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Research Article

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Phytochemical study and evaluation of the anti-radical activity of the hydroethanolic extract of the leaves of *Urena lobata* L. (Malvaceae)

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Abstract

The plant remains an inexhaustible source of new molecules or active ingredients responsible for the pharmacological effect of several pathologies. Free radicals being the cause of deterioration of human health, man resorts to medicinal plants from the Congolese pharmacopoeia. It is for this purpose that the present study aims to determine the chemical composition of polyphenolic compounds and to evaluate the anti-radical activity of the hydroethanolic extract and different fractions of the leaves of *Urena lobata* L. The experiment was carried out using the methods of liquid-liquid fractionation, thin layer chromatography and dosage of major chemical families to assess the presence and contents of these compounds. The free radical DPPH was used as a standard by the qualitative method by thin layer chromatography and by the quantitative method on spectrophotometer to evaluate the antiradical power. Our results reveal that high yields are observed in the ethyl acetate and aqueous fractions respectively of 13.5 and 8.2. The identification of chemical families revealed the presence of phenolic compounds and high contents are observed in the aqueous fraction for polyphenols respectively at 4807 mgEAG/100gMs and 258 mgEC/100gMs for flavonoid compounds. Indeed, the hydroethanolic extract and the aqueous fraction showed strong activity in reducing DPPH at IC50s of 1.66 mg/ml and 2.03 mg/ml. In conclusion, the study affirms the use of this plant in traditional Congolese medicine.

Keywords: Phytochemical study, anti-radical activity, hydroethanolic extract and *Urena lobata L.*

1. Introduction

Phytotherapy is experiencing extraordinary popularity throughout the world; it is impossible to see it as just a fashion phenomenon. Of course, our time is deeply marked by the search for a healthier life, a return to nature, to essential values. But the success of herbal medicine can be explained above all by the level of technical and scientific mastery that we now achieve in this field. Chemistry, pharmacology, and agronomy have made it possible, through progress, to develop therapeutic and galenic forms that are safer, more suitable, and more effective [1]Through its action, today plant medicine becomes a response to diseases such as stress, diabetes, high blood pressure, microbial infections and many others. Alongside these diseases, free radicals are involved in the etiology of a large number of pathologies which are now one of the major problems [2]. Free radicals are metabolic byproducts of oxidative processes. They are highly reactive and potentially damaging chemical species. Cells protect themselves from this using a variety of enzymes or endogenous chemicals that trap free radicals [3].During oxidative stress, untrapped free radicals induce tissue damage. This is why the contribution of medicinal plants remains a major issue in the fight against the various damages that a xenobiotic can create in the face of a health problem. It is with this in mind, the present study proposes to carry out a phytochemical study and evaluate the anti-racal potential of the hydroethanolic extract and different fractions of the leaves of the species *Urena lobata* L. in order to promote the Congolese pharmacopoeia

2. Materials and Methods

2.1. Plant material

The harvest of *Urena Lobata*.L leaves took place on APRIL 20, 2022 in the department of Brazzaville, more precisely in district no. 9 Djiri and then identified at the National Institute for Research in Exact and Natural Sciences (INRSEN). After harvesting, the leaves were dried in the laboratory at room temperature and

2.2. Methods

2.2.1. Liquid-liquid fractionation of hydroethanolic extract

The hydroethanolic extract was prepared by maceration of 100g of *Urena lobata* L. leaf powder in 1000 ml (50% v/v) for 72 hours. The macerated obtained was then filtered and then fractionated. The liquid extract obtained after evaporation of the ethanol subsequently undergoes a liquid-liquid separation on the scale of three types of solvents based on the gradient of increasing polarity (hexane, ethyl acetate and water). The fractions obtained are concentrated using a rotary evaporator then used for quantitative analysis. The ethyl acetate fraction is used for the isolation test of flavonoid compounds [4].

2.2.2. Detection by thin layer chromatography

The solvent extracts were qualitatively analyzed by Thin Layer Chromatography (TLC) on silica gel with an eluent composed of ethyl acetate/formic acid/water (8/1/1). The plates were revealed with Neu [5], followed by observation at 365 nm.

2.2.3. Determination of chemical families in extracts and fractions F1, F2 and F3.

To quantify two families were targeted, these are total polyphenols and flavonoids.

Determination of total polyphenols

The dosage was carried out as follows: to 0.1 ml of plant extract introduced into a test tube, 0.9 ml of distilled water was added; 0.9 ml of Folin-Ciocalteu reagent (1N); then immediately 0.2 ml of a Na2CO3 solution (20%). The mixture obtained was incubated at room temperature for 40 minutes protected from light. The absorbance was then measured with a spectrophotometer at 725 nm against an ethanol solution used as a blank. Note that a calibration line was previously carried out before analysis with gallic acid under the same conditions as the samples to be

analyzed. The results obtained were expressed in mg gallic acid equivalent per 100 grams of dry matter (mgEGa/100 gMs) [5].

Determination of total flavonoids

The total flavonoids were also measured using a spectrophotometer, as follows: 250 µl of the extract and 1 ml of distilled water were successively introduced into a test tube. At the initial time (0 minutes), 75 µl of a NaNO2 solution (5%) was added, followed by 75 µl of AlCl3 (10%) 5 minutes later. After 6 minutes, 500 µl of NaOH (1N) and 2.5 ml of distilled water were successively added to the mixture. The absorbance of the mixture obtained was directly measured with a UV-visible spectrophotometer at 510 nm and the results were expressed in mg Rutin equivalent per 100 grams of dry matter (mgERu/100g Ms). A calibration curve was developed with standard Rutin solutions prepared at different concentrations [5].

2.2.4. Dosage of anti-radical compounds

The measurement of the anti-radical activity of the hydroethanolic extracts of the plant was carried out by the DPPH test according to the protocol described by Hannebelle T.2006 [6].The solution test was carried out by mixing 10 ml of a DPPH solution at 10mg/250ml with 100µl of the extract to be tested at different concentrations (10mg/ml, 5mg/ml, 2.5mg/ml, 1.2mg/ml , 0.612mg/ml). The values obtained were measured at 517nm using a spectrophotometer after 40 min of incubation away from light.The inhibition percentage is calculated as follows:

$$I\% = \frac{Absorbancedublanc - Absorbancedutest}{Absorbancedublanc} \times 100$$

Abs0: absorbance of the blank against a solution of DPPH with methanol
Abst: absorbance of the test

3. Results and Discussion

3.1 Chemical analyzes

3.1.1. Liquid-liquid fractionation

Liquid-liquid fractionation using a gradient of increasing polarity gave three fractions whose physicochemical properties vary depending on the nature of the fraction (Table I).

Table 1: Nature and physical properties of fractions

Nature of the Fraction	Color	Physical nature	Smell	Yield
Hydroethanolic extract	Black	Solid	Little Aromatic	5.2
F1 (hexane)	Black	Pasty	Aromatic	2.6
F2 (Ethyl acetate)	Brown	Solid	aromatic	13.5
F3 (Water)	Light brown	Solid	aromatic	8.5

After separation by decantation on a funnel following the aforementioned polarity gradient, the physicochemical properties of which vary depending on the fractions. These are color, physical state, odor and yield. It should be noted that the color changes from black for the extract and fraction 1 to brown for the rest of the fractions. The physical state of the fractions varies from solid to pasty intermediate. The presence of the pasty physical nature confirms the character of this plant which could be justified by the presence of terpene compounds of aromatic plants. The high yield observed on the acetate fraction could be explained by its chemical composition in genin or aglycone type flavonoids.

3.1.2. Thin-layer chromotography.

The chromatogram of the hydroethanolic extract and the different fractions (F1, F2 and F3) of the leaves of *Urena lobata*. L are represented in the figure below (figure 8). These profiles show a series of spots of different colors obtained after spraying the plate with Neu and viewing with a

UV lamp at 366 nm, which materializes the presence of several chemical compounds.

The orange-yellow fluorescence at the frontal retentions (0.07 and 0.1) respectively in the hydroethanolic extract and the aqueous fraction marks the presence of quercetin derivatives and relates to a substitution on the B ring of a flavonol in 3' and 4' position Orthodihydroxylated.

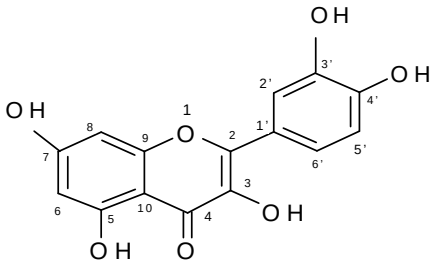

Figure 1: Structure 3',4', 5,7 tetrahydroxyflavonol: Quercetin [7]

The traces of bluish-white fluorescence respectively in the hydroethanolic extract and the aqueous fraction at the frontal retentions (0.75) materialize the presence of flavonones with luteolin derivatives.

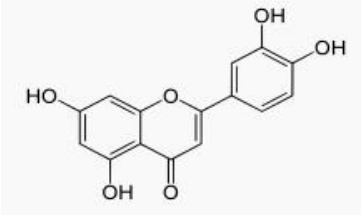

Figure 2: Structure 3' 4', 5, 7-trihydroxyisoflavone : Lutéoline[7]

The spots of blue fluorescence respectively in the hydroethanolic extract and the aqueous fraction at the frontal retentions (0.4, 0.6 and 0.82) clearly highlight in the extract and less clearly in the aqueous fraction F3 , could be attributed to flavonol derivatives hydroxylated in the 3' position

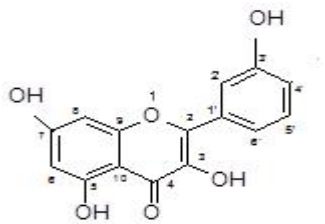

Figure 3 : Structure 3', 5, 7-trihydroxy flavonol : Kaempferol [7]

The red fluorescence at the respective frontal retention (0.82) in the hexanic fraction materializes the presence of a polar type flavonoids. After analyzing these results, these different types of compounds highlight compounds with a fairly significant power for the reduction of free radicals and would have several pharmacological effects. Our results are consistent with those obtained by Dixa Singh et al., (2016) [8]. who characterized and isolated flavonoid compounds in the leaves of the species *Urena lobata* L.

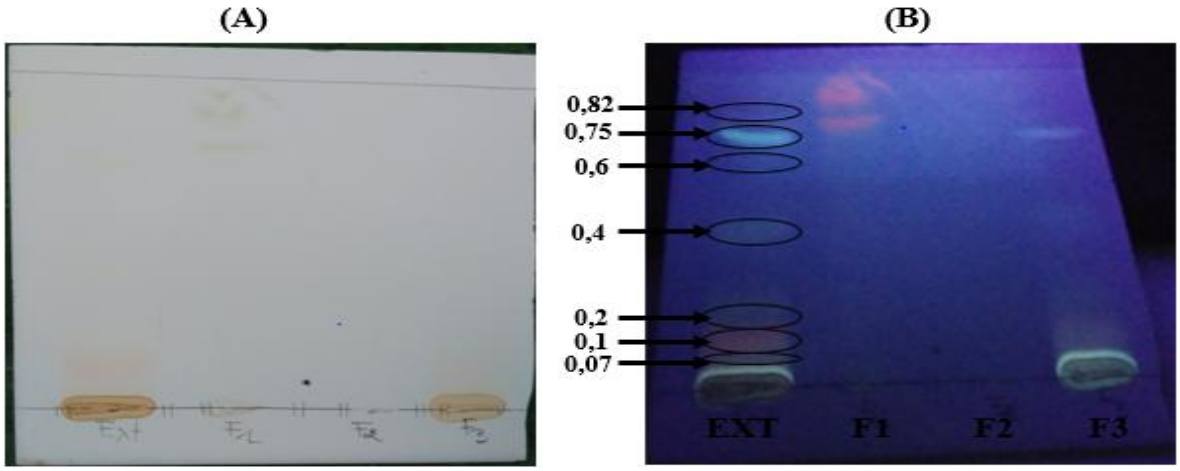

Figure 4: Chromatographic profile of the hydroethanolic extract and fractions of *Urena lobata* L leaves
Ext: Hydroethanolic extract; F1: hexane fraction; F2: ethyl acetate fraction; F3: aqueous fraction
Eluent: Ethyl acetate/formic acid/water (8/1/1); Developer: Neu; Plate (A): Highlighting of compounds in visible light; Plate (B): Demonstration of the compounds after revelation with Neu and visualization at UV visible 366 nm.

Table 2: Frontal ratio of the main spots of the extract and fractions of Urena lobata L leaves.

Sample Types	Number of constituents	Developers		Likely structures NEU
		DPPH (Jaune sur fond violet)	NEU UV-365 nm	
Hydroethanolic extract	07	CJC (0,1 ; 0,2 ; 0,4 ; 0,6 ; 0,75 et 0,82) Très nette	FB (0,4 ; 0,6 et 0,82)	Flavonoïdes(Flavonol) dérivés du kaempferol
			FJO (0,07 et 0,1)	Flavonoïdes (Flavonol) dérivés du quercetin
			FBB(0,75)	Flavonone (dérivé de la lutéoline)
Hexane fraction	02	CJC (0,7 et 0,8)	FJO (0,7 et 0,8)	Flavonoïdes(Flavonol) dérivés du kaempferol
Ethyl acetate fraction	05	Très moins nette	FB (0,1 ; 0,2 et 0,3) FBB (0,5 et 0,6)	Dérivés Acide phénols Flavonone (dérivé de la lutéoline)
Aqueous fraction	05	CJC (0,1 ; 0,2 ; 0,4 ; 0,6 et 0,75)	FJO (0,07 et 0,1)	Flavonoïdes (Flavonol) dérivés du quercetin
			FB (0,4 et 0,6)	Flavonoïdes(Flavonol) dérivés du kaempferol
			FBB(0,75) Très nette	Flavonone (dérivé de la lutéoline)

FJO: Yellow-Orange Fluorescence; FVC: Light Green Fluorescence; ( ): frontal retention CJC: Light yellow color; FB: Blue fluorescence; BCF: Light blue fluorescence.

3.1.3. Dosage of major chemical families, polyphenols and total flavonoids

The results of the quantitative analyzes by UV-visible spectrophotometer of the hydroethanolic extract and the fractions of the leaves of *Urena Lobata*. L, studied are represented below (figure 9). In this composition we see that the leaves are quantitatively richer in phenolic compounds (total polyphenols and flavonoids). At the end of this quantification, the aqueous fraction showed high contents of polyphenolic compounds at 4807 mgEAG/100gMs. However, the hydroethanolic extract remains dominant in flavonoid compounds at a value of 258 mgEC/100g Ms. Low contents of polyphenolic and flavonoid compounds are observed in the ethyl acetate fraction with respective values of 84.4 mgEAG/100gMs and 71.50 mgEC/100g Ms. The hexane fraction presents the fraction which is less rich in these compounds. phenolic because of its eluent during liquid-liquid separation.cc The richness observed at the level of the hydroethanolic extract and the aqueous fraction would favor these two entities to react on oxidative stress in the present study. Previous studies by several authors, such as Kanwal et al., (2010) [9], have shown that polyphenols play numerous roles, including protection against biotic and abiotic attacks and antifungal activity. Ono et al., (1990) [10] show that the effectiveness of this large family has antiviral and antioxidant properties as highlighted by Rahmat, (2012) [11] and Amessis-Ouchermoukh et al., (2014) [12]. The work of Benhouda et al. (2014) [13] showed that total phenol contents vary depending on the type of solvent used for extraction; which then justifies the difference in contents in the present study.

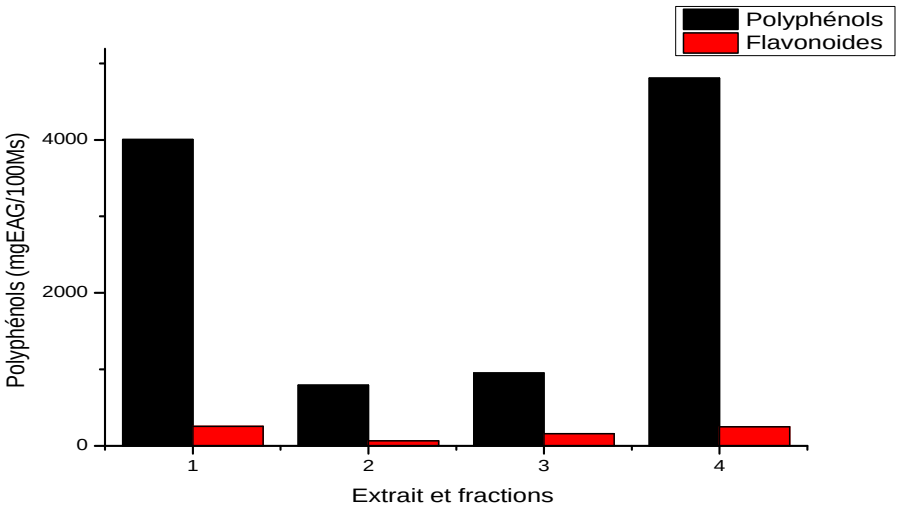

Figure 5: Contents of total polyphenols and flavonoids
1: Hydroethanolic extract; 2: hexane fraction; 3: ethyl acetate fraction; 4: aqueous fraction

3.2. Anti-radical effect of the hydroethanolic extract and different fractions

3.2.1. Anti-radical effect by thin layer chromatography (TLC).

The evaluation of the activity on free radicals by the thin layer chromatographic method made it possible to obtain the results below (Figure 10). As a result of this figure, the intensity and number of bands with yellow streaks on a purple background allows us to state that the species *Urena lobata* L has chemical substances with power on free radicals. These results obtained confirm those found by Andzi Barhé et al (2020) [5] on the same species with the alcoholic fractions obtained by solid-solid fractionation using the open column chromatographic method. This chromatogram therefore confirms that recorded by the Neu and visualized with the

visible UV lamp which highlights phenolic compounds having a strong anti-radical power. Indeed, these substances have been confirmed according to the research work of Benbrook C., (2005) [14] who works to increase the antioxidant content of foods through agriculture and food processing and Imam, M.Z et al., (2011) [15] confirms that the high levels of these compounds in plant extracts have an effect on free radicals in the body.

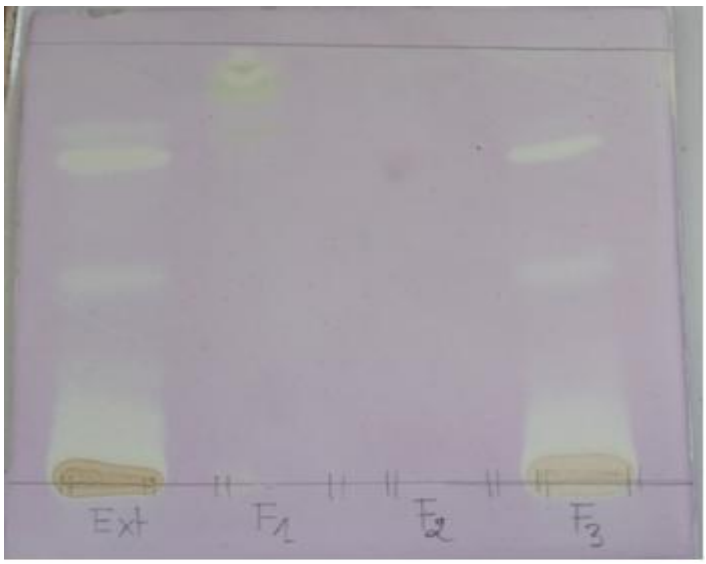

Figure 6: Chromatographic profile of the hydroethanolic extract and fractions of Urena lobata.L leaves
Eluent: Ethyl acetate/Formic acid/Water (8/1/1). **Developer:** DPPH
Ext: Hydroethanolic extract; **F1:** hexane fraction; **F2:** ethyl acetate fraction; **F3**: aqueous fraction

3.2.2. Anti-radical effect by the spectrophotometric method

The activity was carried out by the spectrophotometric method, measured at 517 nm, showed that the strongest anti-radical activities concern the hydroethanolic extract and the aqueous fraction at the respective values of 1.66 mg/ml and 2.03 mg/ ml. Low activities are noted at the level of the hexane and ethyl acetate fractions. These high activities of the hydroethanolic extract and the aqueous fraction could be explained by fairly high contents of phenolic compounds. Our results obtained are consistent with those of Andzi Barhé et al., (2016) [16] on the species *Hibiscus sabdariffa* who showed the correlation of total phenol contents with this anti-radical activity. The work of Mbaihougadobé et al (2017) [17] showed that hydroalcoholic extracts better extract phenolic compounds and demonstrate good anti-radical activity. The same authors also showed that the water-alcohol solvent mixture is a better solvent for plant extraction than alcohol. Studies carried out by Ouzid et al (2018) [18] show that this type of solvent plays an important role in the extraction of phenolic compounds and anti-radical molecules. Finally, the work of Bekara et al. (2016) [19] confirms that this activity could be attributed mainly to the polyphenols present in the plant. The presence of flavonoids in the plant makes it an important ingredient in the traditional medicinal system. Since flavonoids are associated with antioxidant activity, their presence in the plant makes them an important food material according to the work of Dixa Singh et al., (2016) [8].

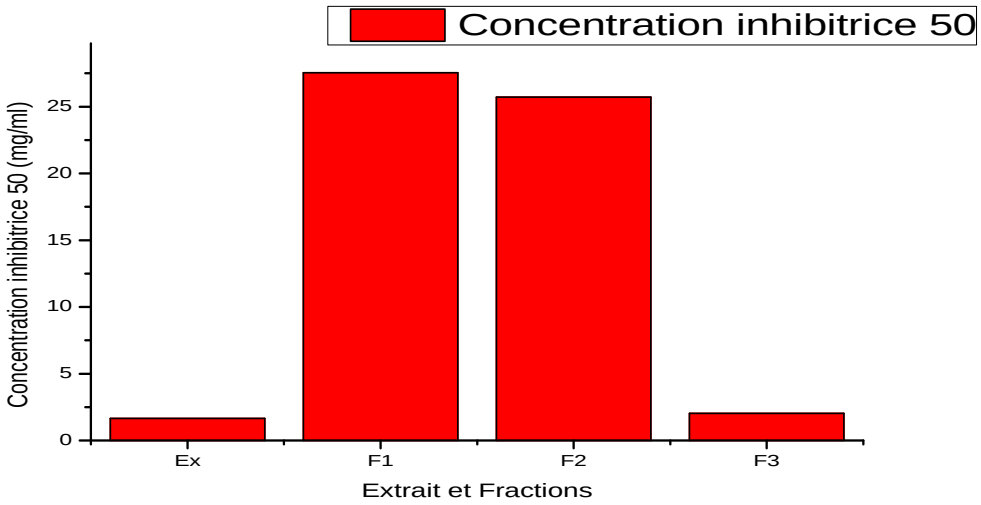

Figure 7: Anti-radical activity of the extract and fractions
Ex: Hydroethanolic extract; F1: hexane fraction; F2: ethyl acetate fraction; F3: aqueous fraction

Conclusion

In short, the work carried out on the species *Urena lobata* L. has revealed several potentials contained in this plant. The extraction of the hydroethanolic extract and separation of the different fractions by the liquid-liquid chromatographic technique showed the high yields observed, in the ethyl acetate and aqueous fractions of 13.5 and 8.5% respectively. The profile chemistry of phenolic compounds in general and flavonoids in particular showed the presence of compounds such as Kaempferol, Quercetin and Luteolin, by detection on a thin layer.high contents of polyphenolic compounds are observed in the aqueous fraction and the respective hydroethanolic extract in the order of 4807 and 4130 mgEAG/100gMs. However, the hydroethanolic extract remains dominant in flavonoid compounds at a value of 258 mgEC/100g Ms. Thanks to these compounds present in the aqueous fraction and the hydroethanolic extract, the anti-radical potential or power was demonstrated with an inhibitory concentration 50 (IC50) of 1.66 mg/ml and 2.03 mg/ml. Finally, the richness of this plant in phenolic compounds attests to its use and its potential in Congolese phytotherapy for responses to public health problems.

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