HPLC profile and antioxidant activity of *Lantana rhodesiensis* extract

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Abstract

The aim of this investigation was to determine the chemical composition of *L. rhodesiensis* Moldenke extracts used in traditional medicine from Burkina Faso and evaluated their antioxidant activities. Four fractions coming from ethanol-water total extract were made such as n-hexane (n-H), ethyl acetate (EA), n-butanol (n-BuOH) and aqueous fractions (AqF). These fractions were used to evaluate the chemical composition using HPLC-GC/MS-DAD methods. Also, phenolic and flavonoid contents were quantified and the whole of these proportionings was directed against the antioxidant activity through the anti-DPPH*, anti-total antioxidant capacity (TAC) and anti-iron reducing power (IRP). The best antioxidant activity was obtained with the radical DPPH* (0.05 μg/mL) and total antioxidant capacity (7.34 mg EAA/mL). 8 polyphenolic compounds were highlighted in HPLC-GC/MS method, against 10 in HPLC-DAD. This study makes it possible to justify the various uses of this specie in traditional medicine.

Keywords: chromatographic profil, antioxidant, *Lantana rhodesiensis*, traditional medicine.

Résumé

Cette étude vise à déterminer la composition chimique des extraits de *Lantana rhodesiensis* Moldenke, une espèce utilisée dans la médecine traditionnelle au Burkina Faso et à évaluer les activités antioxydantes. Quatre fractions sur la base d'extrait éthanol-eau ont été faites à savoir : n-hexane (n-H), acétate d'éthyle (AE), n-butanol (n-BuOH) et la fraction aqueuse (FAq). Nous avons évalué les teneurs en composés chimiques de ces fractions par les méthodes CLHP-CG/SM-DAD. Aussi, les acides phénoliques et les flavonoïdes ont été quantifiés dans ces extraits. Nous avons également utilisé 3 méthodes pour évaluer les activités antioxydantes. Il s'agit de la capacité antioxydante totale, le pouvoir réducteur des ions ferreux et la méthode antiradicalaire par le DPPH. La meilleure activité a été obtenue par celle du radical DPPH avec 0,05 μg/mL et de celle de la capacité antioxydante totale (7,34 mg EAA/mL). 8 composés polyphénoliques ont été mis en évidence par la méthode CLHP-CG/SM contre 10 dans celle CLHP-DAD. Les résultats de cette étude pourraient justifier en partie les diverses utilisations de cette plante dans la médecine traditionnelle.

Mots-clés : profil chromatographique, antioxydant, *Lantana rhodesiensis*, médecine traditionnelle.

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Introduction

According to BAKASSO (2009), 75% of the pharmaceutical products in the world derive from plants. In 2002, WHO estimated that almost 80 % of African population use medicinal plants. Indeed, this high reliance on medicinal plants could be explained by the insufficiency and the inaccessibility of health care facilities, especially the high price of essential drugs. In these last decades, researchers are trying to justify the traditional usefulness of these plants in human health through several techniques (FENG *et al*., 2012 ; MARZENA, 2012 ; ROUIMI, 2017).

Significant ethnopharmacological knowledges are reported on the traditional useness of these medicinal plants. NACOULMA in year 1996 reported a list of medicinal plant from Burkina Faso with potent properties against several illness such as: high blood pressure, cancer, diabetes, parasitic diseases, heart failure, malaria, smallpox, liver pathologies, gout and mental disorders. The former studies on phytochemistry showed that methanolic and aqueous extracts are rich in tannins (BANGOU *et al*., 2011 ; PIERO *et al*., 2015). Also, various compounds were highlighted in these species of plant. It acts without being exhaustive: flavonoids, alkaloids, sterols, terpenoids, cardiac glycosides, phylobatannins, resins, bound anthraquinones, 5,6,7,3',4',5'-hexamethoxyflavone and its analogue, 5-hydroxy-6,7,3',4',5'-pentamethoxyflavone, stigmasterol, phytol, ursolic acid, camphene glycol, camphor, trans-hydrate of sabinene, piperiterone, p-cymene, linalool, α-pinene, camphene, β-pinene, limonene, cineole, α-terpenoid and β-cubebene, potassium, calcium, manganese, iron, lead and zinc (SAWADOGO *et al*., 2012 ; PIERO *et al*., 2015 ; SAWADOGO *et al*., 2015 ; BANGOU *et al*., 2017a). Pharmacological investigation showed that the plant isn't genotoxic, and it induced a very significant antiproliferative effect against cancer cells with 94 % (SAWADOGO *et al*., 2015). Other investigations demonstrated that *L. rhodesiensis* extracts contain anthraquinones which have previously been reported to lower blood glucose (ARIKA *et al*., 2015).

In this present study, we characterize the phytochemical composition of *L. rhodesiensis*, and evaluate the antioxidant activities of this plant.

I. Plant material

Aerial parts (stem-leaves) of *L. rhodesiensis* from Burkina Faso, were collected between May and October 2013 at Gampela (25 Km East of Ouagadougou). The plant was been identified by Professor Millogo-Rasolodimby at the Department of Biology of the University of Ouagadougou. The Voucher specimen was deposited in the OUA herbarium of the CIB (Centre d'Information sur la Biodiversité), UFR/SVT of the University of Ouagadougou.

1.1. Reagents and standards

Acetonitrile (HPLC grade), water (HPLC grade), ethanol (HPLC grade), ethyl acetate (analytical grade), hexane (analytical grade), sulfuric acid (analytical grade), and sodium phosphate were purchased from J. T. Baker (Xalostoc, Mexico). 2,2-Diphenyl-1-picrylhydrazyl (DPPH*), aluminum chloride, ammonium molybdate, and the references quercetin, quercitrin (quercetin-3-rhamnoside), caffeic acid, and ascorbic acid were purchased from Sigma-Aldrich (St. Louis Missouri, USA). The standards kaempferol-3,7-*O*-diglucoside, quercetin-3-*O*-[rhamnosyl-(1-6)-galactoside], kaempferol-3-*O*-[rhamnosyl-(1-6)-glucoside] came from Apin Chemicals Limited

(Abingdon, Oxon, UK). Trichloroacetic acid and ferric chloride were obtained from Merck (Darmstadt, Germany). Potassium ferricyanide was purchased from Fermont (Monterry, Mexico).

1.2. Preparation of extracts

Polyphenols and flavonoids were extracted from dry ground (stem-leaves) (4 g) by maceration in 40 mL of ethanol 80 % (v/v) for 24 hours in darkness at room temperature, and the mark was re-extracted with ethanol 20 % (v/v). The extracts were centrifuged (5000 rpm) for 10 min, at room temperature, and the supernatant was separated. The pellet of the extract with ethanol 20 % was re-extracted in 100 mL of 20 % ethanol (v/v) for 3 hours, centrifuged under the same conditions, and the supernatant decanted. Both supernatants were combined to form the total extract. From this total extract, it was conducted a liquid-liquid fractionation with n-hexane, n-butanol and ethyl acetate to obtain the n-hexane fraction (n-HF), the ethyl acetate fraction (EAF), then-butanol fraction (n-BF) and the aqueous fraction (AqF).

1.3. Determination of phenolic content

Folin-Ciocalteu method was used for measurement of total content of phenolic compounds according to the method describe by NURMI *et al*. (1996), through a linear regression analysis from a standard curve of gallic acid ($Y = 0.003x + 0.016$; $R^2 = 0.997$). Briefly, 250 μl extract was mixed with 2.5 ml of deionized water. After, 125 μL Folin-Ciocalteu reagent was added and the mixture is kept for 5 min. Finally, 375 μL of 20 % Na_2CO_3 was added. After 2 hours incubation at room temperature, the absorbance was measured at 760 nm with a Spectroscopic Analysis Mecasys (Optizen). Three replicates of each sample were analyzed.

1.4. Determination of flavonoid content

Flavonoid content was determined according to LAURANSON-BROYER and LEBRETON (1993) by linear regression analysis using a standard curve of quercetin: $Abs_{425nm} = 0.025x + 0.014$ [Quercetin], correlation coefficient $R^2 = 0.998$. The absorbance was immediately registered after the addition of aluminum chloride, at 425 nm, using a Spectronic Genesys 2 spectrophotometer (Rochester, New York, USA). The flavonoid content in each sample was expressed as μg of quercetin equivalents/ g of dry extract. The determination of flavonoid content was performed through three independent assays in triplicate.

1.5. HPLC-MS/GC and HPLC-DAD Analysis

HPLC-DAD and HPLC-MS/GC analysis were used to determine the secondary metabolite profile of the extract (BANGOU *et al*., 2017b). Briefly, an aliquot of the extract was re-dissolved with some drops of ethanol-water, and the volume was supplemented to 5 mL of acetone 100 %. The extract was centrifuged (5000 rpm) for 10 min, at room temperature, and the supernatant was separated. HPLC-DAD analysis was performed according to the method describe by BANGOU *et al*. (2017b). Concerning the HPLC-MS/GC (Clarus 500 Perkin Elmer) analysis, 1 mL of supernatant was taken using flame ionization detector (FID). Injection volume (0.5 μL), column (Elite 5), carrier gas (He), recording time lies between 2-22 mn and the temperature of ionization lies between 60 to 220 °C. Structural identification was obtained by direct comparisons of retention times and INST1 spectra of resolved compounds.

1.6. Total antioxidant capacity

The total antioxidant capacity (TAC) of each sample was evaluated through the method developed by PRIETO *et al*. (1999), in which the molybdate (VI) is reduced by the antioxidant into Mo (V), forming a green phosphate/Mo (V) complex at acidic pH. Aliquots (100 μL) of each sample were prepared and combined with 1 mL of a sulfuric acid solution (0.6 M), sodium phosphate (28 mM), ammonium molybdate (4mM) and incubated at 95°C for 90 min. After reaching room temperature, the absorbance of each sample was registered at 695 nm against a blank prepared as indicated for the samples but adding ethanol instead of the sample. The reference quercetin was analyzed in the same manner. TAC was expressed as mg ascorbic acid equivalents. Ascorbic acid curve: A_{695} = 3,678x - 0,092[ascorbic acid], correlation coefficient R^2 = 0,998, constructed with ascorbic acid between 1.0 and 30.0 mg.mL^{-1}. The analysis was done for independence aliquots of the samples from three pools of samples.

1.7. Free radical scavenging activity

The DPPH* method reported by CAMPOS *et al*. (1994) was used to evaluate the free radical scavenging activity. Four to five flavonoid concentrations of each sample were individually added to a DPPH* solution (40 μg.mL^{-1} in ethanol) to maintain a final volume of 1 mL. The decrease in absorbance was determined at 523 nm after 10 min. The DPPH* concentrations of samples were plotted to determine by linear regression, the efficient concentration at 50 %, defined as the amount of antioxidant needed to decrease by 50 % the initial DPPH* concentration (EC_{50}). The following calibration curve, made with DPPH* between 6.25 and 100 μg.mL^{-1}, was used to calculate the DPPH* concentration (μg.mL^{-1}) in the reaction medium: A_{523} = 0,030x + 0,001 [DPPH*], correlation coefficient R^2 = 0,999. Antioxidant activities were expressed in terms of EC_{50} in μg.mL^{-1}. The analysis was separately done for the samples from three pools samples.

1.8. Iron reducing power

The iron reducing power (IRP) method reported by YANG *et al*. (2008) was used to evaluate the iron reducing power of each simple. Aliquots (1 mL) of each sample were combined with 2.5 mL (phosphate buffer, 0.2 M, pH 6.6), 2.5 mL (potassium ferricyanide, 30mM) and incubated at 50 °C for 20 min. After, 2.5 mL trichloroacetic acid (0.6 M) was added and the mixture was centrifuged (2000 rpm for 10 min). From the upper layer, 2.5 mL of solution was removed and distilled water (2.5 mL) and ferric chloride (0.5 mL, 6 mM) were added to it. The absorbance at 700 nm of the formation of ferrous ions (Fe^{2+}) was registered after 10 min. The highest absorbance values indicated the greatest capacity of reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. Four flavonol concentrations (10-400 μL combined with the proper volume of ethanol to reach 1 mL as final volume) of each sample (respective concentrations of flavonols calculated from standard curve of quercetin) were evaluated. The reducing power was expressed in terms of EC_{50} (mg.mL^{-1}). The evaluation was separately done for the samples from three pools of samples.

1.9. Statistical analysis

Statistical analysis: All assays were carried out in triplicates and results are expressed as Means ± Standard Deviation (SD) calculated with Excel 2007. Statistical comparisons were done with the XLSTAT7.5.2, using Spearman correlation. Differences were considered to be significant at $p<0.05$.

II. Results

2.1. Phytochemical analysis

2.1.1. Phenolic and flavonoid contents

The total phenolic and total flavonoid contents in the different fractions are consigned in table III. This table III shows that for phenolic contents, results decrease in the following order EAF > AqF > n-HF > n-BF. With respect to the flavonoid contents, the following order was observed EAF > n-BF > n-HF > AqF. The EAF highlight the highest content in total phenolic content (126.07 ± 10.74 mg EAG/g) and in total flavonoid content (25.71 ± 0.05 mg EQ/g). These results show that ethyl acetate solvent extracts more compounds than any other solvent used. This suggests that the secondary metabolites of *L. rhodesiensis* may have intermediate polarity.

2.1.2. HPLCs analysis

HPLC-DAD method applied to the total extract and fractions enable us to highlight 10 compounds (Table I). HPLC-GC/MS method applied to the same fractions enabled us to highlight 8 compounds (Table II). Figure 2 also indicates the probable characteristics of these compounds. Metabolites highlighted on this level could explain the various activities of *L. rhodesiensis* observed especially with TAC. Indeed, former investigations showed that the flavonoids are good antioxidant candidates and more precisely those poly-hydroxylated such as the derivative of 7-hydroxy-5,8,3´,4´-tetramethoxyflavone (BANGOU *et al*., 2017a). The HPLC-GC/MS analysis by enabled us to highlight terpenes which will justify in our case, the antioxidant activities (Table II). Other authors showed that luteolin (TSIMOGIANNIS and OREOPOULOU, 2004) and rosmarinic acid (PENCHEV *et al*., 2010) are antioxidant compounds. MEVY *et al*. (2007) reported that β-caryophyllen, 1,8-cineol and the germacren are antioxidant compounds.

Table I: Wavelength characteristic of the phenols acids and flavonoids detected.

Number of compound	Compound	RT (min)	λmax (nm)
Elar18	Derivative of 7-hydroxy-5,8,3´,4´ -tetramethoxyflavone	54,102 ± 0,052	255sh, 273, 342
Elar19	Genkwanin	31.126	270, 331
Elar20	2,4-dihydroxybenzoic acid	23,844 ± 2,333	240sh, 296sh, 326
Elar21	Derivative of Luteolin-7,3´ -di-O-glucuronide	36,795 ± 0,923	250, 278, 337
Elar22	Rosmarinic acid	36.201	250sh, 290sh, 328
EAFlar	Derivative of 6-hydroxyluteolin -7-O-(xylosyl(1-2)xyloside)	52.554	234sh, 253sh, 273, 344nm
EAFlar	Derivative of genkwanin	51.456 ± 0.75	266, 290sh, 331nm
EAFlar	Derivative of saponarin	42.596 ± 0.228	282, 345nm
n-BFlar	Derivative of acacetin-7-O-(rhamnosyl(1-2)glucoside)	47.744	282, 339nm
n-BFlar	Derivative of chrysoeriol-6,8-di-C-glucoside	36.142 ± 0.086	252sh, 269, 340nm

E: Ethanol-water, EAF: Ethyl acetate fraction, n-BF: n-butanol fraction; lar: *Lantana rhodesiensis*

Table II: Retention time of the essentials oils detected.

Species	Number of compound	Retention time (RT)	Compounds
L. rhodesiensis,	(1)	7.571	β-myrcene
	(2)	8.052	Benzene,1methyl-4-(1-methylethyl),
	(3)	8.474	1,4-cyclohexadiene 1-methyl-4-(1-méthylethylidiene),
	(4)	10.642	Thymol
	(5)	10.717	Phenol, 2-methyl-5-(1-méthylethyl)
	(6)	11.636	Bi-cyclo [7,20] undec-4-ene 4,11,11-triméthyl-8-methylene
	(7)	12.720	Caryophyllene oxide,
	(8)	13.773	1-methylene-2β-hydroxymethyl-3,3-Dimethyl-4β-(3-methylBut-2-en

2.1.3. Antioxidant activities

Three methods such as: Free radical scavenging activity, Iron reducing power and Total antioxidant capacity were used to evaluate the antioxidant activities of *L. rhodesiensis* total extract and fractions.

2.1.4. Free radical scavenging activity

The anti-DPPH activity of an extract result in its capacity to trap free radicals. In this assay, four fractions from total extract were evaluated. Results are presented as EC_{50} and were reported in the Figure 1A. The anti-DPPH activity of fractions vary between 0.05 to 1.21 μg/mL. Among these results, the EAF gave the strongest anti radical activity (0.05 ± 0.00 μg/mL) and the lowest was obtained with the AqF (1.21 ± 0.03 μg/mL).

2.1.5. Iron reducing power

The results of the reducing power of the fraction are indicated in the Figure 1B. These results were ranged between 0.35 to 2.21 μg/mL. The best reducing power was obtained with the n-HF fraction (0.35 ± 0.02 μg/mL). The n-BF has showed the weakest activity with 2.21 ± 0.01 μg/mL.

2.1.6. Total antioxidant capacity

The results for the antioxidant capacity of the fractions are ranged between 3.95 to 7.34 mgEAA/mL (Figure 1C). The following order 7.34 ± 0.07 (EAF) > 7.14 ± 0.18 (n-HF) > 6.17 ± 0.15 (n-BF) > 3.95 ± 0.01 (AqF) mgEAA/mL was observed. This assay also shows that EAF highlighted the best antioxidant capacity.

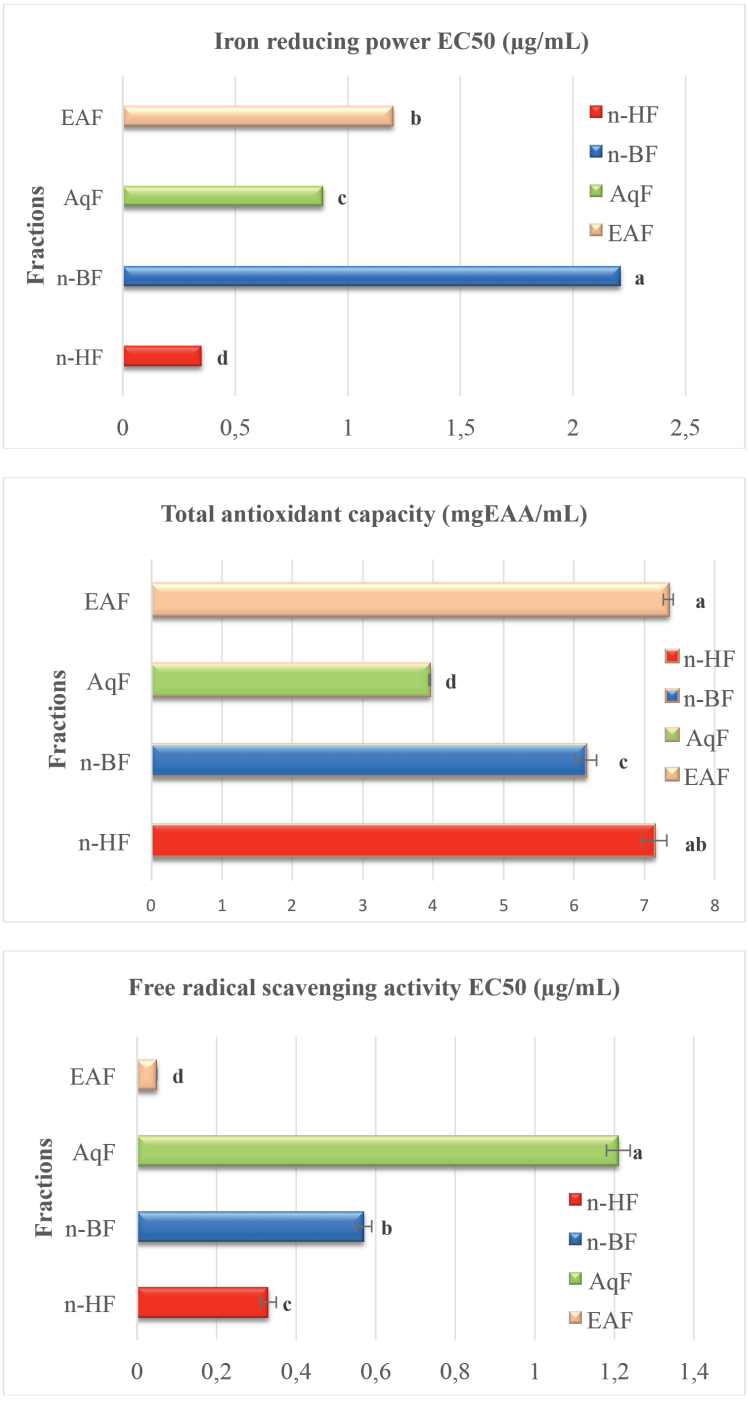

Figure 1: Antioxidant activities obtained using the IRP (Figure 1A), TAC (Figure 1B) and DPPH (Figure 1C) methods on *Lantana rhodesiensis* fractions. n-HF: n-hexane fraction; n-BF: n-butanol fraction; EAF: ethyl acetate fraction. Values are mean ± SD (n = 3). Different letters indicate significant difference ($p < 0.05$).

Table III: Polyphenolic compound contents.

Species	Fractions	Phenol contents (mg EAG/g dry extracts)	Flavonoid contents (mg EQ/g dry extract)
(Lantana rhodesiensis)	n-HF	91.76 ± 1.39^{d}	7.80 ± 0.04^{g}
	n-BF	90.52 ± 11.2^{d}	12.71 ± 0.02^{d}
	AqF	102.43 ± 14.55^{bc}	5.80 ± 0.04^{j}
	EAF	126.07 ± 10.74^{a}	25.71 ± 0.05^{b}

III. Discussion

3.1. Relationship between antioxidants activities and polyphenolic compounds estimated

Free radical scavenging activity in comparison with polyphenolic compounds content, one can suppose that there is a strong contribution of ethyl acetate fraction. But, radical DPPH* inhibition seems to be an unspecified content, but rather in term of quality of the secondary metabolites (MEDA *et al*., 2013). The results of iron reducing power comes to confirm that the antioxidant activity isn't a function forcing related to the content of polyphenolic compounds, but of the quality of the secondary metabolites (MEDA *et al*., 2013). We observe a phenomenon completely different from the two preceding reactions on total antioxidant capacity. Indeed, all occurs as if there was a correlation between polyphenolic content and antioxidant activity. In this present case, we observe a correlation between totals phenolic and antioxidants activities. What corroborates with the investigations of BANGOU *et al*. (2017b). On the other hand, MEDA and collaborators in 2013 had found an influence of the butanolic fractions.

The interest of the relation investigation between antioxidants activities and phytochemical is sought to understand the real implication of each group of compounds implied in the observed activities. In comparison with table IV, we can say that there is a very weak correlation between content of phenolic compound and antioxidants activities except for that of the flavonoids and of the radical scavenging activity (56.29 %). This controversy was the subject of several publications (MANZI *et al*., 2004 ; MEDA *et al*., 2005 ; GURSOY *et al*., 2009 ; AGBAFOR and NWACHUKWU, 2011 ; COMPAORE *et al*., 2011 ; BANGOU *et al*., 2012 ; MEDA *et al*., 2013). It is thus clear that the activities observed are functions of the quality of the compounds.

Table IV: Relationship between antioxidant activities, total phenolic and flavonoid content.

R^2	IRP	TAC	DPPH*
TP	0.0057	0.051	0.1574
TF	0.0917	0.3878	0.5629

Molecular studies on the antioxidant activity made it possible to understand that not only it was qualitative, but especially due of the hydroxylation in C3 position of the molecule of quercetin (GREENHAM *et al*., 2003 ; BANGOU, 2012). Thus, SAWADOGO and collaborators in 2015 characterized 2 poly-hydroxylated molecules in the extracts of *Lantana rhodesiensis* which

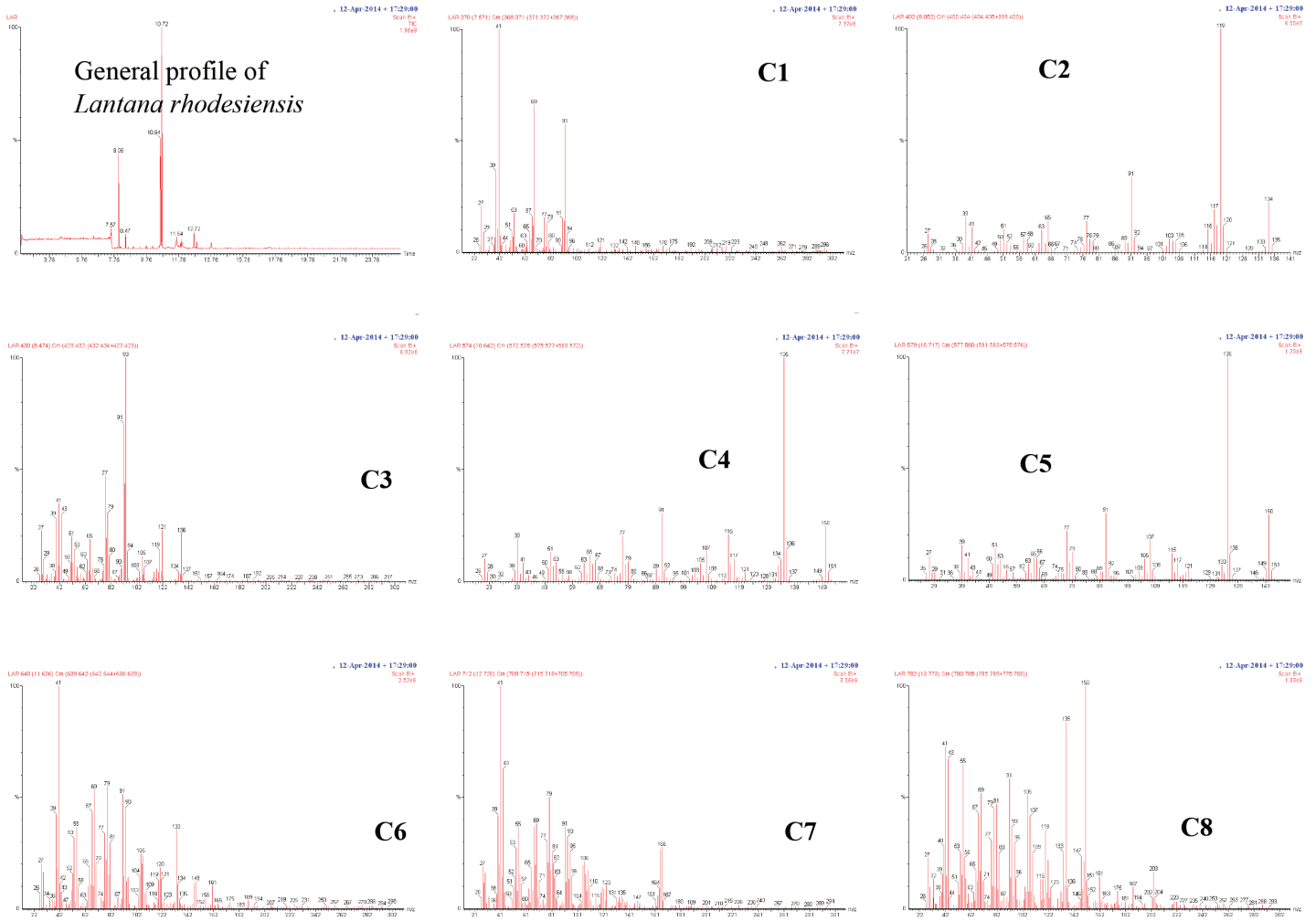

Figure 2: Spectrum of essentials oils detected.

would be potentially antioxidant. It is about 5,6,7,3',4',5'-hexamethoxyflavone and its analogue, 5-hydroxy-6,7,3',4',5'-pentamethoxyflavone.

Our study enabled us to highlight compounds at essential oils (β-caryophyllen, 1,8-cineol, germacren: Table II and Figure 2) and polyphenolic compounds (Table I) which are known for their antioxidant activities (PASCUAL *et al*., 2001 ; SAWADOGO *et al*., 2015 ; BANGOU *et al*., 2017b). As other term, the biological molecules would act by synergy in the plants extracts (BANGOU *et al*., 2017a).

Conclusion

Antioxidant activity was investigated together with polyphenolic compounds. Four types of fractions of ethanol-water were evaluated for polyphenolic quantification. Among these fractions, ethyl acetate fraction presented the best activity on total antioxidant activity, anti-DPPH*, and iron reducing power. By means of the literature we established the importance of balance between oxidant and antioxidant in the alive cells. Indeed, it was demonstrated that the excess of antioxidants or oxidants can involve negative effect on human body. With the sight of polyphenolic compounds quality highlighted, one could justify the various activities observed and the many uses in traditional medicine. Further analysis of these species should be focused to (1) the isolation and characterization, (2) the evaluation of the capacity of the extracts and/or compounds isolated from its extracts to increase the rate of the superoxide dismutase, the catalase and of the glutathione in the human organism.

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