic extract was mixed with chloroform and 3 mL of concentrated sulfuric acid. A reddish-brown color at the interface indicates a positive result for the presence of terpenoids.

According to the Keller-Kiliani method, 1 mL of methanolic extract was introduced into 2 mL of chloroform and 1 mL of sulfuric acid was added. The observation of a brown ring at the interphase indicates the presence of glycosides.

5 mL of the methanolic extract was added to a test tube containing 2.5 mL of ammonia (NH_4OH) solution (20%), and the mixture was shaken. The appearance of a more or less red color indicated the presence of free anthraquinones (Wan *et al*., 2023).

2.5.2 Polyphenols, Flavonoids, Tannins, Saponins Tests

A volume of 0.5 mL of concentrated HCl and a few flakes of magnesium (Mg) were added to a test tube containing 2.5 mL of the methanolic extract. The presence of flavonoids was detected by the red color of the reaction medium, which developed after 3 minutes (Oscar Ditchou Nganso *et al*., 2020).

A mass of 1.5 g of dry plant material was added to a test tube containing 10 ml of 80% MeOH. After 15 minutes of stirring, the extracts were filtered and transferred to dry tubes. The addition of 1% $FeCl_3$ to the solution turned blue-black, indicating the presence of gallic tannins (Hamadou *et al*., 2020, 2022).

A volume of 2 mL of extract was added to a test tube containing 5 mL of distilled water. After vigorous shaking, the formation of a stable foam that persisted for 15 minutes indicated the presence of saponins (Hamadou *et al*., 2020, 2022; Oscar Ditchou Nganso *et al*., 2020).

2.5.3 Antioxidant Activity

The antioxidant activity of *Cola acuminata* extracts was assessed using a multi-faceted approach, employing a range of in vitro assays to comprehensively evaluate their free radical scavenging potential. The assays utilized in this study were the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay, and the hydroxyl radical scavenging assay (HRSA).

The DPPH assay is a widely used method for assessing the antioxidant activity of plant extracts. The assay is based on the principle that DPPH, a stable free radical, reacts with antioxidants to form a stable diamagnetic molecule, resulting in a decrease in the absorbance of the reaction mixture. In this study, the DPPH assay was performed by mixing 1 mL of the extract (at various concentrations) with 1 mL of 0.1 mM DPPH solution in methanol. The reaction mixture was then incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition of DPPH radical was calculated using the formula:

$$\% \text{ inhibition} = [(A0 - A1) / A0] \times 100,$$

Where A0 is the absorbance of the control (DPPH solution without extract) and A1 is the absorbance of the test sample (DPPH solution with extract). The IC50 value, which represents the concentration of the extract required to scavenge 50% of the DPPH radicals, was calculated from the dose-response curve.

The ABTS assay is another widely used method for assessing antioxidant activity. The assay involves the generation of ABTS radicals, which are then scavenged by antioxidants present in the extract. In this study, the ABTS assay was performed by mixing 1 mL of the extract (at various concentrations) with 1 mL of ABTS radical solution (generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate). The reaction mixture was then incubated in the dark for 6 minutes, and the absorbance was measured at 734 nm using a UV-Vis spectrophotometer. The percentage inhibition of ABTS radical was calculated using the same formula as for the DPPH assay.

The HRSA is a sensitive assay that measures the ability of antioxidants to scavenge hydroxyl radicals,

which are highly reactive and damaging to cellular components. In this study, the HRSA was performed by mixing 1 mL of the extract (at various concentrations) with 1 mL of a reaction mixture containing 0.1 mM $FeSO_4$, 0.1 mM EDTA, and 1 mM H_2O_2. The reaction mixture was then incubated at 37°C for 30 minutes, and the absorbance was measured at 532 nm using a UV-Vis spectrophotometer. The percentage inhibition of hydroxyl radical was calculated using the same formula as for the DPPH assay.

The results of the antioxidant activity assays were expressed as percentage inhibition and IC50 values. The IC_{50} values were calculated using a non-linear regression analysis, and the results were compared with those of the positive control, butylated hydroxytoluene (BHT). The data were analyzed using statistical software, and the results were presented as mean ± standard deviation (SD) of triplicate measurements.

2.6 Quantitative Composition of Phytochemicals

2.6.1 Polyphenols, Flavonoids, Tannins, Saponins Contents of Extract

Folin-Ciocalteu phenol was determined according to the protocol described by (Singleton e*t al*., 1999)(Anhwange *et al*., 2022). The Folin-Ciocalteu reagent is a yellow mixture of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and phosphotungstic acid ($H_3PW_{12}O_{40}$), which, upon reduction during the oxidation of phenols, produces a mixture of blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}). The blue color obtained has a maximum absorption at 750 nm and is proportional to the amount of phenolic compounds present in the medium.

In acidic medium and in the presence of $AlCl_3$, flavonoids complex, giving a red color with an absorption maximum at 430 nm. The content of flavonoids present in the sample is proportional to the intensity of the color according to the following reactions: between quercetin and $AlCl_3$ (Hamadou *et al*., 2022).

In acidic media, tannins react with vanillin to form a red complex with an absorption maximum at 500 nm, the color intensity of which is proportional to the tannin content of the sample (Bainbridge *et al*., 1996).

2.6.2 Alkaloids, Terpenoids Contents of extract

The determination of alkaloids and terpenoids contents in *Cola acuminata* extracts was performed using a combination of qualitative and quantitative methods.

Alkaloids Determination

The presence of alkaloids in the extracts was detected using the Dragendorff's reagent test. This test is a widely used qualitative method for detecting the presence of alkaloids in plant extracts. The test involves the reaction of the extract with Dragendorff's reagent, which is a mixture of bismuth nitrate and potassium iodide. The formation of a reddish-brown precipitate or coloration indicates the presence of alkaloids.

To perform the test, 1 mL of the extract was mixed with 1 mL of Dragendorff's reagent, and the mixture was observed for the formation of a precipitate or color change. The intensity of the color or precipitate was used to estimate the relative amount of alkaloids present in the extract.

For quantitative determination, the extracts were subjected to spectrophotometric analysis using a UV-Vis spectrophotometer. The absorbance of the extract was measured at a specific wavelength (typically between 200-400 nm), and the alkaloid content was estimated using a calibration curve prepared with a standard alkaloid compound.

Terpenoids Determination

The presence of terpenoids in the extracts was detected using the Salkowski test. This test involves the reaction of the extract with chloroform and sulfuric acid, resulting in the formation of a reddish-brown coloration or precipitate, which indicates the presence of terpenoids.

To perform the test, 1 mL of the extract was mixed with 1 mL of chloroform, followed by the addition of 1 mL of concentrated sulfuric acid. The mixture was then observed for the formation of a reddish-brown coloration or precipitate.

For quantitative determination, the extracts were subjected to spectrophotometric analysis using a UV-Vis spectrophotometer. The absorbance of the extract was measured at a specific wavelength (typically between 200-400 nm), and the terpenoid content was estimated using a calibration curve prepared with a standard terpenoid compound.

Quantification of Alkaloids and Terpenoids

The quantification of alkaloids and terpenoids was performed using a calibration curve prepared with a standard compound. The calibration curve was constructed by plotting the absorbance of the standard compound against its concentration. The concentration of alkaloids and terpenoids in the extracts was then estimated by interpolating the absorbance of the extract on the calibration curve.

The results were expressed as mg/g of dry extract, and the data were analyzed using statistical software. The results were presented as mean ± standard deviation (SD) of triplicate measurements.

2.7 Statistical Data Analysis

The data obtained from the various experiments were analyzed using statistical software (SPSS version 25.0, IBM Corporation, Armonk, NY, USA). All

experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA) to determine the significance of differences between the means of different groups. The Tukey's post-hoc test was used to compare the means of different groups, and the differences were considered significant at $p < 0.05$.

The IC_{50} values were calculated using a non-linear regression analysis, and the results were expressed as mean ± SD. The correlation between the phytochemical contents and antioxidant activities was analyzed using Pearson's correlation coefficient. The data were also subjected to descriptive statistical analysis, including mean, SD, and coefficient of variation (CV). The CV was used to assess the precision of the measurements.

All statistical analyses were performed using SPSS version 25.0, and the results were considered statistically significant at $p < 0.05$. The results of the statistical analysis were used to interpret the data and draw conclusions about the phytochemical profile, antioxidant activity, and bioactive potential of *Cola acuminata* extracts.

3. RESULTS AND DISCUSSION

3.1 Dry matter and Ash Contents and Extraction Yield

The results presented in Tables 1 and 2 provide valuable insights into the dry matter and ash contents, as well as the extraction yields of *Cola Acuminata* using various solvents. The dry matter content of *Cola Acuminata* was found to be 89.39±0.48%, which is within the range reported for other plant materials (Avanza *et al*., 2021; Yutharaksanukul *et al*., 2024). The ash content, on the other hand, was 4.57±0.03 a relatively low mineral a relatively low mineral content, consistent with values reported for other plant-based materials (Sharma *et al*., 2018).

The extraction yields obtained using different solvents (Table 2) reveal that methanol (MeOH) was the most effective solvent, yielding 20.26% of the initial mass, followed by ethyl acetate (EtAcO) with 11.54%, and hexane with 7.70%. These results are in line with previous studies that have demonstrated the efficacy of MeOH in extracting a wide range of compounds from plant materials (Sultana *et al*., 2007); (Do *et al*., 2014). The varying extraction yields can be attributed to the differences in solvent polarity and the solubility of the extracted compounds (Markom *et al*., 2007). The relatively high yield obtained with MeOH suggests that *Cola Acuminata* contains a significant amount of polar compounds, which is consistent with the presence of phenolic and alkaloid compounds reported in other studies (Atawodi *et al*., 2003).

The extraction yields obtained in this study are also comparable to those reported for other plant materials using similar solvents (Spigno *et al*., 2007), (Cacace & Mazza, 2003). For instance, (Spigno *et al*., 2007) reported extraction yields ranging from 15 to 25% using MeOH for various grape pomace samples. The results of this study demonstrate the potential of *Cola Acuminata* as a source of valuable compounds, and further studies are warranted to characterize the extracted compounds and explore their potential applications.

Table 1: Dry matter and ash contents

Sample	Dry matter Content (%)	Ash Content (%)
Cola Acuminata	89.39±0.48	4.57±0.03

Table 2: Extraction yields of *Cola Acuminata* using various solvents

	Sample	MeOH Extract	EtAcO Extract	Hexane Extract
Mass (g)	150	30.40	17.32	11.55
Yields (%)	-	20.26	11.54	7.70

3.2 Phytochemical Profile

The phytochemical profile of *Cola acuminata* extracts, obtained using various solvents, is summarized in Table 3. The analysis reveals a diverse array of bioactive compounds, including alkaloids, phenols, quinones, flavonoids, saponins, tannins, terpenoids, and glycosides. The presence and relative abundance of these compounds are influenced by the solvent employed for extraction.

Table 3: Phytochemical profile of *Cola acuminata* extracts

Metabolites	Alkaloids	Phenols	Quinone	Flavonoids	Saponins	Tannins	Terpenoids	Glycosides
Hexane Extract	+	++	++	+	+	++	+ +	+
MeOH Extract	+	+++	+ ++	+++	+	+++	++	+
EtAcO Extract	+	++	+++	+ +	+	++	+++	+ ++

The methanol extract (MeOH) exhibited the highest phytochemical diversity, characterized by elevated levels of phenols (+++), flavonoids (+++), and tannins (+++). This suggests that MeOH is particularly effective for extracting polar compounds, aligning with findings from (Stalikas, 2007). The abundance of these

compounds correlates with the reported antioxidant and pharmacological activities of *Cola acuminata* (Atawodi *et al*., 2003).

In contrast, the ethyl acetate extract (EtAcO) demonstrated significant concentrations of terpenoids (+++) and quinones (+++). This indicates that EtAcO is suitable for extracting semi-polar and non-polar compounds, as noted by (Markom *et al*., 2007). The presence of these phytochemicals suggests potential applications in antimicrobial and antifungal activities.

The hexane extract, while exhibiting lower phytochemical diversity compared to the MeOH and EtAcO extracts, still contained notable amounts of phenols (++) and tannins (++). This indicates that hexane can extract some polar compounds, albeit less efficiently than MeOH (Do *et al*., 2014). The presence of quinones (++) in the hexane extract also suggests potential antimicrobial and antiviral properties (Haslam, 1996).

Notably, alkaloids (+) were identified in all three extracts, highlighting their biological significance. Alkaloids are known for their diverse pharmacological activities, including anti-inflammatory and antimicrobial effects.

Overall, the findings of this study align with previous reports on the phytochemical composition of *Cola acuminata* (Atawodi *et al*., 2003), (Esimone *et al*., 2007). The diverse range of secondary metabolites present in the different solvent extracts underscores the potential of *Cola acuminata* as a valuable source of bioactive compounds. These compounds may have various applications in the pharmaceutical, food, and cosmetic industries, particularly in the development of products with antioxidant, anti-inflammatory, and antimicrobial properties. Further research is warranted to isolate and characterize the specific compounds responsible for these activities, as well as to explore their practical applications.

3.3 Phytochemical Contents

The results presented in the Figure 2 illustrate the phytochemical composition of different extracts from *Cola acuminata*, specifically focusing on the levels of polyphenols, flavonoids, tannins, and saponins.

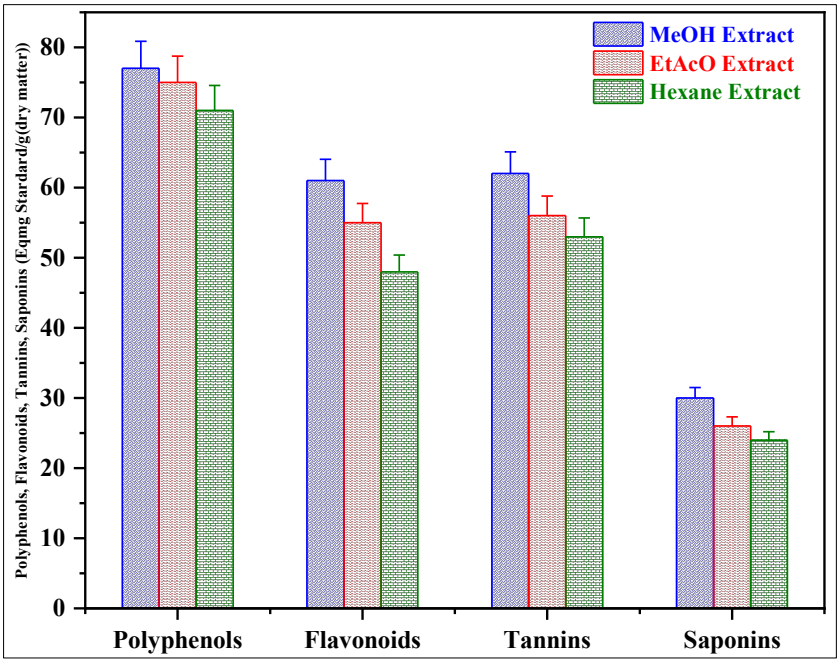

Figure 2: Phytochemical composition of different extracts from *Cola acuminata*

Polyphenols

The methanol extract (EM) exhibits the highest concentration of polyphenols (74.77 g equivalent gallic acid/g MS), followed closely by the acetone extract (EAE) (70.64 g equivalent gallic acid/g MS) and the hexane extract (EH) (71 g equivalent gallic acid/g MS). The elevated levels of polyphenols in the EM suggest its potential as a source of antioxidants, as polyphenols are well-documented for their ability to scavenge free radicals and mitigate oxidative stress (Costa *et al*., 2025). This finding aligns with previous literature indicating that polar solvents effectively extract polyphenolic compounds due to their solubility (Wink, 1997).

Flavonoids

The flavonoid content is notably highest in the EM (55.52 g equivalent quercetin/g MS), with the EAE and EH extracts showing lower levels (51.07 g and 48.22 g equivalent quercetin/g MS, respectively). Flavonoids are recognized for their numerous health benefits, including anti-inflammatory and cardioprotective effects. The substantial concentration of flavonoids in the EM highlights its potential for therapeutic applications,

reinforcing the importance of selecting appropriate solvents for the extraction of bioactive compounds (Mastellone *et al*., 2023).

Tannins

The tannin content in the extracts reveals that the EM again leads with 53.79 g equivalent tannic acid/g MS, followed by the EAE (51.79 g equivalent tannic acid/g MS) and EH (50.38 g equivalent tannic acid/g MS). Tannins are often associated with astringent properties and have been linked to various health benefits, including antimicrobial and antioxidant activities (Haslam, 1996). The high levels of tannins in the EM suggest its potential utility in traditional medicine and food preservation.

Saponins

The saponin content is highest in the EAE (29.53 g equivalent diosgenin/g MS), compared to EM (26.49 g equivalent diosgenin/g MS) and EH (24 g equivalent diosgenin/g MS). Saponins are known for their surfactant properties and potential health benefits, including cholesterol-lowering effects and immune system modulation. The elevated levels of saponins in the EAE may indicate its potential for specific therapeutic applications, such as in the formulation of functional foods (Timilsena & Phosanam, 2023), (Mazza & Uc, 2007).

The methanol extract of *Cola acuminata* shows a robust profile of polyphenols, flavonoids, and tannins, suggesting strong antioxidant and therapeutic potential. The acetone extract, while slightly lower in some parameters, displays noteworthy saponin content, indicating diverse bioactivity across the different extracts. Further studies are warranted to elucidate the potential use these extracts as color dyeing and explore the potential applications of these extracts in leather tanning; food coloring, and textile industry.

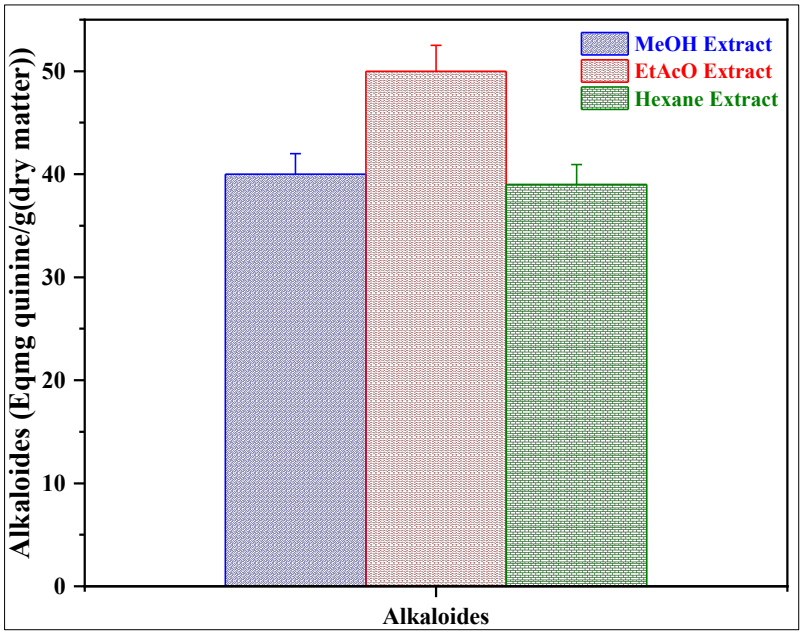

Figure 3: Alkaloid content of different extracts from *Cola acuminata*

The results presented in the Figure 3 highlight the alkaloid content of different extracts from *Cola acuminata*, specifically focusing on the methanol extract (EM), acetone extract (EAE), and hexane extract (EH).

The acetone extract shows the highest concentration of alkaloids at 49.77 g equivalent quinine/g MS, surpassing both the methanol extract (40.20 g equivalent quinine/g MS) and the hexane extract (38.64 g equivalent quinine/g MS). This finding is significant, as alkaloids are well-known for their diverse pharmacological properties, including antimicrobial, analgesic, and antitumor activities (Bribi, 2018). The elevated alkaloid content in the EAE suggests that acetone is particularly effective in extracting these bioactive compounds, potentially due to its ability to solubilize a broader range of alkaloids compared to the other solvents used.

The presence of alkaloids in *Cola acuminata* may contribute not only to its medicinal applications but also to its potential use in textile dyeing processes. Alkaloids can offer vibrant colors and may have mordant properties, enhancing dye fixation on fabrics (Sharma *et al*., 2018). Furthermore, the antioxidant properties associated with alkaloids may play a role in protecting textiles from fading and degradation, thereby extending the longevity of dyed materials.

The findings indicate that the acetone extract of *Cola acuminata* possesses a superior alkaloid profile, which could be harnessed for both medicinal and industrial applications, particularly in natural dye

formulations. Future research should explore the specific alkaloids present in the extracts and their mechanisms of action, as well as their effectiveness in different dyeing processes.

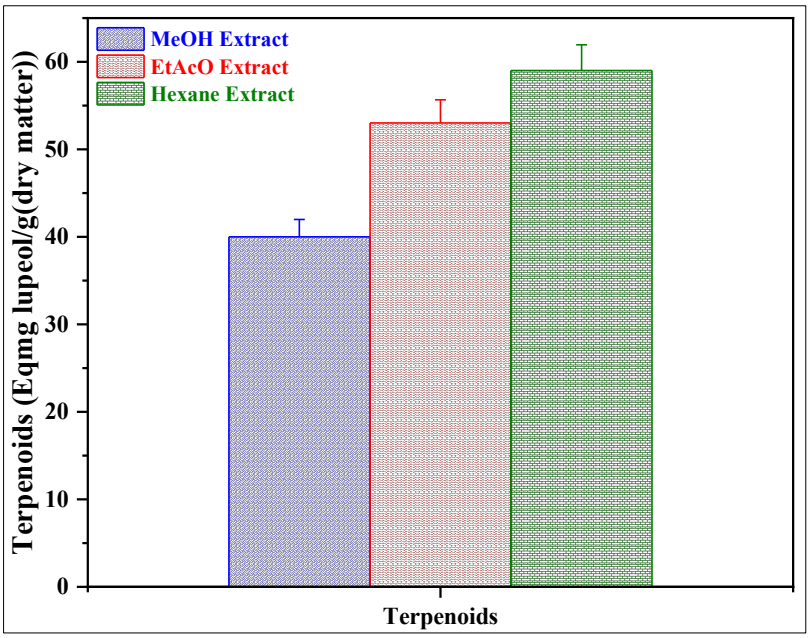

Figure 4: Terpenoid content of various extracts from *Cola acuminata*

The results illustrated in the Figure 4 indicate the terpenoid content of various extracts from *Cola acuminata*, specifically comparing the methanol extract (EM), acetone extract (EAE), and hexane extract (EH). Notably, the hexane extract displays the highest concentration of terpenoids at 58.64 g equivalent lupene/g MS, followed closely by the acetone extract at 52.77 g equivalent lupene/g MS, while the methanol extract shows a lower concentration of 40.20 g equivalent lupene/g MS. The elevated terpenoid levels in the hexane extract suggest that non-polar solvents like hexane are particularly effective in extracting these lipophilic compounds. Terpenoids are a vast class of organic chemicals derived from plants, known for their diverse biological activities, including anti-inflammatory, antimicrobial, and antioxidant properties (Gozari *et al*., 2020). The significant presence of terpenoids in *Cola acuminata* could contribute to its traditional medicinal uses, offering potential therapeutic benefits.

Additionally, terpenoids play a crucial role in the flavor and fragrance of plants, which may enhance the appeal of *Cola acuminata* extracts in various applications, including food, cosmetics, and aromatherapy. Their potential use in natural dyeing processes should also be considered, as some terpenoids may impart color and improve the overall quality of textile dyes (Ghosh *et al*., 2018).

The hexane extract of *Cola acuminata* exhibits the highest terpenoid content, indicating its potential for diverse applications in pharmacology and industry. Future studies should focus on characterizing the specific terpenoids present in these extracts and evaluating their individual contributions to the biological activities observed and also their substantial use as color dyeing.

3.4 Antioxidant Activity

HRSA (Hydroxyl Radical Scavenging Activity)

The methanol extract (MeOH) of *Cola acuminata* (Figure 5) demonstrates the highest inhibition percentage at 57.43%, significantly surpassing the hexane (44.02%) and acetone (AcOEt) extracts (56.74%). This heightened activity suggests that the MeOH extract may harbor more effective hydroxyl radical scavengers, likely due to a greater concentration of phenolic compounds recognized for their antioxidant capabilities (Oscar Ditchou Nganso *et al*., 2020; Wangso *et al*., 2022). The solubility of these compounds in polar solvents may further enhance the MeOH extract's efficacy in inhibiting hydroxyl radicals.

ABTS (2,2'-Azinobis(3-Ethylbenzothiazoline-6-Sulfonic Acid) Assay)

In the ABTS assay (Figure 5), the MeOH extract again exhibits superior performance with an inhibition rate of 65.59%, compared to hexane (47.95%) and AcOEt (47.93%). This assay evaluates the antioxidant capacity to quench the ABTS radical cation, highlighting the extract's ability to donate electrons. The pronounced activity of the MeOH extract may indicate a rich presence of flavonoids or tannins, both of which are known to effectively scavenge ABTS radicals (Khanum *et al*., 2024; Leutcha *et al*., 2025).

DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Assay

The DPPH assay results (Figure 5) reveal that the MeOH extract achieves a notable inhibition rate of 62.88%, significantly exceeding the hexane (38.56%) and AcOEt (54.10%) extracts. This assay is a standard method for evaluating the free radical scavenging ability of antioxidants. The remarkable activity of the MeOH extract may reflect its diverse phytochemical profile, encompassing various antioxidants that can donate hydrogen atoms to neutralize free radicals (Leutcha *et al*., 2025).

FRAP (Ferric Reducing Antioxidant Power)

Finally, in the FRAP assay (Figure 5), the MeOH extract again leads with a significant value of 66.16 meq vit C/mL, underscoring its reducing power. This assay measures the ability of antioxidants to convert ferric ions to ferrous ions, illustrating their potential in redox reactions. The higher reducing power of the MeOH extract suggests a more substantial presence of reductive compounds, such as ascorbic acid or polyphenols (Benzie & Strain, 1996).

The methanol extract of *Cola acuminata* consistently exhibits superior antioxidant activity across all assays, reaffirming its potential as a source of natural antioxidants. Future research should aim to identify and quantify the specific phytochemicals responsible for these activities, as well as to explore their mechanisms of action. Such investigations could pave the way for developing functional foods or nutraceuticals targeting oxidative stress-related diseases.

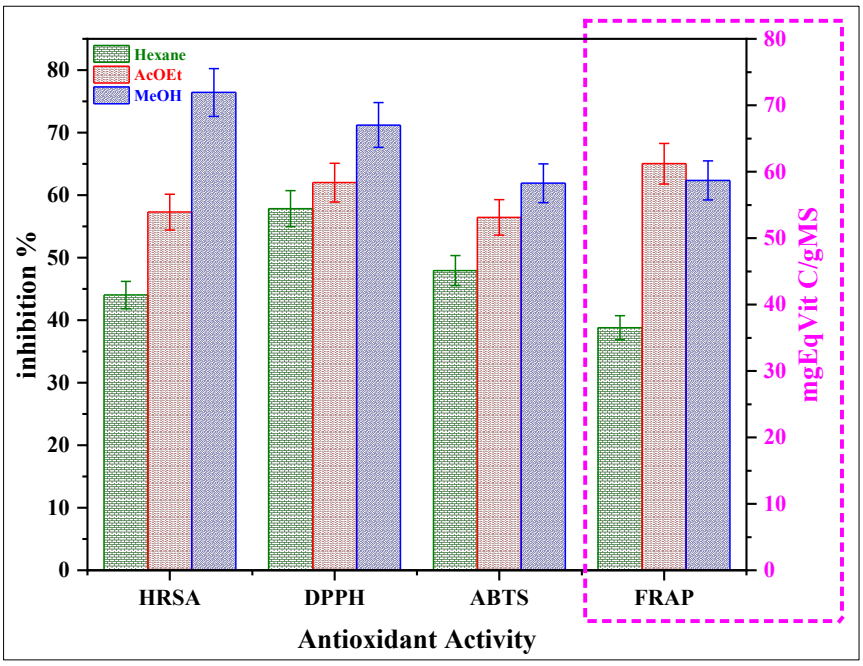

Figure 5: Antioxidant activity of various extracts from *Cola acuminata*

Analysis and Commentary on IC50 Results for *Cola Acuminata* Extracts

The Figure 6 presents the percentage of inhibition for the methanol extract (E.MeOH) of *Cola acuminata* and Butylated Hydroxytoluene (BHT) across two antioxidant assays: HRSA (Hydroxyl Radical Scavenging Activity) and ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid): The aim is to calculate the IC50 values, which represent the concentration of an antioxidant required to inhibit 50% of free radical activity.

HRSA Results

In the HRSA assay, the methanol extract demonstrates a gradual increase in percentage inhibition with increasing concentration, reaching 69.42% at 500 µg/mL. In comparison, BHT, a well-known synthetic antioxidant, shows a similar trend, achieving a maximum inhibition of 75.25%. The IC50 for E.MeOH can be estimated from the concentration that corresponds to approximately 50% inhibition, which appears to be around 300 µg/mL based on the data. This result indicates that while E.MeOH is effective, it may require a higher concentration than BHT to achieve comparable inhibition, suggesting that BHT is a more potent scavenger of hydroxyl radicals under the conditions tested.

ABTS Results

In the ABTS assay, E.MeOH shows an increase in inhibition from 35.89% at 100 µg/mL to 62.53% at 500 µg/mL. The IC50 for E.MeOH can likewise be estimated to be near 300 µg/mL, indicating a similar trend as observed in the HRSA assay. BHT again demonstrates superior performance, achieving 75.25% inhibition at the highest concentration, which reinforces the notion that synthetic antioxidants may have higher efficacy than natural extracts in certain assays.

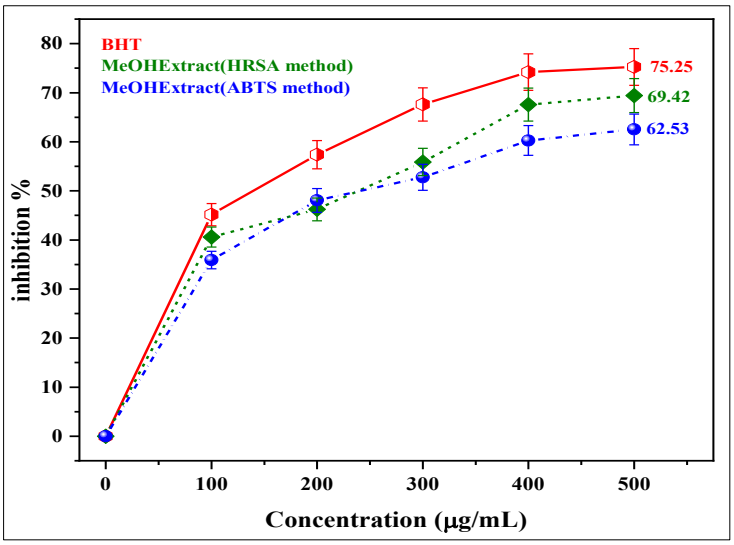

Figure 6: Percentage of inhibition for the methanol extract (E.MeOH) of *Cola acuminata* and Butylated Hydroxytoluene (BHT) across two antioxidant assays

4. CONCLUSION

The findings of this study underscore the significant phytochemical diversity present in *Cola acuminata* extracts obtained through various solvents, particularly methanol, ethyl acetate, and hexane. The methanol extract exhibited the highest concentrations of phenolic compounds, flavonoids, and tannins, correlating with its superior antioxidant activity across multiple assays, including HRSA, ABTS, and DPPH. The estimated IC50 values indicate that the methanol extract requires a concentration of approximately 300 µg/mL to achieve effective inhibition, which, while notable, is less potent than the synthetic antioxidant BHT. Moreover, the ethyl acetate extract revealed substantial levels of terpenoids and quinones, highlighting its potential for antimicrobial applications. Although the hexane extract showed lower phytochemical diversity, it still retained significant antioxidant properties, particularly due to the presence of phenols and tannins. The consistent presence of alkaloids across all extracts further emphasizes the coloring potential and biological properties relevance of *Cola acuminata*, suggesting its potential in developing natural dye products, antioxidant, anti-inflammatory, and antimicrobial properties. Overall, this study establishes *Cola acuminata* as a promising source of natural dye and bioactive compounds for applications in the tanning, pharmaceutical and food industries. Future research should focus on the utilization of extracts towards leather tanning, along with an exploration of their action on skin, to fully harness the coloring potential of this plant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Statement - Studies in Humans and Animals: Not applicable

Data Availability: Data will be made available on request.

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Highlights

- *Cola acuminata* extracts exhibit diverse phytochemical profiles and coloring properties.
- Methanol extract shows superior antioxidant activity across multiple in vitro assays.
- Different solvents extract distinct phytochemicals with potential dyes properties.
- Alkaloids are present in all extracts, indicating potential anti-inflammatory and antimicrobial activities.
- *Cola acuminata* is a promising source of natural dye molecules and bioactive compounds.

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