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Identification of chemical families and radical scavenging activity of extracts from plant organs of *oncoba welwitschii* (Oliv.)

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Abstract
In Africa, particularly in Congo-Brazzaville, plant dependencies play a very important role in the well-being and living environment of citizens; this is for example the case of *Oncoba welwitschii* (Oliv.) whose different parts are used for the treatment of certain pathologies. This study was conducted to identify the chemical families and evaluate the antioxidant activity of extracts of *Oncoba welwitschii* (Oliv.). Total phenols were determined using Folin-Ciocalteu's reagent and flavonoids using AlCl3. The antioxidant activity was evaluated by DPPH methods. Phytochemical screening of leaves, flowers, barks and roots revealed the presence of alkaloids, flavonoids, terpenes and sterols, coumarins, tannins, nitrogen compounds, polyphenols and sugar. The results of the flavonoids assay showed that the ethanolic extracts of the leaves are the richest in flavonoids (126.72±10.86mg EQ/g), than the extract of the flowers, barks and roots. The polyphenol content is highest in the bark (826.08±12.45 mg GAE/g), followed by the leaves (794.31±15.10 mg GAE/g). The results reveal that the flowers and barks are rich in terpene compounds and have greater antioxidant activity than the leaves, which have a higher content of flavonoids and polyphenols. The presence of these compounds in this plant could justify its use in traditional care.

Keywords: *Oncoba welwitschii*, phytochemical screening, flavonoids, polyphenols, antioxidant activity, DPPH

1. Introduction
Nowadays, natural products are an important source for the search for new active compounds against many diseases. The therapeutic use of plants is an integral part of the traditions of all cultures. The medicinal valorization of these practices notably involves the identification and isolation of new molecules. This is the case of *Oncoba welwitschii* (Oliv.), a plant presumed to have amazing medicinal properties such as: antiseptic, antimicrobial. It is used in the treatment of syphilis, gonorrhea, sepsis, urinary and genital or intestinal infections. Subsequent work by Silou [1], as well as by Radna *et al*., [2], reveals that the seeds of this plant are very rich in vegetable oil (about 39.3% to 42.0%), active ingredients cited as chaulmoogric acid which has a characteristic optical power [2]. It is for this reason that we are interested in directing our work on this plant. Indeed, our work consisted in identifying certain chemical families and evaluating the antioxidant activity of extracts from the leaves, flowers, barks and roots of *Oncoba welwitschii* in order to find a scientific basis that could explain and confirm the use of this plant in traditional medicine.

2. Materials and Methods
The work began with the identification of the plant at the Institute of Research in Natural Sciences (IRSN) of Brazzaville in Congo, made by Doctor Jean-Marie MOUTSAMBOTE (Ethno botany); then, a survey of the uses and also the geographical distribution zones of the plant.

2.1. Plant material
The parts of the *Oncoba welwitschii* (Oliv.) plant used in our studies were harvested in Brazzaville in September 2021 in the Republic of Congo. The study was carried out on samples of leaves, flowers, bark and roots of *Oncoba welwitschii* (Oliv.), which were dried in the sun for 72 hours (three days) and then ground with an electric grinder until obtaining of a powder.

The storage of the material for possible subsequent studies is done carefully away from light and humidity.

2.2. Preparation of extracts
10g of each sample (leaves, fruits, barks and roots) were prepared in a hydro-alcoholic mixture (50mL of water and 50 mL of ethanol), 24 hours later, filtered, added to the hydro-ethanoic solution 20 mL of chloroform is obtained and the mixture is separated into two (02) phases (chloroform phase and a hydro-alcoholic phase). At the end, each solution is concentrated, the Fe1, Fr1, Et1, Ra1 fractions (hydro-alcoholic fractions) and Fe2, Fr2, Et2, Ra2 (chloroform fractions) are obtained. With Fe = leaves, Fr = flowers, Et = barks and Ra = roots.

2.3. Phytochemical screening of Oncoba welwitschii (Oliv.) extracts
The identification of the chemical families was carried out by thin layer chromatography according to the methods of Harbornes and Wagner [1, 3]. Seven chemical families were researched, namely: alkaloids, flavonoids, terpenes and sterols, amino acids, tannins, saponins and sugars. The operating conditions are described in Table 1.

2.4. Dosage of flavonoids
The flavonoid content of extracts and fractions obtained from leaves, flowers, barks and roots of *Oncoba welwitschii* was determined by the method described by Ono *et al*., [4]. This method consists of adding 0.5 mL of an $AlCl_3$ solution (2% in absolute ethanol) to 0.5 mL of sample. After one hour of incubation at room temperature, the absorbance is measured at 420 nm. The total flavonoid content is calculated in terms of quercetin equivalent by reference to the calibration curve.

2.5. Dosage of polyphenols
The total polyphenol content of extracts and fractions obtained from leaves, flowers, barks and roots of *Oncoba welwitschii* was determined by the method described by Sokman *et al*.,[5] and Milcent *et al*.,[6]. These authors used the FolinCiocalteu method to calculate the polyphenol content of a plant extract. This method consists of adding 2.5 mL of a FolinCiocalteu solution (0.2N) to 0.5 mL of sample. After stirring for 5 minutes, 2 mL of a sodium carbonate solution (75 g/l) are added. After 2 hours of incubation at room temperature, the absorbance at 760 nm is measured. The results are expressed in terms of gallic acid equivalents using a calibration curve.

2.6. Anti-radical activity
The anti-free radical activity was demonstrated by DPPH (1,1-Diphenyl-2-picrylhydrazyl) test. The extract to be tested is deposited on a thin layer (silica plate) and the chromatogram is developed in the following solvent system: But/AcOH/AcOEt/H_2O (9/6/3/2). These show up as a light yellow color on the chromatogram.
All experimental procedures were performed in triplicate and their mean values (± standard deviation) were given.

3. Results and Discussion
3.1. Results
3.1.1. Phytochemical screening
The result of the phytochemical screening of the extracts of leaves (Fe), flowers (Fl), barks (Et) and roots (Ra) obtained by the analysis of chromatograms 1 to 8, show the presence of alkaloids, flavonoids, terpenes and sterols, coumarins, proteins and amino acids and sugars (Figure 1).
The brown spots on the deposit line of chromatogram 1 revealed with the iodo-mercuric reagent are characteristic of the alkaloids; they are present only in the x and y extracts. The fluorescent blue spot of chromatogram 2 after visualization with 10% NaOH characterizes the coumarins. The flavonoids are present in all the extracts, they are revealed with Neu in the form of blue, blue-yellow, orange spots of chromatogram 3. Chromatograms 4 and 5 revealed with sulfuric anysaldehyde show pink, blue-green and gray spots, characteristics of terpenes and sterols. Chromatogram 6, revealed with sulfuric naphthol-2, highlights saponosides (greenish yellow spot), sterols (gray spots) and sugars (blackish gray spots). The black spots of chromatogram 7 revealed with 2% iron trichloride characterize the tannins and polyphenols. Ninydrine highlights proteins and amino acids in the form of pink spots on the chromatogram 8. Kabena *et al*., [7] have highlighted on the Bas-Congo variety alkaloids, tannins, anthocyanins, polyphenols and quinones by precipitation and tube staining reactions. In our study quinones were not sought as well as anthocyanins[8]. The work of Ngoua Meye Misso *et al*. [9], on the Gabon variety, the tube reactions revealed an abundance of coumarins, saponosides, polyphenols and alkaloids [9]. Our results are closer to those of Ngoua Meye Misso *et al*. [9]. The difference lies in the abundance of flavonoids, sugars and the presence of proteins and amino acids as well as the method used. Indeed, tube reactions are less specific because of false positives than thin layer chromatography. This can also be justified by chemotypy because the chemical composition varies with the medium.
Polyphenols have the role of protection against biotic and abiotic attacks, antifungal activity, antiviral activity, antioxidant activity and anti-inflammatory activity [10]. The presence of alkaloids can explain various biological activities. As for terpenes, they are used as additives in the food and cosmetics industries [11] and several of them have biological activities: antimicrobial, insecticidal, anti-carcinogenic, anti-inflammatory, anesthetic and antihistamine (mono and sesquiterpenes), diuretic, neuroprotective. Mention may also be made of the anti-tumor and cytotoxic properties of diterpenes (taxol), and antioxidant activities attributed above all to phenolic diterpenes. The presence of coumarins can translate or explain the antifungal, antibacterial, antiviral, anti-inflammatory, anti-tumor and anticoagulant effect of certain extracts.

3.1.2. Assay of flavonoids and polyphenols in extracts of leaves, flowers, barks and roots of Oncoba welwitschii
3.1.2.1. Dosage of flavonoids
The flavonoid content of the extracts of leaves, fruits, barks and roots of *Oncoba welwitschii* was calculated in terms of quercetin equivalent by reference to the calibration curve according to the values of absorbance (A) according to the expression: $A_1 = 0.0096C_1 + 0.0339$; $R^2 = 0.9992$.
Where C_1 is the concentration (mg/mL), by means of which the flavonoid content in the leaves is evaluated at 126.72 ± 10.86 mg EQ/g of mass of dry matter (DM) or 1.27%; in the flowers, it is 32.53 ± 1.22 mg EQ/g MD or 0.33%; in the bark, the content is 70.44 ± 2.81 mg EQ/g DM or 0.70% and in the roots, it is evaluated at 25.60 ± 1.94 mg EQ/g or 0.26% (Table 2).

3.1.2.2 Dosage of polyphenols

The total polyphenol content was obtained by the curve of the calibration line of quercetin as a function of the absorbance values (A_2) according to the equation: $A_2 = 0.025C_2 + 0.0243$; $R^2 = 0.9946$.

Where C_2 is the concentration (mg/mL), by means of which the polyphenol content in the leaves is evaluated at 794.31 ± 15.10 mg GAE/g of mass of dry matter (DM) or 0.79%; in the flowers, it is 473.24 ±6.13 mg GAE/g MS or 0.47%; in the bark, the content is 826.08 ±12.45 mg GAE/g DM or 0.83% and in the roots, it is evaluated at 223.13 ± 5.83 mg GAE/g or 0.22% (Table 3).

3.1.3. Anti-radical activity

The anti-radical activity was demonstrated by DPPH test: The fraction to be tested was deposited on a thin layer (silica plate); the chromatogram is developed in the following solvent: But/AcOH/AcOEt/H_2O (9/6/3/2). Active spots show up as a light yellow color on the chromatogram (Figure 2).

4. Discussion

The objectives of this work were to carry out a study on the identification of certain chemical families and to highlight the antioxidant activity of ethanolic extracts from the roots, barks, flowers and leaves of *Oncoba welwitschii*. The molecules present in the different plant organs are important for the treatment of certain pathologies and an injudicious use of the organs of medicinal plants could compromise the preservation of biodiversity. The phytochemical screening carried out on the different organs of *Oncoba welwitschii* a showed that the leaves, flowers, barks and roots of this species contain flavonoids, alkaloids, nitrogen compounds, tannins, sugar and terpenes and sterols. However, note the absence of coumarins in the three parts of the plant (leaves, flowers and bark). Alkaloids are present in the flowers, but they are absent in the other organs of the plant (Figure 1). The work of Kabena *et al.*, [7], also showed that the leaves of the same species from the DRC (Democratic Republic of Congo) also contain flavonoids, alkaloids, polyphenols, tannins, anthocyanins and others [12]. Coumarins are strongly present in the species from Gabon, but they are absent from *Oncoba welwitschii* in the species from the Democratic Republic of Congo. The results obtained from leaves, flowers, roots and bark in Gabon are similar to those of Kabena *et al.* [7], which revealed almost the same composition of secondary metabolites in the species harvested (roots and barks) from the Democratic Republic of Congo [7]. Nevertheless, these authors found a low content of alkaloids [9, 10]. The richness of the species *Oncoba welwitschii* at these large groups of active chemical compounds could then justify the traditional use of this plant to treat many diseases such as: syphilis, gonorrhea, sepsis, urinary and genital infections or intestinal [2]. Indeed, other authors have also shown that the different types of chemical compounds found in the bark, roots and leaves of this plant have proven therapeutic effects.

The results of the flavonoid assay (Table 2) showed that the ethanolic extracts of the plant are rich in these compounds. It should nevertheless be noted that the ethanolic extracts of the leaves are the richest in flavonoids with a value of 126.72 ± 10.86 mg EQ/g. The ethanolic extract of the flowers, barks and roots contain less flavonoids (32.53 ± 1.22 mg EQ/g; 70.44 ± 2.81 mg EQ/g) compared to ethanolic root extracts (25.60 ± 1.94 mg EQ/g). Several factors can influence the flavonoid content. Various studies have shown that external factors (geographical and climatic factors), genetic factors, but also the degree of maturation of the plant and the duration of storage has a strong influence on the flavonoid content [13, 3, 14].

The analysis of Table 3 showed that the polyphenol content varies slightly from one ethanolic extract of one plant organ to another. It is the bark which recorded the highest content of polyphenols (826.08 ± 12.45 g GAE/100g), after the leaves of (794 ± 15.10 g GAE/100g), followed by flowers (473 ± 6.13 g GAE/100g), the roots are the least rich in polyphenols (223.13 ± 5.83 g GAE/100g). By expressing, for the leaves, flowers, barks and roots of *Oncoba welwitschii*, the percentages of total flavonoids of each extract relative to the mass of total phenols obtained are respectively 12.34%, 10.56% and 11.38%.

The ethanolic extract of the leaves, flowers, barks and roots are active after confirmation with DPPH. The presence of reducing agents in the plant extracts causes the reduction of the Fe^{3+} ion to the Fe^{2+} form. The reducing power of *Oncaba welwitschii* extracts is probably due to the presence of hydroxyl groups in the phenolic compounds, which can serve as electron donors. Antioxidants are considered reducers and in activators of oxidants [13].

Table 1: Experimental summary of screening

Chemical family	Elution system	Revelation and observation
Alkaloids	AcEt/MeOH/(12% NH_4OH) (9/1/1)	Iodo-mercuric reagent, visible observation.
Terpenes and sterols	Toluene-AcOEt (8/2)	Sulfuric anisaldehyde + heating at 110°C, visible observation
Coumarins	toluene/ AcOEt (9/3)	10% NaOH, Observation under UV at 365nm
Amino acids	But/AcOH/AcOEt/H2O (9/6/3/2)	Application of ninhydrin reagent by spray outlined under UV light at 365nm
Tannins	$CHCl_3$/ AcOEt/MEC (10/2/2)	FeCl3 at 2%, visible observation
Flavonoids	$CHCl_3$/ AcOEt/MEC (10/2/2)	Neu, observation under UV at 365nm
Sugars	V/formic acid/AcOH/water (5/1/1/5)	Naphthol-2 sulfuric heated to 110°C, visible observation

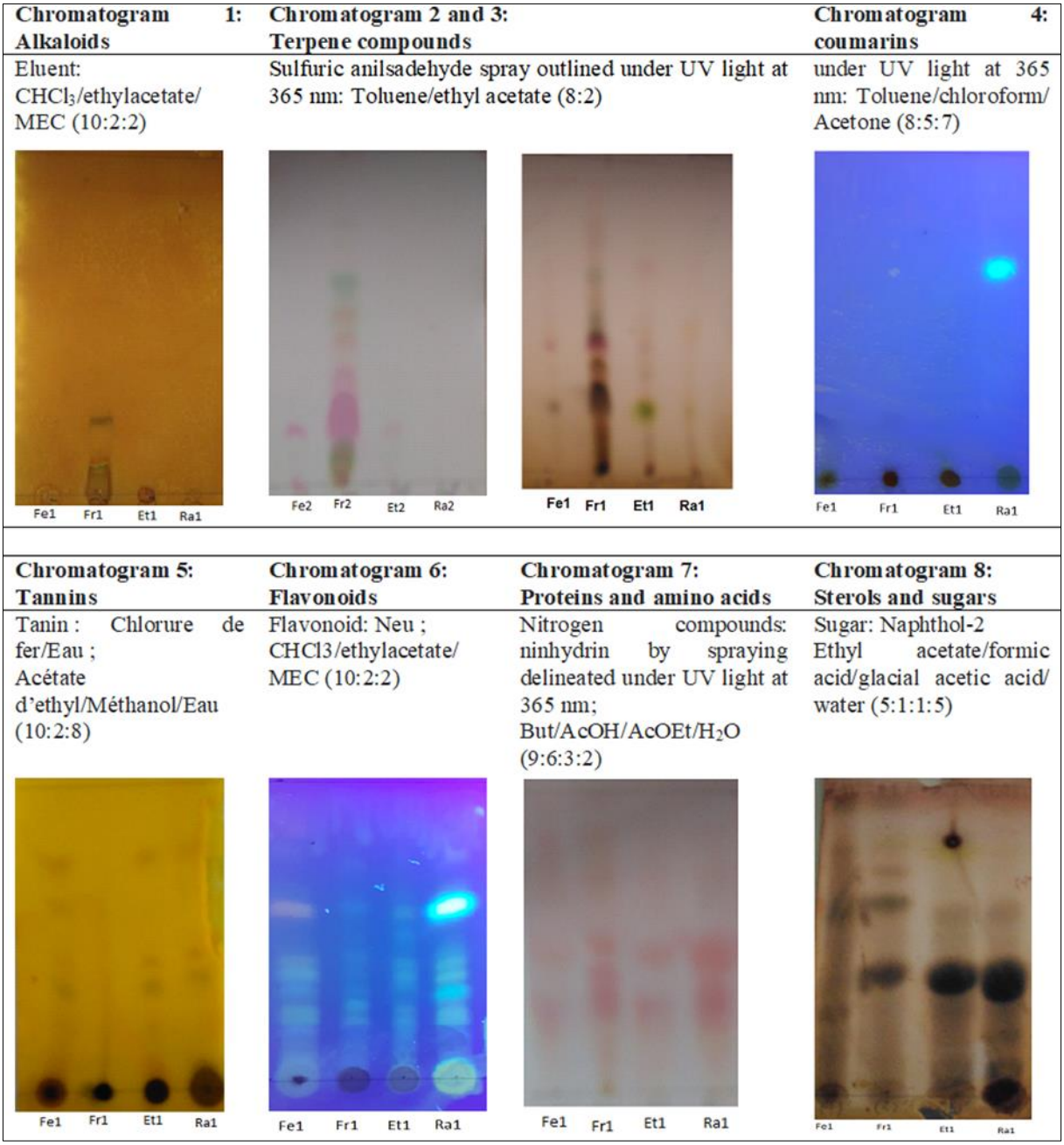
AcOEt: Ethyl acetate: MeOH: Methanol; NH_4OH: Ammonia; Purpose: Butanol; AcOH: Acetic acid; H_2O: Water; $CHCl_3$: Chloroform; DUDE

Table 2: Flavonoid content

Types of organs	Content mg EQ/g
Leaves	126.72 ±10.86
Flowers	32.53 ± 1.22
Bark	70.44 ± 2.81
Roots	25.60 ± 1.94

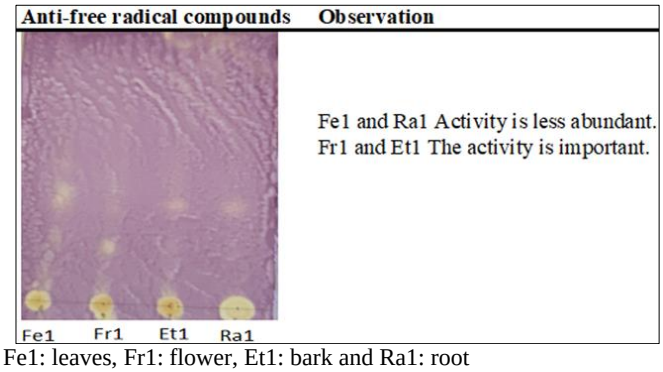
Table 3: Polyphenol content

Types of organs	Content mg EAG/g
Leaves	794.31 ± 15.10
Flowers	473.24 ± 6.13
Bark	826.08 ± 12.45
Roots	223.13 ± 5.83



Fe1: leaves, Fr1: flower, Et1: bark and Ra1: root

Fig 1: Summary table of chromatograms



Fe1: leaves, Fr1: flower, Et1: bark and Ra1: root

Fig 2: Chromatogram of anti-radical activity

Conclusion

Despite the biological and medicinal importance of *Oncoba welwitschii*, this species has previously only been studied from a botanical and ecological point of view. Phytochemical screening showed the presence of alkaloids, coumarins, tannins, terpenes and nitrogen compounds. In addition, free saponins and quinones are present in the Fr1, Fe1, and Ra1 fractions of *Oncoba welwitschii*. However, the present study has demonstrated the great richness of the plant in polyphenols, natural products of considerable interest in the pharmacological field. This work brings, therefore, a phytochemical contribution to the knowledge of *Oncoba welwitschii*, and thus makes it possible to better understand the pharmacodynamic properties of the extracts of this plant. It would therefore be very interesting to exploit these extracts for the search for their active principles, responsible for their pharmacological properties.

Authors' contributions

This work was carried out in collaboration among all authors. Authors NLAC, MYF, MS and OPR designed the study and wrote the protocol. Authors MYF and GT managed the literature searches, Authors NLAC, MYF and BSG wrote the

first draft of the manuscript. All authors read and approved the final manuscript.

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