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Research Article

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Phytochemical tests and evaluation of antioxidant and xanthine oxidase inhibitory activities of *Commiphora pedunculata (Kostschy et peyr) (Burseraceae)* stem bark extracts

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I. Abstract

The present work deals with the research of chemical constituents and evaluation of antioxidant and xanthine oxidase inhibitory activities of *Commiphora pedunculata* (Kostschy et peyr) stems bark extracts. In order to carry out this work, the extractions were made by three techniques namely infusion, decoction and maceration with the various solvents of increasing polarity such as cyclohexane, dichloromethane, methanol and methanol-water. Phytochemical screening reveals the presence of compounds such as alkaloids, flavonoids, saponosides, and catechic tannins in the methanol extracts, infusion and decoction of *Commiphora pedunculata* tested. Terpenes and steroids are revealed in the cyclohexane extract. The quantitative analyses present the maximum values measured in the Methanol extract of total phenols contents with a level of 0.358 mg EAG/g ES, total flavonoids of 0.466 mg EQ/g ES, total anthocyanins of 4.673 mg EAG/g ES. For biological activities, the extracts of the plant showed antioxidant and xanthine oxidase inhibitory activities. These results obtained, are due to the polyphenolic compounds highlighted in the extracts of this plant.

Keywords: Chemical constituents, Antioxidant activities, Xanthine oxidase and *Commiphora pedunculata*

II. Introduction

Since the dawn of time, plants have been used in various ways by humans. According to the World Health Organization, nearly 80% of the population depends on traditional medicine for primary health care (WHO, 2002). In Chad, the majority of the population has first resorted to traditional care through medicinal plants before considering modern medicine for reasons of lack of health structures, long distances from hospitals, insufficient number of health care personnel and especially lack of financial means to take care of health care.

Free radicals would be at the origin of several pathologies among which we can mention arthrosis, asthma, cancer, diabetes, heart disease, atherosclerosis (Ikram Hamlaoui, 2014; Sarr et *al*., 2015). Xanthine oxidase catalyzes the two terminal steps of purine base catabolism, namely the conversion of hypoxanthine to xanthine and xanthine to uric acid (Garat A, 2009). This reaction is responsible in several pathologies such as diabetes, cardiovascular problems, hyperuricemia and gout. Xanthine oxidase is one of the main sources of these free radicals. Just as there are compounds that can scavenge free radicals, allopurinol is the main inhibitor of xanthine oxidase. However, these compounds create many side effects, allopurinol for example generates superoxides (Berry and Hare, 2004).

Polyphenols and especially flavonoids extracted from plants have powerful antioxidant properties capable of inhibiting the formation of free radicals, chelating metal ions and opposing the oxidation of macromolecules including xanthine oxidase (XO) and hypoxanthine (HXO) (Lespade, L., Bercion, S., 2010).

In the perspective of the discovery of compounds or extracts with antioxidant and antiradical potential, we oriented our study on one of the plants with proven antioxidant properties. It is *Commiphora pedunculata.*

The plant *Commiphora pedunculata* (kostschy et peyr) Engl. is a shrub often thorny of about five (5) meters in height, exceptionally reaching ten (10) meters of the family Burseaceae. It is widely spread on the African continent and especially distributed in the dry regions of Africa, from Senegal to Ethiopia to South Africa. In Chad, it is found in the south of the country and north of Lake Chad (Jean César; Chatelin Cyrille, 2019). Traditionally, it is used throughout the world. *Commiphora pedunculata* is used in the treatment of gout (Pierre Bonnet, et al. 2008; M. Séverin, 2017), inflammation and other diseases. (L.Lagnika, 2005). Resin is used in fumigation to perfume and disinfect homes (Michel Arbonnier, 2009). The use of bark against dysentery could be justified by the antibacterial properties reported by Akor and Anjorin (2009), the astringent and antibacterial properties of tannins are reported by Bruneton (2009). The use of the leaves against cough is justified by the antitussive properties reported (Adebayo et al 2006). Antimicrobial activity of hexane, ethyl acetate and methanol extracts of C. *pedunculata* stem bark has been reported (Sallau et *al* 2014). Adebayo et *al*. (2006); Akor and Anjorin (2009) showed that the leaves contain tannins, alkaloids, triterpenes, sterols and phenolic compounds.

The main objective of this study is to valorize *Commiphora pedunculata* (Kostschy et peyr) as a plant with antioxidant and antiradical potential, through a phytochemical study, an evaluation of the antioxidant activity and the determination of the xanthine oxidase of different extracts.

III. Materials and Methods

III. 1. Plant material

The plant material of our study is the bark of Commiphora pedunculata stems. They were collected in the Western Logone region during the month of April 2021. They were washed, cut, dried in the sun and light, then ground and pulverized. The powder obtained was transported to the laboratory of natural substances of the Faculty of Exact and Applied Sciences of the University of N'Djamena for possible analysis.

III.2 Methodology

III.2.1. Extraction

In order to carry out the phytochemical analyses and to evaluate the antioxidant activities and the inhibitory activity of xanthine oxidase, the extractions were made by three following techniques:

Infusion: 20g of *Commiphora pedunculata* stem bark powder in 200 mL of distilled water boiled at a ratio of 1/10 (w/v) for 15 min. The whole was cooled and filtered under reduced pressure then the filtrate was kept in the refrigerator at 4°C for possible studies.

Decoction: 2g of powder of the plant material was put in 100 mL of distilled water. The whole was boiled for 30 minutes. Filtration was carried out under reduced pressure after cooling and the filtrate was kept in the refrigerator at 4°C for possible studies.

Maceration: solvents of increasing polarity (cyclohexane, Dichloromethane, ethyl acetate and Methanol) were used for these extractions. For each extract, the operation is done in doublet to optimize the extraction. First, 50 g of the plant material powder was introduced into 50 mL of the solvent. The whole is stirred for 24 hours. After filtration, the residue was put into the same amount of solvent and stirred for 2 hours. Extracts scored 1 and 2 are pooled, concentrated with a rotavapor, labeled and stored in the refrigerator for analysis. The residue is taken up in the increasing order of solvent polarity following the same protocol. Thus, we have the extracts with cyclohexane, dichloromethane, ethyl acetate and methanol.

III.2.2. Chemical analysis

III.2.2.1. Phytochemical screening

The identification of the chemical families of the plant extracts was done by associating two methods: the coloring test and the thin layer chromatography (TLC).

Reactions in tubes

The chemical tests for the characterization of the main chemical groups were carried out on the extracts of Commiphora pedunculata stems (kostschy et peyr). The detection of the main chemical groups in the extracts was performed following the protocols described and used at the Department of Chemistry of the University of N'Djamena (Yaya, 2014).

Expression of reaction results in tubes

The research of chemical constituents was carried out by reactions. The results are classified according to the sign (+) for the very positive tests and the sign (-) for the negative tests.

Thin layer chromatography (TLC)

To confirm the results of the tube reactions, phytochemical screening was performed by thin layer chromatography (TLC) on ALUGRAM Xtra SIL G/UV254 plates according to the methods described by Hildebert Wagner and Sabine Bladt (Wagner and Bladt, 1996).

Thin layer chromatography (TLC) is a qualitative method to confirm the results of tube characterization reactions. Extracts were separated using several specific solvent systems. The identification of the compounds was carried out according to 2 methods: on the one hand under the UV lamp at 366 nm and on the other hand by the observation of the color of the spots resulting from the separation after the revelation by the specific reagents. The revelation was made by means of specific chemical developers in particular the sulphuric anhisaldehyde and the aluminium trichloride to 2% in the methanol.

III.2.3. Quantitative analyses

III.2.3.1. Preparation of the solution for the determinations

The extracts used in this study were prepared at a concentration of 2 mg/mL. The solution obtained is kept protected from light until its use.

III.2.3.2. Determination of total phenolic compounds

The determination of total polyphenols was carried out spectrophotometrically, according to the colorimetric method using the Folin-Ciocalteu reagent (Mokhtar Annaba. 2011). In test tubes, 2 mL of each solution is introduced, followed by the addition of 5 mL of Folin-Ciocalteu reagent, after 8 minutes 4 mL of an aqueous solution of sodium carbonate Na2CO3 at (7.5%) (w/v) were added. The whole set is incubated in the dark for 30 minutes at room temperature. The blank is prepared in the same way but replacing the 2 mL of extract with 2 mL of distilled water. The absorbance of each solution was read using a Shimadzu UV-1601 double beam spectrophotometer at a wavelength of 765 nm. A calibration curve was performed under the same operating conditions using gallic acid at different concentrations (0 to 1000 µg/ml). The results are expressed as microgram equivalents of gallic acid per gram of dry extract (µg EAA/g ES).

III.2.3.3. Determination of total flavonoids

The quantification of flavonoids was performed by a method based on the formation of a very stable complex, between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of flavonoids (Nassir Tajudden et *al*,. 2005). The protocol used is based on the one described by (Kim et al. 2004). In a glass hemolysis tube, 7 mL of extract, or standard, or distilled water for the control was added and then 5 mL of AlCl3 was added. The mixture was incubated in the dark for 15 minutes. The absorbance was read immediately at 510 nm against the control. A methanolic solution of quercetin was prepared at different concentrations to allow the calibration curve to be plotted.

III.2.3.4. Determination of total monomeric anthocyanins: differential pH method

a) Preparation of solutions

A 0.025 M aqueous potassium chloride solution was used as the first reagent. The pH of the solution was then adjusted to 1 using a 6 N hydrochloric acid solution.

A 0.4 M aqueous sodium acetate solution served as the second reagent. The pH of the solution was then adjusted with 6 N hydrochloric acid solution to the value of 4.5.

b) Procedure

The protocol used is described in the work of (Aïra Rezaire, 2012). Six glass hemolysis tubes were used per extract at different given concentrations. The contents of three of them were diluted with the potassium chloride solution, while the other three are diluted with the sodium acetate solution. The volume of the extract should not exceed 20% of the total volume. The tubes were then incubated in the dark for 15 minutes and the absorbances were read at 520 nm and 700 nm against the control containing distilled water.

Expression of results

This is a differential test. Thus the final absorbance is obtained by the difference of the absorbance measured at different wavelengths:

Abs= Abs520- Abs700pH1- Abs520- Abs700pH4,5

The calculation of the concentration of total monomeric anthocyanins (TMA) provides results that are expressed in mg/l :

AMT= Abs x MxDFx1000εxl

With M = 449.2 g/mol, molar mass of cyanidin-3-glycosylated
DF : the dilution factor of the analyzed sample
l : the length of the measuring cell in cm
ε = 26.900 $M^{-1}cm^{-1}$: the molar extinction coefficient of cyanidin-3-glycosylated

The total monomeric anthocyanins (TMA) are then expressed in mg/100 g dry matter (DM):

TMA in mg EEC3G/g DM = total monomeric anthocyanins (mg/L)Sample concentration

TM in mgEEC3G100g dry matter =TMA in mgg dry matter×100×extraction yield

III.2.3. Biological analysis of the extracts

III.2.3.1-Evaluation of the antioxidant activity

Many methods are used to evaluate the antioxidant activity of extracts. Most of these methods are based on the coloration or discoloration of a reagent in the reaction medium. These redox reactions involve either proton transfer or electron transfer. The DPPH and FRAP tests are used in this study.

Evaluation of the antioxidant activity of extracts by the DPPH method

This method is based on the reduction of the stable radical species DPPH- in the presence of a hydrogen donor antioxidant (AH), which results in the formation of a non-radical form. In the presence of free radical scavengers, the purple DPPH- reduces to yellow DPPH.H.

The antioxidant activity of the extracts of the different plants was determined by mixing 0.16 ml methanolic solution of DPPH at the concentration of 0.04 mg/ml with 8 ml of extracts at concentrations ranging from 0 to 1 mg/ml. The absorbance reading was taken at 517 nm after 30 min of incubation in the dark.

The percentage of DPPH radical inhibition was calculated according to the following relationship:

DPPH inhibition %= 1-essay OD blanc OD x100

All measurements are replicated at least three times to minimize errors. The comparison of inhibitions was done using quercetin solutions in the same concentrations (D.Kim, et al. 2003).

Evaluation of antioxidant power by ferric ion reduction (FRAP)

The reducing power of an extract is associated with its antioxidant power. The reduction of iron produced by the extracts is determined according to the method described by Oyaizuand repeated by Nabila (Nabila et al. 2012). It is based on the reduction of ferric ion Fe3+ to ferrous iron Fe2+.present in the K3Fe(CN)6 complex. Thus, one milliliter of extracts of different concentrations (from 0.018 to 2.0 mg/mL) was mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH=7) and 2.5 mL of a 1% K3Fe(CN)6 potassium ferricyanide solution. The whole is heated in a water bath at 50°C for 20 min. Then 2.5 mL of 10% trichloroacetic acid was added to stop the reaction. The tubes were centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of supernatant was diluted with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl3 aqueous solution. The absorbance of the reaction medium was read at 700 nm using a double beam UV spectrophotometer (Shimadzu 1601 spectrophotometer) against a blank that had undergone the same preparation, replacing the extract with distilled water. The activity of the extracts was compared to that of controls, gallic acid, whose absorbance was measured under the same conditions as the extracts, but at lower concentrations. An increase in absorbance corresponds to an increase in the reducing power of the extracts tested (Singleton and Rossi, 1965).

III.2.3.2. Evaluation of the inhibitory activity of xanthine oxidase

To reduce the excess of uric acid in the body, the enzyme, xanthine oxidase intervenes by catalyzing the oxidation of hypoxanthine and xanthine into uric acid. During this oxidation, molecular oxygen acts as an electron acceptor producing superoxide radicals and hydrogen peroxide (Terada et al. 1990).

The reagents used in this manipulation were prepared in a buffer solution pH = 7.5 obtained by mixing 46 mL of 0.15M Na2HPO4 with 4 mL of 0, 1M citric acid):

Xanthine oxidase (4.6 unit/0.57 mL): 155 µL dilution of this enzyme to 5000 µL with buffer solution pH = 7.5 (working concentration 0.25 unit/mL);

Xanthine: 2.28 mg of xanthine in 400 µL of 0.1M NaOH with buffer solution pH = 7.5;

Allopurinol: 1mg/mL in buffer solution pH = 7.5;

This volume includes the plant extract (at concentrations of 90.8, 181.8, and 363.6 µg/mL) or allopurinol (at concentrations of 10, 30, and 50µg/mL), phosphate buffer solution pH = 7.5, and xanthine oxidase solution. The different volumes of reagents used are recorded in Table 2. (Yaya, 2014) (Yaya, 2014)

Table 1: Volumes (µL) and concentrations (µg/mL) of reagents used.

Sample name and concentration (µg/mL)	Plant extract (Ci=2mg/mL)	Xanthine oxidase (Ci=0.25unit/mL)	Allopurinol (Ci=1mg/mL)	Buffer pH 7.5	Xanthine (Ci=0.6Mm)
Plant extract 1 (363.6)	200	100	-	200	600
Plant extract 2 (181.8)	100	100	-	300	600
Plant extract 3 (90.8)	50	100	-	350	600
Allopurinol:ALL1 (50)	-	100	200	370	600
Allopurinol ALL2 (30)	-	100	150	350	600
Allopurinol ALL3 (10)	-	100	100	400	600
Control negative	-	100	-	400	600

After incubation at the temperature of 25°C for 3 minutes, of the reaction mixture, the reaction was triggered by adding the xanthine solution to the mixture. The inhibitory activity of XO was evaluated by measuring the absorbance at 295 nm using a spectrophotometer (Thiombiano et al., 2014).

The percentage of XO inhibitory activity per samples analyzed and allopurinol is calculated according to the equation:

%inhibition= OD control-0D samples OD control×100
OD control: activity of XO without the extracts and/or allopurinol,
OD samples: activity of XO in the presence of the samples and/or allopurinol.

IV. Results and Discussions

IV.1 Chemical analysis

IV.1.1. Phytochemical screening

A phytochemical screening was done on the extracts obtained during the extractions to identify the different families of compounds present.

IV.1.1.1. Reaction in tubes

According to the family of molecules sought, the test was carried out on specific extracts according to the protocol used. For polyphenols, it is the 5% infusion which is used. These colorimetric tests are based on the interaction of certain functions with the reagents used. In the following table 3, is designed the results of different colorimetric tests.

Table 2: The result of the photochemical screening

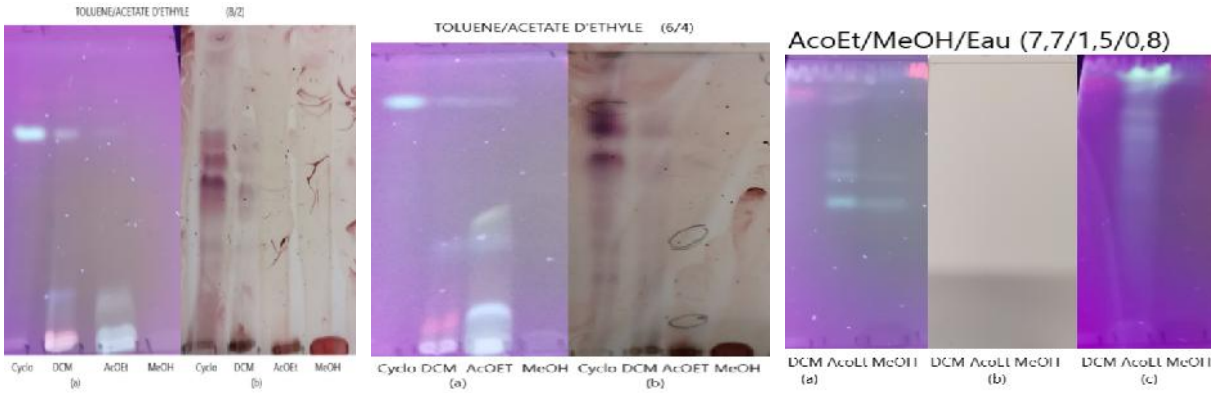
Extracts	Chemical Families	Reagents	Observations	
Cyclohexane	Terpenes/steroids			+
Methanol	Alkaloid	Dragendorff	-	-
		Mayer	Red	+
Infused	Flavonoids		Orange	+
	Tannin	$FeCl_3$	Red	+
		Stiansy	Red	+
Decoction	Saponosides		Foam	+
Dichloromethane	Cardiotonic heteroside	-	-	-

+ Presences and - absences

Phytochemical screening reveals the presence of compounds such as alkaloids, flavonoids, saponosides, and catechic tannins in the methanol extracts, infused and decoction of Commiphora Pedunculata tested. Terpenes and steroids are revealed in the cyclohexane extract. But there is absence of cardiotonic heterosides in the dichloromethane extract. These results corroborate to those obtained by Sallau and collaborators (Sallau, M.S et al., 2014) where polar compounds are evidenced in polar extracts and terpenes/steroids are detected in petroleum ether extract as we also observed in cyclohexane extract.

IV.1.1.2. Thin layer chromatography

All the results of this analysis are recorded on the following figures where the solvent systems are marked.



I II III

Figure 1: I and II (a) UV 366nm revelation and (b) anisaldehyde revelation and heating to 105°C; III (a) UV 366 nm revelation, (b) visible observation and (c) UV 366 nm observation after sputtering with $AlCl_3$

At 366 nm, some compounds such as phenolic acids, flavonoids, coumarins, saponosides alkaloids etc. appear as blue and grey fluorescent spots and the red color that of anthocyanins. From figures I and II, the Rf of the blue spots of the ethyl acetate extract, shows the presence of terpenes with alcohol or ester functions and the presence of terpenes/steroids by the red colors. The chromatograms of III, confirms the presence of polyphenols which could be simple coumarins and flavonoids other than flavonols and flavones (Wagner and Blad, 1996). These results confirm those obtained from the coloring reactions in Table 2.

Previous studies have shown the presence of flavonoids. Flavonoids have even been isolated and one of which is known epicatechin from the soluble ethyl acetate fraction of the methanol extract of stem barks (Nasir Tajuddeen, et al. 2015). From Figure 1, blue spots are clearly seen before and after revelation with aluminum trichloride. This would confirm the presence of polyphenolic compounds in the extracts of the analyzed samples. Although all the families highlighted in the tube reactions were not confirmed by TLC, we can say that the different extracts of the plant would contain most of them. In particular the polyphenols which must be quantified.

IV.2. Quantitative analyses

IV.2.1. Determination of total phenolic compounds

The quantitative analyses of total phenols, were determined from the equation of the linear regression of the calibration curve, plotted using gallic acid as standard (Figure 2). The values obtained are expressed as mg GAE/g ES and mg GAE/g DM.

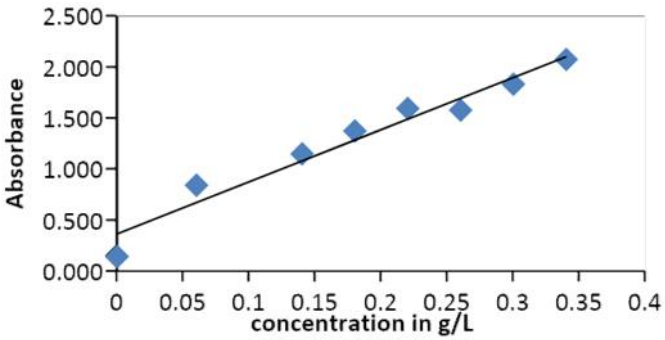


Figure 2: Calibration curve of gallic acid

Table 3 presents the results of the quantification of total polyphenols.

Table 3: Results of polyphenol determination

	Solvent extracts of increasing polarity		Crude extracts		
	Cyclohexane	Methanol	Methanol-water	Infused	Decocted
Quantity of total polyphenols in mg GAE/g DM	0.293±0.001	0.358±0.025	0.345±0.002	0.321±0.010	0.343±0.009

The total polyphenol contents of the different extracts ranged from 0.293 to 0.358 mg GAE/g DM. The highest concentration of phenols was measured in the methanol extract, with a level of 0.358 mg GAE/g DM, compared to the other samples, where we record contents in the order of 0.343 and 0.321 and 0.293 mg GAE/g DM respectively for the decoctate, infused.

IV.2.2. Determination of total flavonoids

The levels of flavonoids in the extracts were calculated from the calibration curve, plotted using quercetin as standard (Figure 3).

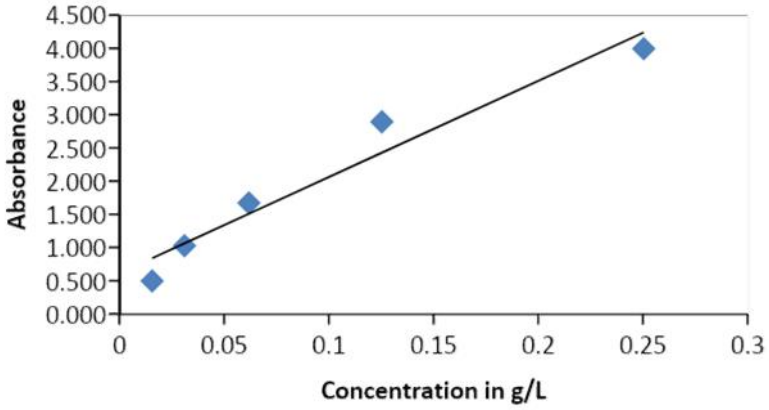


Figure 3: Calibration curve of quercetin

Figure 3 allowed to quantify the content of Total Flavonoids in the extracts of the plant. The quantities obtained from the different extracts tested are placed in Table 4.

Table 4: Result of the determination of Flavonoids

	Extracts with solvents of increasing polarity		**Crude extracts**		
	Cyclohexane	Extract Methanol	Methanol-water	Infused	Decocted
Quantity of total flavonoids (mg QE/g DM)	0.326±0.072	0.466±0.039	0.449±0.002	0.392±0.006	0.452±0.009

The results presented in Table 4 show that the total flavonoid contents vary considerably between the different extracts. The methanol extract has a maximum flavonoid content of 0.466 mg EQ/g DM, followed by the decoctate which has as content 0.451 mg EQ/g DM and the methanol-water extract 0.449 mg EQ/g DM. The infusion has acontent of 0.392 mg EQ/g DM. While the lowest flavonoid content was found in the cyclohexane extract of 0.326 mg EQ/g DM.

IV.2.3. Anthocyanin determination: differential pH method

The spectrophotometric method of differential pH allows a measurement of the absorbances of anthocyanins and other interfering compounds in relation to their functions. It is based on the measurement of the absorbance of extractive solutions diluted with buffer solutions of pH = 1 and pH = 4.5.

Anthocyanins are reversibly transformed under the influence of pH. The structural change associated with the modification of the chromophores determines the different color of the anthocyanin solutions as a function of pH. The colored form (oxonium) predominates at pH 1 and the colorless form (hemiacetal) at pH 4.5. The results of the assay are shown in the following table 5.

Table 5: Anthocyanin assay results

	Extracts with solvents of increasing polarity		Extraits bruts		
	Cyclohexane	Extract Methanol	Methanol-water	Infused	Decocted
Quantity of Anthocyanin in mg QE/g DM	0.334±0.334	4.673±2.334	1.836±0.16	1.335±0.60	0.723±0.255

The analysis reveals that the methanol extract has the highest anthocyanin content of 4.673 ± 2.334 mg QE/g DM, followed by the methanol-water extract of 1.836 ± 0.16 mg QE/g DM, then the infused extract of 1.335 ± 0.60 mg QE/g DM and the decocted extract of 0.723 ± 0.255 mg QE/g ES and finally the cyclohexane extract of 0.334 ± 0.334 mg QE/g DM. These results show that all the *Commiphora pedunculata* extracts studied contain anthocyanins. So we can use this plant as a source of anthocyanins.

IV.3. In vitro analysis of extracts

IV.3.1. *In vitro* analysis of antioxidant activities of the extract

The absorbance values of the extracts read at different concentrations allowed us to calculate the percentage of radical inhibition using DPPH tests. The results allowed us to draw the curves recorded in figure 4 below. The standard used is quercetin.

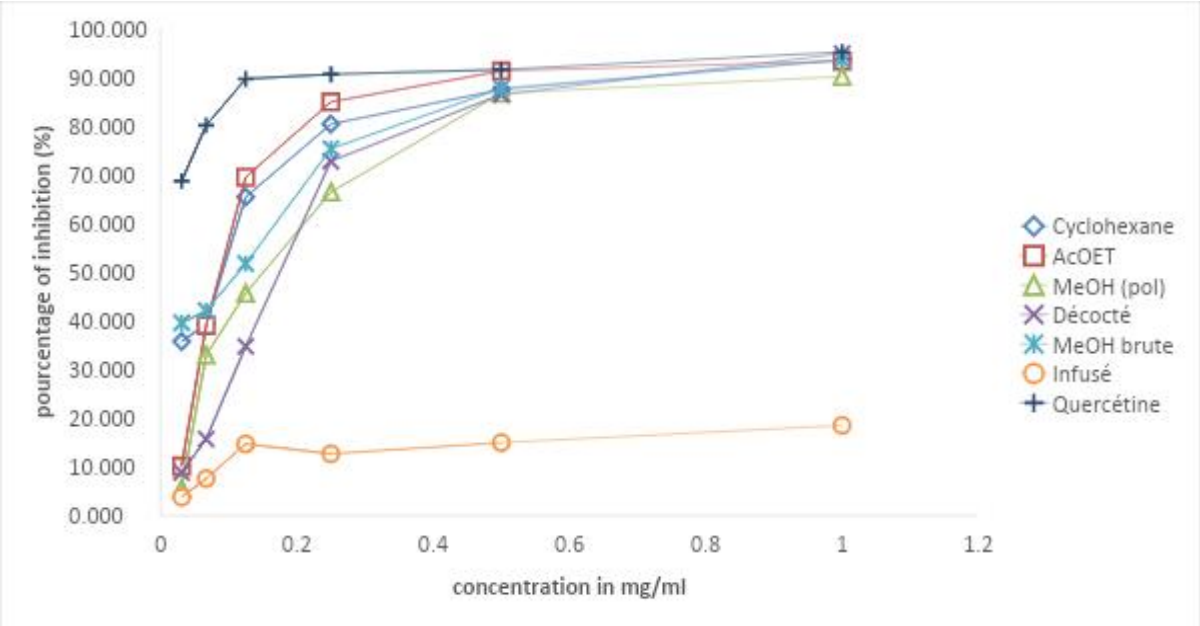


Figure 4: Antioxidant content per DPPH

These results refer to values representing the means of two measurements ± standard deviation. The figure shows the percentages of DPPH radical inhibition in descending order as follows: after queketin, the reference molecule, comes the ethyl acetate extract, the cyclohexane extract, the crude methanol extract, the decoctate, the methanol-water extract and the infused one.

Finally, it can be said that the study of the antioxidant activity of *Commiphora pedunculata* showed the free radical scavenger potential of this plant which could be considered as a source of natural antioxidants. The antioxidant activity observed would be due to their high polyphenol and anthocyanin content.

The high content of polyphenols in the cyclohexane extract could explain in large part its better antioxidant capacity compared to the others.

IV.3.2-Analysis of the antioxidant power by reduction of the ferric ion (FRAP)

The evaluation of the reducing power of the extracts also showed a better activity of the bark extract as shown in Figure 5.

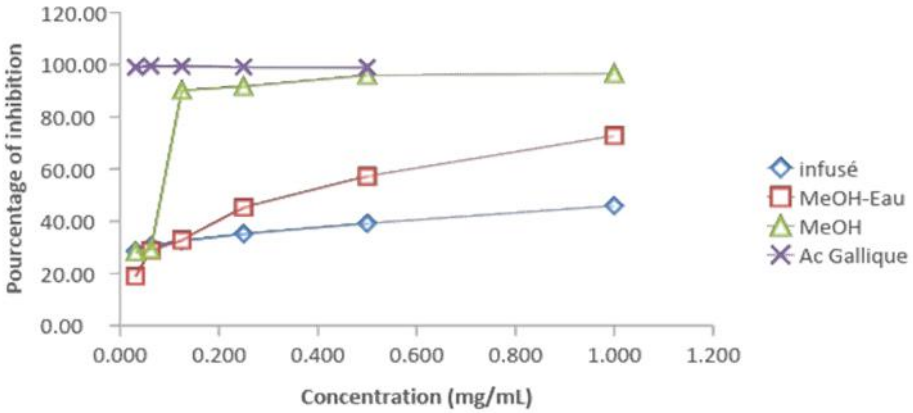


Figure 5: Ferric ion reduction test

The results of the ferric ion reduction test show that the methanolic extract obtained after prefractionation, gives a better inhibition followed by the hydromethanolic extract. For all samples, at the concentration higher than 0.60 mg/ml), the inhibition percentages exceed 50%.

In this antioxidant activity, we see in these figures that the percentages of inhibition of the infused are lower than the other samples at concentrations above 0.20. On the other hand, for concentrations closer to 1mg/ml, the inhibition of the other extracts is close to that of the reference molecules. These results are explained by the presence of flavonoids, ploypheno1s, tannins highlighted in the qualitative analysis of phytochemistry, These results corroborate the results of Gulçin and his collaborators and those found in the literature (Bruneton J, 2009; Gulçin et al., 2010).

IV.3.3-Xanthine oxidase assay

In this assay, the methanol-water and crude methanol extracts and then the Infused were tested. Allopurinol was used as a positive control. The measurement of the activity was performed using a spectrophotometer. The absorbance reading was taken at 295 nm against the blank which does not contain the enzyme solution. The percentage of xanthine oxidase (XO) inhibitory activity was determined from the absorbance versus time (s) curve. The results of this activity are shown in Figure 6, giving the percentage of inhibition as a function of the concentrations of each plant extract.

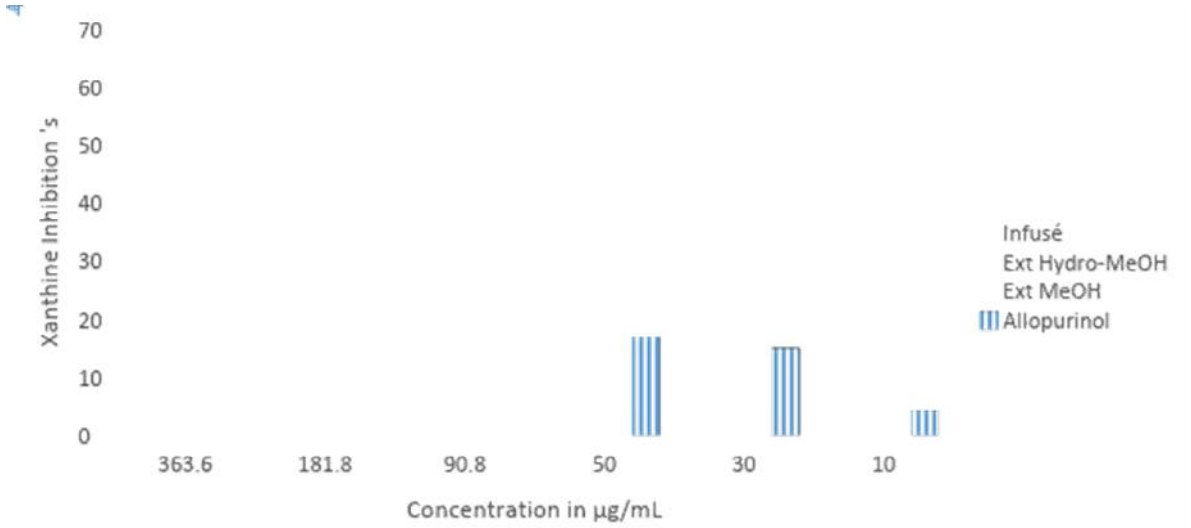


Figure 6: Percentage inhibition of xanthine oxidase

The methanol extract at the concentration of 90.8 µg/mL has an inhibition that approaches those of the others at higher concentrations. This could be explained by the fact that this prefractionated extract contains the phenolic compounds probably more than the flavonoids.

Previous studies have shown that flavonoids, tannins as well as coumarins are good inhibitors of XO (Iio et al., 1985; Hatano et al., 1990).

The results obtained from the biological activities although in vitro, are due to the polyphenolic compounds evidenced in the extracts of this plant. Flavonoids for example do not only inhibit and deactivate free radicals but also participate in the neutralization of oxidizing enzymes such as xanthine oxidase and chelate metals depending on their chemical property.

Conclusion

The present work on phytochemical tests and evaluation of antioxidant and xanthine oxidase inhibiting activities of *Commiphora pedunculata* (kostschy et peyr) (Burseraceae) stem extracts allowed us to have sufficient results to valorize this plant as a source of antioxidants and inhibitors of xanthine oxidase.

The qualitative analysis reveals the presence of alkaloids, flavonoids, saponines, and catechic tannins in the methanol extracts, infusion and decoction of *Commiphora pedunculata*. Terpenes and steroids are revealed in the cyclohexane extract. These results are confirmed by analysis on thin layer chromatography.

Quantification shows that the crude methanol, methanol-water, infused and decoction extracts are rich in polyphenols and flavonoids. For anthocyanins, the results show that all the extracts of *Commiphora pedunculata* studied contain anthocyanins. So, we can use this plant as a source of anthocyanins.

For biological activities, the extracts of the plant showed antioxidant and xanthine oxidase inhibiting activities. These results are due to the polyphenolic compounds found in the extracts of this plant. The flavonoids for example do not only inhibit and deactivate free radicals but also participate in the neutralization of oxidizing enzymes such as xanthine oxidase and chelate metals depending on their chemical property.

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