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Quantification of Total Phenolics, Flavonoids, Tannins, Anthocyannins and Antioxidant Activities of *Cola urceolata K. Schum*

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ABSTRACT: *Introduction:* *Cola urceolata K. Schum* is a plant of the *Sterculiaceae* family of the genus *Cola*. It is a species widely used in traditional medicine for its therapeutic properties and in food as a nutrient.
Objectives: As part of the valuation of medicinal plants from the Central African Republic, the interest of this study is focused on ethnobotanical surveys, phytochemical analyzes and the antioxidant activity (DPPH) of *Cola urceolata K. Schum*.
Methods: For the extraction, four solvents of different polarities (Cyclohexane: CYHA; Dichloromethane: DCM; Ethyl Acetate: EtOAc; Methanol: MeOH) were performed. Phytochemical tests were based on color reactions and chromatographic analyzes. The 96-well plate reader was used for quantitative analyzes of polyphenols, tannins, flavonoids and anthocyanins.
Results: The results of the extraction showed that the methanol extract has the best yield of 8.21% followed by the cyclohexane extract 3.88%. The polyphenol content was better than the other quantified compounds. It varies between 8.90 ± 1.06 and 15.09 ± 1.58mg of gallic acid equivalent (EqAG). Qualitative screening showed the presence of alkaloids, anthocyanins, sterols and triterpenes. The inhibition of DPPH by the different extracts gave mean values which varied between 4.65±2.85 and 15.17±4.60%. The results of principal component analysis (PCA) carried out using the «FactoMineR» and «factoextra» packages of the R software circle of correlation of variables.
Conclusion: The phytochemical screening confirms the presence of certain molecules which can confer on *Cola urceolata K. Schum* its therapeutic virtues.

KEYWORDS: *Cola urceolata K. Schum,* Total Polyphenols, Total anthocyanins, antioxidant activity.

1 INTRODUCTION

Plants are known for their production of natural substances. These products have multiple interests, particularly in the food, cosmetics and pharmaceutical industries. Medicinal plants have various biological and pharmacological activities. From the 1940s until today, natural molecules or molecules derived from natural molecules used on the oncology market represent 55% [1]. Plants are reservoirs of a wide variety of secondary metabolites: alkaloids, phenolic compounds and terpenoids [2]. A natural antioxidant in plant resources can protect biological systems from oxidative stress [3]. Polyphenolic compounds are among the molecules sought for their physiological and pharmacological activities [4]. They are natural compounds widely distributed in the plant kingdom. Their role as natural antioxidants is generating increasing interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases. Polyoxygenated steroles and triterpenes have selective cytotoxicity and anti-tumor properties [5].

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In view of the beneficial effects assigned to polyphenols and its derivatives, alkaloids and other natural compounds, our research is directed towards the class of chemical molecules endowed with biological activities.

Medicinal plants used in the Central African pharmacopoeia to treat many pathologies were targeted. The aim of this work was to quantify the content of polyphenols and other compounds of therapeutic interest. Also, to realize the antioxidant activity of the extracts of the retained plant. After ethnobotanical surveys and the literature review, a plant was selected for this study, it is *Cola ureceolata K. Schum.*

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The 7th arrondissement of Bangui is located on the right bank of the Oubangui River in the Central African Republic. It covers an area of more than 1792 ha = 17.92 / km2 located with a coordinate of 4° 22’32 nord, 18 ° 36’12. The choice is made on *Cola urceolata Sterculiaceae*, after ethnobotanical surveys followed by bibliographical summaries. The leaves were harvested at the edge of the Landja river in the 7th arrondissement in the Central African Republic on March 25, 2021. The plant was identified at the Center for Studies and Research in Pharmacopoeia and Traditional African Medicine (CERPHAMETA) by Doctor Olivia SEMBOLI. The harvested parts were dried in the open air at the LAARSN Laboratory for ten days.

2.2 METHODS

2.2.1 EXTRACTION

The methods of Worowounga et al [6] and Gbogbo et al [7] were used with modifications. One hundred grams (100g) of powder from the leaves of *Cola urceolata K. Schum* was extracted successively with four solvents of increasing polarity (Cyclohexane: CYHA; Dichloromethane: DCM; Ethyl Acetate: EtOAc; Methanol: MeOH). For each extraction, 100g of powder is placed in 400mL of cyclohexane for 4 hours at room temperature. After filtration, the extract is evaporated using a rotavapor at 35°C. The residues are taken up with the following solvent.

2.2.2 PHYTOCHEMICAL STUDIES

The major families of secondary metabolites were sought by colored reactions in a tube according to the classical methods of characterization on the powder of the plants studied. Alkaloids were identified by Mayer's tests, tannins by the $FeCl_3$ test, anthocyanins by the ammonia reaction, saponins by the foam test. As for the triterpenes and steroids, the Liebermann-Burchard test was used [8].

2.2.3 DETERMINATION OF TOTAL POLYPHENOLS

The method of Namkona et al [9] to determine the content of polyphenols was applied by modification. A volume of 20 µL of extract is added to 100 µL of Folin reagent solution (0.2 N). The plate containing the solutions is shaken for 30 seconds followed by a 5-minute incubation protected from light. After the incubation, 80 µL of Na_2CO_3 are added. The mixture is stirred again for 30 seconds with 15 minutes incubation at room temperature. The absorbance is read on a plate reader at 620 nm. Gallic acid is the standard used for this test. If the extract absorbs, the whites of the extract are subtracted from the read absorbances of the extracts. The results were expressed in milligram of catechin equivalents per g of dry weigh (mg CE/g dr).

2.2.4 LES TANNINS

For the determination of the condensed tannin content extracts of *Cola urceolata K. Schum*, the Namkona method was used with modification [9]. 50 µL of extract solution was mixed with 150 µL of vanillin (1% in 7 M H_2SO_4) in an ice bath and then

incubated at 25 °C. After 15 min, the absorbance of the solution was read at 500 nm. Results were expressed as mg catechin equivalents per g of dry weigh (mg CE/ g dw).

2.2.5 LES ANTHOCYANES

Two buffer solutions were prepared: The first solution consisted of hydrochloric acid and potassium chloride (pH 1.0 and 0.2 M respectively). The second buffer solution was a mixture of acetic acid and sodium acetate (pH 4.5 and 0.2 M respectively). Briefly, 100 µL of the buffer solution was added to 100 µL of extract. The reading was made on two wavelengths at 450 and 620 nm after 15 min of incubation [9]. The following equation was applied for the calculation: A = [(A450 – A620) pH 1.0 - (A450 – A620) pH 4.5].

The results were expressed in milligram of cyanidin-3-glucoside equivalents per g of dry weigh (mg C3GE/ g dw).

2.2.6 FLAVONOIDS

Quercetin was used as reference compound to allow drawing the standard curve. A volume (100 µL) of the diluted extract (3 mg/ mL) was mixed with 100 µL of solution aluminum trichloride $AlCl_3$ (2%) in MeOH. After an incubation of 15 min, the absorbance was measured at 415 nm [9]. The results were expressed in milligram of quercetin equivalents per g of dry residue (mg QE/g dr).

2.2.7 DPPH RADICAL SCAVENGING ACTIVITY

The DPPH solution (0.35M) was dissolved in MeOH. This solution was diluted 1/10 times. The extract (3mg / mL) of *Cola urceolata K. Schum* was dissolved in DMSO. In the well, 180 µL of DPPH is added to 20 µL of *Cola urceolata K. Schum* extract. The mixture is stirred for 30 seconds and followed by an incubation of 25 minutes. The absorbance is measured at 450nm [9]. Quercetin is used as a benchmark. Percentage scavenging activity was calculated using this formula: % of DPPH inhibition= $[(A_b-A_a) /A_b] \times 100$. Where Aa and Ab are the absorbance values of the test and of the blank, respectively was used as a standard.

2.2.8 STATISTICAL ANALYSES

Principal component analysis (PCA) was performed using the "FactoMineR" and "factoextra" packages of the R software circle of correlation of variables.

3 RESULTS AND DISCUSSION

Ethnobotanical investigations carried out on the *Cola urceolata K. Schum* plant have given the following results: maceration of the leaves is used in the treatment of chronic sorcerer's poisoning, treatment of chronic wounds, abdominal pain and the treatment of diabetes. The work of Apema et al [10] confirms the use of this plant in the treatment of diabetes.

3.1 YIELD OF EXTRACTION

The extraction yield varied between 0.93 and 8.21% (Figure 1). Methanol extract has the best yield of 8.21% followed by cyclohexane extract of 3.88%. Extraction data for *Cola urceolata K. Schum* are not available in the literature.

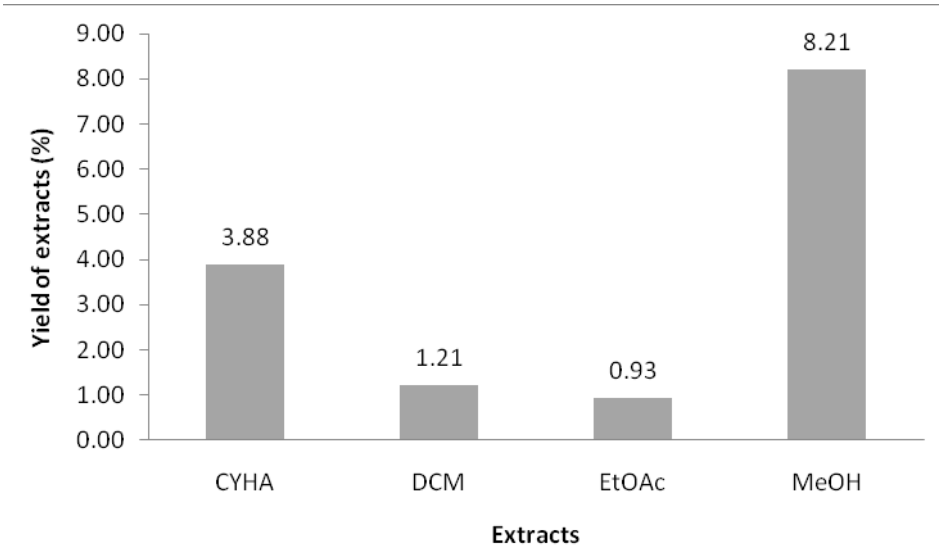
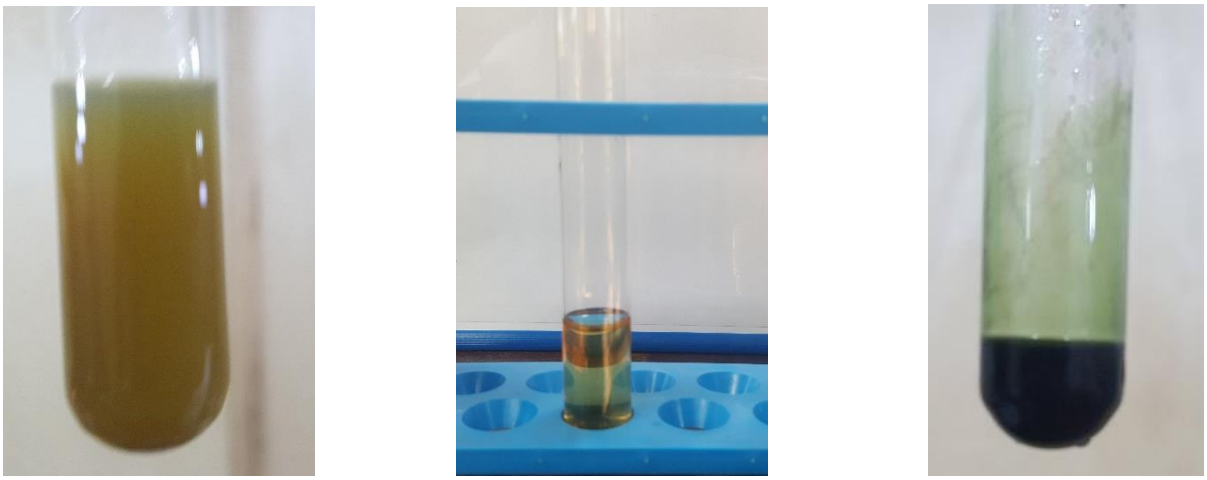


Fig. 1. Rendement d'extractionde Cola urceolata K.Schum

3.2 PHYTOCHEMICAL SCREENING

The formation of a cloudy precipitate in the tube indicates the presence of alkaloids (Figure 2). The tube, which has a colouration which is accentuated by acidification and which turns blue-purplish in a basic medium, indicates the presence of anthocyanins. A formation of the brownish red ring at the contact zone of the two liquids followed by a supernatant layer which turns green was formed, it is revealed the presence of sterols and tri-terpenes in the leaves of *Cola urceolata K.Schum*.



Alkaloids *Anthocyanins* *Sterols and triterpenes*

Fig. 2. Qualitative analysis results

Qualitative analysis results indicated the presence of alkaloids, anthocyanins, sterols and triterpenes. We noted, however, the absence of the tannin families and the saponisides (Table1). Phytochemical studies of four *Cola* species of the *Sterculiaceae* family (*Cola acuminata, Cola nitida and Cola gigantea*), shows that alkaloids are present in all the four species [11].

Table 1. Phytochemical screening of extracts from different parts of leaft Cola urceolata K.Schum

Extract	Alkaloids	Tannins	Anthocyanins	Saponosides		Sterols and triterpenes
				Presence	Foam index	
Cola urceolata K. Schum	+++	-	++	-	0	++

3.3 TOTAL POLYPHENOLS, FLAVONOIDS TANNINS AND ANTHOCYANES CONTENTS

The total polyphenol contents of the various extracts vary between 8.90 ± 1.06 and 15.09 ± 1.58 mg of gallic acid equivalent (mg EqAG / g) and are higher than the other families of quantified compounds. They are followed by anthocyanins which vary between 5.36 ± 0.13 and 0.40 ± 1.18 mg EqCya / g. However, the contents of flavonoids and tannins are very low compared to polyphenols from different extracts. In conclusion, the methanol extract contains more polyphenols than the other extracts (Table 2). No data on the content of families of compounds is mentioned in the extracts of *Cola urcelata K. Schum* leaves, therefore our results obtained for the first time are original.

Table 2. Content of polyphenols, flavonoids tannins and anthocyanins of extracts from leaves of Cola urceolata K. Schum

Extract	Polyphenols mg GAE/g dr	Flavonoids mg QE/g dr	Tannins mg CE/ g dr	Anthocyannis mg C3GE/ g dr
CYHA	9.36±0.78	0.01±0.00	nd	8.67±1.83
DCM	8.90±1.06	0.01±0.00	nd	5.29±0.33
EtOAc	15.09±1.58	0.08±0.01	0.06±0.02	5.36±0.13
MeOH	14.2±1.29	0.08±0.00	0.07±0.01	0.99±0.05

nd: not detected; dr: dry residue

3.4 ANTIOXIDANT ACTIVITY

The results of the antioxidant activities showed that the percentages of DPPH inhibition by the extracts of *Cola urceolata K. Schum*were between 4.65 and 15.17% for a concentration of 0.3 mg / mL (Figure 3). The inhibition value of DPPH by the MeOH extract was 15.17 ± 4.60%. EtOAc and DCM extracts of 13.18 ± 4.91 and 9.50 ± 00% respectively were followed. The lowest value is that of CYHA which was 4.65 ± 2.85%. The extracts of *Cola urceolata K. Schum* tested against DPPH have very low antioxidant activity, compared to that of quercetin used as the reference, which inhibited at 77.33% for a concentration of 3.33 µg / mL. No data available in the literature on the antioxidant activity of *Cola urceolata K. Schum.*

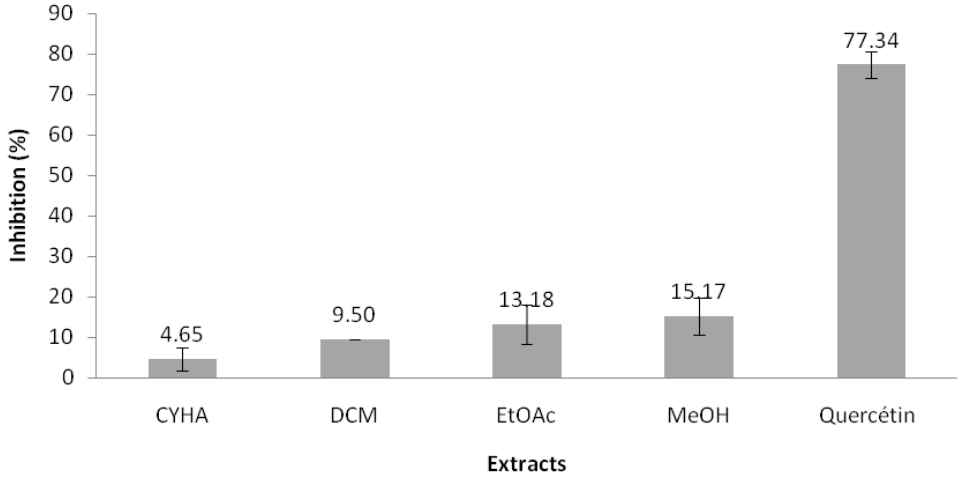


Fig. 3. Antioxidant activity of extracts from Cola urceolata K.Schum

3.5 PRINCIPAL COMPONENTS ANALYSIS

The results of principal component analysis (PCA) carried out using the "FactoMineR" and "factoextra" packages of the R software circle of correlation of variables are in figure 4. Positively correlated variables are grouped together. There are a positive correlation between polyphenols and flavonoids.Variables that are close to the center of the graph are less important for the first components.

Principal component analysis (PCA) was performed to understand how the TPC, TFC and TAC contribute to the biological activity (anti-DPPH) of the plant extracts.

Principal components (PC), PC1 and PC2 were showed 79 and 11.13% of the total data variance, respectively (Fig. 4).

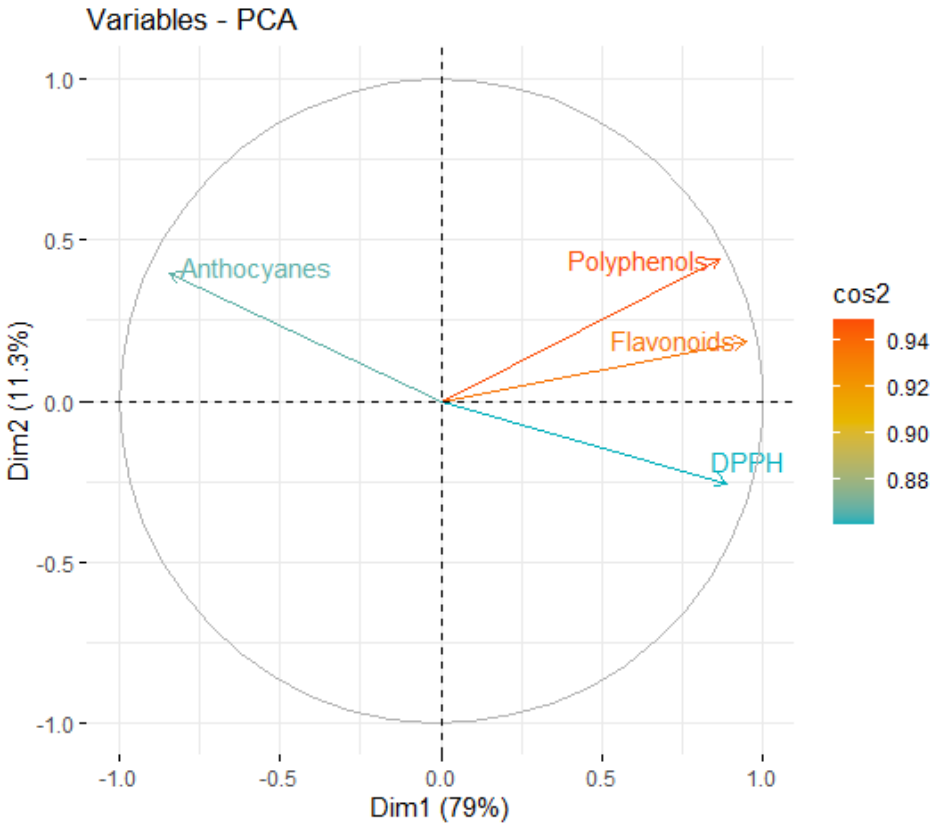


Fig. 4. ***Principal components analysis "FactoMineR" and "factoextra" packages of antioxidant properties (total phenolic content; total flavonoids content; total anthocyanin content; DPPH: antioxidant activity)***

3.6 PEARSON CORRELATION MATRIX

It shows the correlation values (degree of linear relationship between each pay variable). If the two variables tend to increase and decrease at the same time, the value of the correlation is positive. When one variable increases while the other decreases, the value of the correlation is negative (table 3).

Overall, there was a strong positive correlation between DPPH and TFC. TPC and TFC content positively contributed to an increase of potential inhibition against DPPH with Pearson correlation coefficients (*r*) equal to 0.61 and 0.83, respectively. There was a low negative correlation between TAC-anti-DPPH, TAC-TFC and PPH with an *r*-value of -0.62, 0.67 and -0.72 respectively (Table 3).

This indicates that the flavonoids compounds contribute to the inhibition of this mentioned activity.

Table 3. Pearson correlation matrix (Pearson (n))

	Polyphenols	Flavonoids	Anthocyanes	DPPH
Polyphenols	1.00	0.85	-0.62	0.61
Flavonoids	0.85	1.00	-0.67	0.83
Anthocyanes	-0.62	-0.67	1.00	-0.72
DPPH	0.61	0.83	-0.72	1.00

4 CONCLUSION

The phytochemical screening of extracts from the leaves of *Cola urceolata K. Schuma* revealed the presence of alkaloids, anthocyanins, steroids and triterpenoids. The quantitative analyzes gave a better rate of polyphenolics than those of tannins, flavonoids and anthocyanins. The results of the antioxidant activity of the extracts of the leaves of this plant had nevertheless remained significantly lower than that of quercetin used as a reference. It is therefore very likely that they contain compounds which, when purified, may exhibit activity comparable to that of quercetin. Further research is needed to isolate, purify and identify these constituents. Overall, there was a strong positive correlation between DPPH and TFC.

AUTHOR CONTRIBUTIONS

Experiments and the article writing were done by Xavier Worowounga. Bienvenu Armand Éric Foto, Jean-Laurent Syssa-Magalé supervised the work, validated the experiments, proofread, and refined the article to be ready for publication. Semboli Olivia was performed the treatment and statistical analysis. Armel-Frederic Namkona and Issa-Madongo Mathurin made the collection of plant samples, ethnobotanical investigation in the field. Koueni-Ouakounda Kevin Hermann preparation of extracts and reagents in the laboratory.

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