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Phytochemistry investigation and antioxidant activity of four edible Verbenaceae of Burkina Faso.

Bangou Mindiédiba Jean[1*], Reyes-Martínez Alfonso[2], Coulidiati Tangbadioa Hervé[3], Megalizzi Véronique[4], Nacoulma Odile Germaine[5]

***Corresponding author:**

Bangou Mindiédiba Jean

[1]Laboratoire de Biochimie et de Chimie Appliquées (LABIOCA), Unité de Formation et de Recherche en Sciences et Techniques. Université Ouaga I, 09 BP 848 Ouagadougou 09, Burkina Faso.
[2]Instituto de Silvicultura e Industria de la Madera, Universidad Juárez del Estado de Durango, Boulevard del Guadiana 501, Fraccionamiento Ciudad Universitaria, 34160, Durango, Dgo., México.
[3]Section SVT, UFR/Sciences et Technologies, Université de Koudougou,01 BP 376 Koudougou 01.
[4]Université Libre de Bruxelles. Faculté de Pharmacie, Service de Chimie Analytique Instrumentale et Bio Electrochimie et de Toxicologie cancéreuse Expérimentale. Campus de la Plaine-205/6. Boulevard du Triomphe B-1050 Bruxelles – Belgium.
[5]Laboratoire de Biochimie et de Chimie Appliquées (LABIOCA), Unité de Formation et de Recherche en Sciences et Techniques. Université Ouaga I, 09 BP 848 Ouagadougou 09, Burkina Faso

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Abstract

Lantana rhodesiensis Moldenke, *Lippia chevalieri* Moldenke, *Vitex diversifolia* Bak and *Vitex doniana* Sweet are four species of Verbenaceae family widely used in traditional medicines in Burkina. The aim of this investigation was to study the chromatographic profile of phenols acids and that of the flavonoids as well as the antioxidants activities. Then, three types of extraction were made: acetonic (100%), decoction, ethanol-water(80/20 + 20/80); and fractions of ethanol-water which arehexane fraction (HF), ethyl acetate fraction (EAF), butanol fraction (BF) and aqueous fraction (AqF) of each species. These evaluations were supplemented by the quantification of the metal ions and the characterization by atomic absorption and HPLC-DAD methods of totals phenols acids and totals flavonoids.The whole of these proportioning was directed against the antioxidant activity through the anti-DPPH* (free radical scavenging activity), anti-TAC (total antioxidant capacity) and anti-IRP(iron reducing power).36 flavonoids on 41 were highlighted by the HPLC-DAD method. A derivative glycosylate of quercetin was detected in the *V. diversifolia* extracts. *L. chevalieri* presented the strongest value in terms of ions content with a 55.88 g/kg of calcium.In terms of antioxidant activity, *L. rhodesiensis* and *L. chevalieri* presented the best activities on total antioxidant activity and anti-DPPH, and iron reducing power respectively. It's arise that decoction extract have the best's activities and can justify the traditional uses.

Keywords: chromatographic profile, antioxidant, phenols, flavonoids, metals, spectrometry.

Introduction

Verbenaceae family contains around the world 100 genus and 2600 species. It's represented in intertropical Africa by 100 species divided into 10 genus [1],[2]. Species from three genus of this family such as *Lippia*, *Lantana* and *Vitex* were the subject of our study. *L. rhodesiensis*, *L. chevalieri*, *V. diversifolia* and *V. doniana* are widely used traditionally in the management of several diseases including cancer, inflammatory diseases, arterial

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hypertension, arterial high blood pressure, tumors, liverpathologies, nervous hepatitis, tooth aches, cutaneous diseases, intestinal bilharziose, bucco-anal and digestive candidiasis (Table 1). These plants are as well-known and used in traditional medicines in Burkina Faso [3],[4][5],[6]. Phytochemistry and biological screening of these plants form integral part is our research plan. Several studies were undertaken on these plants: polyphenolic compound contents and chromatographic profiles of phenols acids and flavonoids [1],[4],[7], enzymatic activity [4], [8], antioxidant and antibacterial activities [1],[7],[9],[10].The most elucidated studies on these plants were directed towards their essential oils contents [8],[11],[12],[13]. According to Nwachukwu and Uzoeto [14] acetone, ethanol, methanol, hot and cold water extracts of leaves of *Vitex doniana* have antimicrobial properties which can be pharmaceutically exploited on *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus*. The antinociceptive activity of the ethanolic extract of *V. doniana* has been evaluated [13]. The extract demonstrated significant antinociceptive activity in a dose dependent manner compared to control [15]. Recently, *Vitex diversifolia* exhibited the highest antibacterial activity against clinically isolated human pathogenic bacteria [16].One of our prospects was to evaluate the polyphenolic compound contents and biological activity of these plants. Thus three types of extracts were used in this present study and the best extracts (ethanol-water) was tooselected for the sifting of the phenols acids, flavonoids and the evaluation of their antioxidant potential.

We also aimed to evaluate the antioxidant activity of these different extracts and fractions against free radical scavenging activity (DPPH*), total antioxidant capacity and iron reducing power. Specifically, in total extracts and fractionswe have, (1) measuredthe phenolic and flavonoid compositions, (2) estimated their metals content, (3) studied their chromatographic profile through phenols acidsand flavonoids (4) evaluated their antioxidants activities through three methods.

Table 1: Traditional uses of four (04) medicinal plant species (Verbenaceae)

Species	Traditional uses	Part used	References
Lantana rhodesiensis Moldenke	Arterial hypertension,cancer,diabetes mellitus arrhythmia, parasitic diseases, congestive heart failure,anti-proliferative, malaria, smallpox, lithiases	Fruits, leaves, stem-leaves	[3] [4] [8] [37] [58]
Lippia chevalieri Moldenke	Malaria, diarrheas, arterial high blood pressure, pathologies of the liver, bucco-anal and digestive candidiasis, menorrhagia of the menopause, nervous hepatitis, gout and the mental disorders	Stem-leaves, leaves,	[4] [5] [11] [25] [59]
Vitex diversifolia Bak	Antibacterial, tooth aches, cutaneous diseases, intestinal bilharziose, weaning, antiiflammatory, arterial hypertension, Astringents	Fruits, bark, leaves, Stem-leaves	[6] [60] [61] [62]
Vitex doniana Sweet	Affection of respiratory tracks, tumors, dysentery, diarrhea, painful rules, chicken pox, small pox, evil of chronic stomach, madness, rheumatic pains, inflammatory disorders, Nutritive Sweetener	Fruits, bark, leaves, stem bark	[9] [12] [63] [64] [65] [66]

Experimental

Plant material

Stems leaves of *L. rhodesiensis* and *L. chevalieri*were collected to Boulbi (Saponé), leaves of *V. diversifolia* collected of Komadougou(11 Km North of Fada N'Gourma) and *V. doniana* (leaves) collected of Gampéla (25 Km East from Ouagadougou). All these were collected in Burkina Faso between May and October 2013. The plants were botanically identified by Professor Millogo-Rasolodimby from the Department of Plant Biology at the University of Ouagadougou. The Voucher specimens were deposited in the OUA herbarium of the CIB (Centre d'Information sur la Biodiversité), UFR/SVT of the University of Ouagadougou.

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).

Preparation of extracts

Polyphenols and flavonoids were extracted from 4 g of dry powder from leaves, bark or stem-leaves were extracted by macerationin 40 mL of acetone 100% (acetone extract), ethanol 80% (v/v), or by decoction (decoction extract), for 24 hours in darkness, and at room temperature. The extracts were centrifuged (5000 rpm) for 10 min, at room temperature, and the supernatants separated. The pellets (only for ethanol 80% extract) were re-extracted in 100 mL of 20% ethanol (v/v) for 3 hours, centrifuged under the same conditions, and the supernatant decanted. Both supernatant were combined to form the total extracts (ethanol-water extract). The totals extracts were concentrated to dry for polyphenolic, flavonoids and antioxidants activities. The ethanol-water extract was concentrated to half the volume and then fractioned twice with ethyl acetate (EAF). The pellets were also macerated in 100 ml hexane for 24 hours in darkness (HF). The two organic fractions (ethyl acetate and hexane), the aqueous fractions (AqF), and the separated aliquots of the total extracts, were individually concentrated under vacuum to dryness and then re-dissolved in 5 mL ethanol, aliquots were taken to be used in the determination of flavonoid content, in the HPLC-DAD analysis. The phenolic extractions were prepared from three different pools of samples. The ethanol-water extract was also subjected to sequential liquid-liquid extraction with n-hexane (HF), ethyl acetate (EAF), and n-butanol (n-BF).

Determination of phenolic content

Folin-Ciocalteu method was used for measurement of total content of phenolic compounds according to Nurmi et al. [17], by linear regression analysis from the standard curve of gallic acid (y = 0.003x+0.016; r = 0.997). A 250 µl extract was mixed with 2.5 ml of desiionized water. Afterwards, 125 µL Folin-Ciocalteu reagents were added and the mixture was allowed to stand 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 on a Spectroscopic Analysis Mecasys (Optizen). The phenol contents were expressed as mg gallic acid equivalents g^{-1} dry tissue (mg GAE g^{-1} dry tissue).Three replicates of each sample were analyzed.

Determination of total flavonoid content

Flavonoid content was determined according to Lauranson-Broyer and Lebreton [18] by linear regression analysis from the following standard curve of quercetin: Abs_{425nm} =0.025x + 0.014 [Quercetin], correlation coefficient r = 0.998. The curve was registered after the addition of 60 µL of a freshly prepared 5% (w/v) aluminum chloride solution to 1 mL of quercetin solution (four different concentrations in the range of 100 to 1400 µg/mL). The absorbance was immediately registered after the addition of aluminum chloride, at 425 nm, using a Spectronic Genesys 2 espectrophotometer (Rochester, New York, USA). The flavonoid content in each sample was also registered after the addition of aluminum chloride and the flavonoid content in each sample was expressed as mg of quercetin equivalents g^{-1} dry tissue (mg QE g^{-1} dry tissue). The addition of aluminum chloride produces bathochromic shifts (which can be perceived by a yellow coloration) in flavonoids containing orthodihydroxyl groups, due to the formation of complexes between the aluminium and C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols ; complexes also are formed between the aluminum and the orthodihydroxyl groups in A or B-ring of flavonoids [19]. The addition of aluminium chloride represents a standard procedure for reproducibility [18]. The determination of flavonoid content was estimated individually for three pools of samples.

HPLC-DAD Analysis

To determine the flavonoid profiles, aliquots of each extract (concentrated to dryness and re-dissolved in 5 mL ethanol, as mentioned in the section Preparation of extracts) were analyzed as previously described [20] on a Perkin Elmer Series 200 HPLC system (Shelton, Connecticut, USA) and a Perkin Elmer Brownlee Analytical C18 column (4.6 x 250 mm, 5µm) (Shelton, Connecticut, USA), by an acidified acetonitrile-water gradient. Water adjusted to pH 2.5 with orthophosphoric acid was the solvent A, and acetonitrile was the solvent B, mixed according to the following gradient; starting with 100% A, decreasing to 91% over the next 12 mn, to 87% over next 8 mn, to 67% over the next 12 mn, to 57% over the next 10 mn, and held at this level until the end of the 60 mn analysis. Standard chromatograms were plotted at 260 and 340 nm. Spectral data for all peaks were accumulated in the range 220-400 nm using diode array detection (Perkin Elmer Series 200). Structural identification was obtained by direct comparisons of retention times and UV spectra of resolved compounds with those of standard; two kaempferol glycosides, identified as kaempferol-3,7-O-diglucoside and kaempferol-3-O-[rhamnosyl-(1-6)-glucoside], and one quercetin glycoside, identified as quercetin-3-O-[rhamnosyl-(1-6)-galactoside]. The structural information of compounds, for which standards were not availabe, was obtained from their spectral parameters according to the compilations of Mabry, Markhan and Thomas and Campos and Markham [19], [20].

Quantitative determinations were made by an external standard method, with the commercial reference (quercitrin), by area measurements, using the following standard curve: Area = -0.0046x + 0.0278 [Quercitrin], correlation coefficient r = 0.992. The content of each compound was expressed as µg of quercitrin equivalents g^{-1} dry tissue (g QiE g^{-1} dry tissue). The HPLC-DAD profiles were individually obtained and analyzed for the extracts from three pools of samples.

Total Antioxidant Capacity

The total antioxidant capacity (TAC) of each samples was evaluated through the method developed by Prietoet al. [21], in which the reduction of Mo (VI) to Mo (V) is carried out by the antioxidant, forming a green phosphate/Mo (V) complex at acidic pH. Aliquots (100 µL) of each sample (containing 100 µg.mL^{-1} of flavonols, respective concentrations of flavonols calculated from the standard curve of quercetin) were prepared and combined with 1 mL of a solution constituted of sulfuric acid (O.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 equivalentsmL^{-1} (mg AAE mL^{-1}). Ascorbic acid curve: A_{695} = 3.678x – 0.092 [ascorbic acid], correlation coefficient r = 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.

Free radical scavenging activity

The DPPH* method reported by Campos et al. [22] 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) in such a way so as 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 = 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 pool samples.

Iron Reducing Power

The iron reducing power (IRP) method reported by Yanget al. [23] 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.

Atomic absorption (*Samples preparation*)

Typical analytical procedure of 975.03 to AOAC was used adapted by Perkin-Elmer [24]. Take 1 g of ground dried plant sample and place it in a small beaker. Add 10 mL of concentrated HNO_3 and allow standing overnight. Heat carefully on a hot plate until the production of red NO_2 fumes has ceased. Cool the beaker and add a small amount (2 – 4 mL) of 70 % $HClO_4$. Heat again and allow evaporating to a small volume. Transfer the sample to a 50 mL flask and dilute to volume with distilled water. Caution: always add HNO_3 to the tissue samples and allow this mixture to digest before adding $HClO_4$. $HClO_4$ can react explosively with untreated organic matter.

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 $p<0.05$.

Results and discussion

Biological investigation

Antioxidants activities

Several methods were developed to evaluate antioxidant potential of plants extracts. They are based on the determination of products resulting from oxidation or, on the contrary, measure the effectiveness of a substance to trap radicals, often by giving a form

H• [25].Recent studies showed that there can be differences in the determination of antioxidant activity according to the method used [25],[26]. Thus for better evaluating of antioxidant potential of plant extracts it is necessary to use at least two methods. In the present study we used three methods: total antioxidant capacity (y = 3.6781x - 0.092, r = 0.9985), iron reducing power (y = 2.6486x + 0.0017, r = 0.9988), free radical scavenging activity (y = 0.0309x + 0.0019, r = 0.9996) to evaluate the antioxidant activity of acetone, decoction, ethanol-water extracts and four fractions of ethanol-water of these plants.

Total Antioxidant Capacity

The results of the reduction of molybdate (VI) to molybdate (V) are presented in the Table 2. By taking account of the whole tests (total extracts and fractions), the results are included between 0.85 and 9.34 mg EAA/mL. In a general way the extracts of ethanol-water presented the best reducing activities with an average of 7 mg EAA/mL. More specifically the butanolic fraction of *V. diversifolia* gave the best activity (9.34 Mg EAA/mL). The weakest activities are held by decoction extracts with an average of 0.9 mg EAA/mL. The low value being held by *L. chevalieri* (0.850 mg EAA/mL). At this level we observe a controversy correlation between polyphenolic compound content and molybdate reducing activity. Indeed sometimes a correlation seems existed such as polyphenolic compound content case of ethanol-water and decoctions extracts on the one hand and on the other hand with the reducing activities (Table 2). Often not correlation existing in a similar situation. In fact, with a total phenolic content of 44.74 mg EAG/g extracts (butanolic fraction of *V. diversifolia*), we observed a best activity on TAC than ethyl acetate fraction which gave 131.32 mg EAG/g extracts. These results corroborate with those of Barriada-Bernal et al. [27] who evaluated antioxidant activities against the total flavonoids contents. They don't found a clear correlation betweentotal antioxidant capacity and the total flavonoids content. Indeed, 1210.48µg/g of total flavonoid content gave 4.646 mgEAA. However120.48µg/g < 1210.48µg/g gave 6.113EAA > 4.646 mgEAA. The same results were obtained by Falleh et al. [28].

Table 2: Polyphenolic compounds contents and antioxidants activities of the plants

Species	Type of extracts/Fractions	Phenol contents (mg EAG/g dry extracts)	Flavonoid contents mg EQ/g dry extract)	Free radical scavenging activity EC_{50}(µg/mL)	Total antioxidant capacity (mg EAA/mL)	Iron reducing power EC_{50}(µg/mL)
L. rhodesiensis	Acetone	24.85 ± 1.57^{a}	1.31 ± 0.09^{b}	2.31 ± 0.05^{b}	1.447 ± 0.047^{b}	0.24 ± 0.02^{d}
L. chevalieri		7.86 ± 0.42^{c}	0.90 ± 0.03^{c}	3.49 ± 0.08^{a}	1.337 ± 0.023^{c}	0.08 ± 0.00^{f}
V. diversifolia		4.00 ± 1.03^{d}	1.89 ± 0.13^{a}	1.45 ± 0.02^{d}	1.277 ± 0.036^{cd}	0.10 ± 0.00^{ef}
V. doniana		18.84 ± 1.34^{b}	2.08 ± 0.14^{a}	1.72 ± 0.04^{c}	1.496 ± 0.048^{b}	0.12 ± 0.00^{e}
L. rhodesiensis	Decoction	7.03 ± 0.46^{c}	0.48 ± 0.02^{c}	0.05 ± 0.00^{d}	1.049 ± 0.021^{f}	0.15 ± 0.00^{d}
L. chevalieri		16.10 ± 1.05^{a}	0.77 ± 0.02^{b}	1.30 ± 0.08^{c}	0.850 ± 0.008^{g}	0.06 ± 0.00^{e}
V. diversifolia		12.14 ± 1.62^{b}	1.19 ± 0.03^{a}	8.12 ± 0.20^{a}	0.954 ± 0.028^{f}	0.07 ± 0.00^{e}
V. doniana		18.70 ± 3.05^{a}	1.17 ± 0.06^{a}	2.95 ± 0.06^{b}	0.966 ± 0.004^{f}	0.07 ± 0.00^{e}
L. rhodesiensis	Ethanol-water	69.48 ± 8.07^{a}	3.27 ± 0.40^{a}	12.21 ± 0.95^{b}	8.936 ± 0.646^{a}	0.64 ± 0.01^{c}
L. chevalieri		19.48 ± 1.68^{d}	1.62 ± 0.33^{b}	13.93 ± 0.39^{a}	6.555 ± 0.096^{c}	0.23 ± 0.01^{e}
V. diversifolia		33.48 ± 3.50^{c}	3.23 ± 0.43^{a}	6.07 ± 0.22^{c}	6.357 ± 0.122^{c}	0.31 ± 0.01^{d}
V. doniana		54.14 ± 4.35^{b}	3.58 ± 0.20^{a}	5.04 ± 0.03^{d}	7.441 ± 0.267^{b}	0.28 ± 0.00^{d}
(*L. rhodesiensis*)	n-HF	91.76 ± 1.39^{d}	7.80 ± 0.04^{g}	0.33 ± 0.02^{g}	7.14 ± 0.18^{e}	0.35 ± 0.02^{i}
	n-BF	90.52 ± 11.2^{d}	12.71 ± 0.02^{d}	0.57 ± 0.02^{e}	6.17 ± 0.15^{f}	2.21 ± 0.01^{a}
	AqF	102.43 ± 14.55^{bc}	5.80 ± 0.04^{j}	1.21 ± 0.03^{a}	3.95 ± 0.01^{j}	0.89 ± 0.01^{d}
	EAF	126.07 ± 10.74^{a}	25.71 ± 0.05^{b}	0.05 ± 0.00^{j}	7.34 ± 0.07^{d}	1.20 ± 0.03^{b}
(*L. chevalieri*)	n-HF	40.20 ± 1.34^{h}	6.49 ± 0.08^{i}	0.11 ± 0.01^{i}	4.59 ± 0.15^{h}	0.09 ± 0.00^{m}
	n-BF	73.00 ± 0.34^{e}	6.59 ± 0.12^{i}	0.28 ± 0.01^{h}	4.15 ± 0.05^{i}	0.26 ± 0.00^{k}
	AqF	99.32 ± 0.56^{cd}	7.09 ± 0.06^{h}	ND	3.98 ± 0.03^{j}	0.35 ± 0.02^{i}
	EAF	79.23 ± 1.48^{e}	4.96 ± 0.00^{l}	0.60 ± 0.02^{d}	3.98 ± 0.13^{j}	0.26 ± 0.00^{k}
(*V. diversifolia*)	n-HF	41.803± 1.97^{h}	5.45 ± 0.18^{jk}	0.38 ± 0.00^{f}	2.86 ± 0.02^{l}	0.14 ± 0.01^{l}
	n-BF	44.74 ± 2.17^{gh}	5.39 ± 0.42^{k}	0.99 ± 0.01^{b}	9.34 ± 0.12^{a}	0.30 ± 0.00^{j}
	AqF	57.54 ± 3.64^{fg}	9.23 ± 0.24^{ef}	1.01 ± 0.04^{b}	7.53 ± 0.14^{cd}	0.36 ± 0.00^{i}
	EAF	131.32 ± 4.43^{a}	35.25 ± 0.74^{a}	0.12 ± 0.01^{i}	8.61± 0.16^{b}	0.74 ± 0.02^{f}

Table 2: Continued

Species	ETOH-H2O fractions	Phenol contents (mg EAG/g dry extracts)	Flavonoid contents mg EQ/g dry extract)	Free radical scavenging activity EC_{50}(µg/mL)	Total antioxidant capacity (mg EAA/mL)	Iron reducing power IC_{50}(µg/mL)
(*V. doniana*)	n-HF	110.16 ± 9.51^{b}	8.04 ± 0.08^{g}	1.21 ± 0.02^{a}	3.92 ± 0.12^{j}	0.25 ± 0.00^{k}
	n-BF	60.12 ± 1.32^{f}	17.51 ± 0.05^{c}	0.34 ± 0.01^{g}	6.07 ± 0.13^{f}	0.59 ± 0.02^{h}
	AqF	10.87 ± 0.31^{i}	9.80 ± 0.11^{e}	0.72 ± 0.04^{c}	3.40 ± 0.01^{k}	0.36 ± 0.04^{i}
	EAF	93.54 ± 6.23^{cd}	17.81 ± 0.10^{c}	0.34 ± 0.01^{g}	5.23 ± 0.02^{g}	0.93 ± 0.00^{c}
(References)	Gallic acid				1.26 ± 0.03^{o}	0.92 ± 0.001^{c}
	Cafeic acid				1.73 ± 0.06^{m}	0.84 ± 0.01^{e}
	Ascorbic acid				1.78 ± 0.02^{m}	0.66 ± 0.03^{g}
	Quercetin				1.51 ± 0.01^{n}	0.65 ± 0.04^{g}

Freeradical scavenging activity

The anti-DPPH* activity of the vegetable extracts translates their aptitude to trap the free radicals of the organism. To this end we evaluated three types of extracts and four fractions coming from the ethanol-water total extract (Table 2). Results are evaluated in EC_{50} and were between 0.05 (*L. rhodesiensis*) and 13.93 (*L. chevalieri*) EQ/g dry extracts. The EC_{50} of a curve dose-effect represents the concentration of a compound where 50% of the maximum effect is observed. The antioxidant capacity of a compound is higher as its EC_{50} is small. Among these results, the strongest value was observed with *L. rhodesiensis* in decoction extract and it ethyl acetate fraction (0.05 EQ/g dry extracts). These results are consigned in Table 2 and are similar to those obtained by Reyes-Martinez et al. [29]. These species also presented the strongest totals phenolic and flavonoids contents in their fractions (ethyl acetate fraction). While looking at close the results related to the fractions, one notes that the ethyl acetate fractions presented the best anti-DPPH* activities. We couldn't establish a correlation of these activities with the totals phenolic and flavonoid contents. Indeed on the level of total phenolic contents and ethanol-water extract, *L rhodesiensis* which gave the strongest content (69.48 mg EAG/dry extract) also presented the second weaker DPPH*activity (12.21 µg/mL). Contrary to its decoction content which is 7.03 mg EAG/dry extract but it presented the best activity (0.05 EQ/g dry extracts). Our results are in disagreement with those of Barriada-Bernal et al. [27]. According to our former investigation [9], it was difficult to establish a clear correlation between the flavonoids rate and the DPPH* reduction. Several authors tried to justify this fact [30],[31],[32],[33],[34]. Confer relationship between antioxidants activities and polyphenolic compounds estimated part.

Iron Reducing Power

In the mechanism of phenolic antioxidant activity, the reduction of Fe (III) is often used like an indicator of donor of electrons [35]. Iron reducing power method makes it possible to measure the ability of phenolic compounds to reduce Fe (III) in Fe (II). The ascorbic acid was used as standard for the development of the calibration curve whose regression equation is: $y = 2.6486x + 0.0017$; $r = 0.9988$. The results of this study are indicated in Table 2. Taken account of the three types of extraction the results give values ranging between 0.06 and 0.92 µg/mL. In terms of ions ferric reduction in ions ferrous the strongest values were obtained with *L. chevalieri* extracts with 0.06 µg/mL (decoction) > 0.08 µg/mL (acetone) > 0.23 µg/mL (ethanol-water). The low values are obtained with *L. rhodesiensis* with respectively 0.15 µg/mL (decoction) > 0.24 µg/mL (acetone) > 0.64 µg/mL (ethanol-water). According the results of this study there are not a correlation between total phenolic contents and ion ferric reducing activity. Indeed *L. rhodesiensis* which gave the best totals phenolic content (69.48 mgEAG/g dry extracts) also presented the weakest ions ferric reduction (0.64 µg/mL). That is confirmed by the results obtained with the fractions. Firstly, fractions of *L. rhodesiensis* presented the lowest results (range between 0.35 and 2.21 µg/mL). However this plant gave the best total phenol contents of fraction with an average of 102.70 EAG/g dry extracts. Secondly, for 131.32 mg EAG/g dry extracts of totals phenolic, one has a reducing activity of 0.74 µg/mL. On the other hand with a content of 10.87 mg EAG/g one obtained 0.36 µg/mL of ion ferric reduction. That corresponds to the higher half of the first activity. Finally, we note that ethyl acetate fraction gave the weakest results. Concerning total flavonoid content,we note a correlation between theircontents and ion ferric reducing activity. These results are corroborated [27], [36]. While looking of close ions proportioning using atomic absorption method, we realize that among the four plants species, *L. chevalieri* presented the strongest content of ions ferrous (5.661 g/kg). This result is followed by that of *V. diversifolia* (1.145 g/kg). We can suppose that the ions ferrous reducing activity is correlated by the content of the plants.

Phytochemistry investigation

Phenolic and flavonoid contents

Three types of extraction were used in this study to evaluate phenolic and flavonoids contents. With the continuation we selected the type of extract having given the best contents of

polyphenolic to evaluate the fractions contents. Four types of fractions were evaluated such as hexanic fractions (n-HF), butanolic fractions (n-BF), ethyl acetate fractions (EAF) and aqueous fractions (AqF). Total extracts contents and their fractions were used for the evaluation of the antioxidant activities and the HPLC-DAD analysis.

In a general way, the results of phenolic and flavonoids quantification are decreasing in the order ethanol-water, acetone and decoction. The totals phenolic varied between 4.00 and 69.48 mg EAG/g dry extracts (Table 2). The best values are obtained with the ethanol-water extracts. At this level *L. rhodesiensis* holds the strong value (69.48 mg EAG/g), followed *V. doniana* (54.14 mg EAG/g). The low value is obtained with *L. chevalieri* (4.00 mg EAG/g) of acetone extracts. This value is followed of that of*L. rhodesiensis* (7.03 mg EAG/g) of decoction extracts.

In terms of total flavonoids content, the values vary between 0.48 and 3.58 mg EQ/g dry extracts. The strongest values are obtained with ethanol-water extracts of *V. doniana* = *V. diversifolia* = *L. rhodesiensis* (3.58 mg EQ/g). The weak values are held by the decoction extracts with 0.48 mg EQ/g for *L. rhodesiensis* and 0.77 mg EQ/g for *L chevalieri*. However in terms of flavonoid contribution to total phenolics, the best contribution was observed with acetone extracts such as *V. diversifolia* with 47.25% > *V. doniana* = *L. rhodesiensis* (11.45%). These values are followed so far by the decoction extracts with 9.80%.*L. rhodesiensis* and *L. chevalieri* presented the best activities on total antioxidant activity, anti-DPPH*, and iron reducing power respectively. Former investigation of totals phenolic and flavonoid contents of *L. rhodesiensis* in methanolic extract showed 21.55 ± 0.75 mg GAE/100 mg and 5.09 ± 0.19 mg QE/100 mg extract, respectively [4]. Piero et al. [37] quantified in aqueous extracts of *L. rhodesiensis* (leaves) phenols content and find 685.25 ± 30.77 mg/g gallic acid equivalent, while flavonoids was 187.33 ± 54.97 mg/g. Our results are much lower than those of Piero *et al.* [37] who used aqueous extracts. However, they are better than those of Bangou et al. [4] who used methanolic extracts. Lagnika et al. [38] investigated antibacterial, antifungal, antioxidant activities and toxicity of *Vitex doniana* leaves against dichloromethane, methanol and hydroethanolic extracts. Their results supported the utilization of this plant in infectious diseases and also showed this plant as good sources for antioxidants. Agbafor et al. [10] showed clearly that this best antioxidant activity of *Vitex doniana* is due to it vitamin C content.

Ions quantification

The analysis is related to eight minerals such asCa^{2+}, Fe^{2+}, K^{+}, Mg^{2+}, Na^{+}, Zn^{2+}, Pb and As. No arsenic trace was found in our various plants. With the exception of lead and arsenic, we evaluated minerals for their implications of human physiological activity at low dose. The proportion of arsenic and lead wanted to be evaluating the risk of contamination of the grounds by the mining activities. The care by traditional medicine is generally related to the combinations of plants (phytotherapy), fragment of animals (opotherapy) and minerals (mineral therapy). Then, some oligo elements (Ca^{2+}, Fe^{2+}, K^{+}, Mg^{2+}, Na^{+}, Zn^{2+}) have an essential role in physiological balance and especially for the correct operation of the neurotransmitters [6],[25]. In general way their absences involve disturbances [6],[25]. Thus for example, magnesium deficiency will involve syndromes of neuromuscular hyperirritability, cardiovascular and thrombosis accidents([6],[25]). Its metabolism is also related to those of Ca^{2+} and P. In terms of ions quantification the values are included between $1.1x10^{-4}$ and 55.88 g/kg of dry weight. Calcium presented the best contents with 55.88 g/kg (*L. chevalieri*) > 23.36 g/kg (*L. rhodesiensis*) > 16.87 g/kg for *V. doniana* and *V. diversifolia*. Traces of lead are found on *Vitex* species. In terms of daily need for the ions highlighted, sodium comes at the head with 2g/day > 1.5 g/day (magnesium) and of potassium (0.5 g/day). Lead occupies the last place with 0.0005 g/day.Vunchi et al.[39], analyzed the proximate, vitamins and mineral compositions of *Vitex doniana* fruit pulp. They found importance values of minerals: potassium (16.5 mg/100g), sodium (10.4 mg/100g), calcium (30.27 mg/100g), iron (5.2 mg/100g), Copper (2.7 mg/100g), magnesium (20.10 mg/100g) and phosphorus (16.5 mg/100g). Confrontation of our results shows that the mineral values are higher in the fruits than in the leaves or stem-leaves (Table 3). Indeed, this study concluded that *Vitexdoniana* fruit pulp could be promoted as: carbohydrate and lipid supplements for cereal-based diets inrural communities [39]. Sawadogo et al. [3] have quantified some metal ions (Na^{+} and K^{+}) and showed th.eir implication in the Na^{+}-K^{+}-pump action.

Table 3: Atomic absorption of seven oligo elements detected in the four edible plants

Species	Ca^{2+}	Fe^{2+}	K^{+}	Mg^{2+}	Na^{+}	Zn^{2+}	Pb
L. rhodesiensis	23.36 ± 1.92	0.51 ± 0.00	19.32 ± 0.56	3.23 ± 0.03	0.079 ± 0.001	0.035 ± 0.000	--
L. chevalieri	55.88 ± 1.38	5.66 ± 0.36	16.34 ± 0.79	6.76 ± 0.02	0.057 ± 0.001	0.05 ± 0.00	--
V. diversifolia	16.87 ± 0.05	1.145 ± 0.008	7.20 ± 0.41	5.32 ± 0.12	0.058 ± 0.001	0.037 ± 0.000	$(1.1 \pm 0.35)*10^{-4}$
V. doniana	16.58 ± 0.23	0.27 ± 0.00	10.11 ± 0.11	4.29 ± 0.11	0.06 ± 0.01	0.045 ± 0.001	$(1.9 \pm 0.43)*10^{-4}$

HPLC-DAD analysis

A total of 41 polyphenolic compounds (Figure 1: 1/7-7/7) was detected while excluding the aromatic acids from bases which seem to be transverse to all plant extracts. The HPLC-DAD analysis related to the three types of extracts and fractions made on the basis of ethanol-water extract which presented the best contents of phenolic and flavonoids. The ethanol-water extract

presented more compounds (12 compounds). Only 8 compounds were detected on the fractionslevels. Luteolin was highlighted in the two species of *Vitex* and on the whole types of extracts. In the same way a derivative of quercetin-3-O-arabinoside (Dvidi6 and Avidi28) was highlighted in the decoction extract and in the acetone extract of *V. diversifolia* (Table 4)

Former studies showed that flavonoids are characterized by two bands maximum of absorbance of cycles A and B [1],[19]. More specifically flavones and flavonols are characterized by a band I (cycles A) in the areas 320 - 385 nm and a band II (cycles B) in interval 250 - 285 nm (Mabry *et al.* 1970). Band I of flavones being increasingly shorter from 20 to 30 nm with that of equivalent flavonols. Those of the phenols acids lie between 210 - 273 nm. Any increase in the number of hydroxyls grouping and/or glycosylation and O-methylation inducing bathochromic (displacement of maximum wavelengths towards the right) and hypsochromic (displacement of maximum wavelengths towards the left) effects, respectively [1], [19].

These observations associated at the protocol with elucidation [20] (Structure information from HPLC and on-line measured absorption spectra: Flavones, Flavonols and Phenolic Acids) enable us to distinguish 05 phenolsacids and 36 flavonoids (Table 4 & Figure 1: 1/7-7/7). Former investigations of the methanolic extracts of *L. chevalieri* highlighted 20 composed which have primarily acids phenols [1].

Thus we couldn't find the derivative luteolin and rutin [1] in our extracts. Other researchers showed in the dichloromethane fractions of *L. rhodesiensis*, two flavonoids such as 5,6,7,3',4',5'-hexamethoxyflavone and its analogue, 5-hydroxy-6,7,3',4',5'-pentamethoxyflavone [3]. The most elucidated studies on *L. chevalieri*, *L. rhodesiensis*, *V. diversifolia* and *V. doniana*especially related to their content of essential oils [1],[3], [5],[8].

Which could be the implication of these compounds highlighted in the biological activities observed?

Table 4: Wavelength characteristic of the phenols acids and flavonoids detected (1/2).

Number of compound	Compound	RT (min)	λ_{max} (nm)
Dvido1	Acacetin-7-O-(rhamnosyl(1-2)glucoside)	35.248	269, 330
Dvido2	Luteolin	47.612	253, 267sh, 291sh, 347
Dvido3	chlorogenic acid	25,812 ± 0,034	245sh, 296sh, 323
Dvidi4	Derivative of Acacetin-7-O-[rhamnosyl (1-2) glucoside]	32,122 ± 0,143	271, 330
Dvidi5	Isoorientin	32.358	254sh, 268, 290sh, 348
Dvidi6	Derivative of quercetin -3-O-arabinoside	33,187 ± 0,093	255, 267sh, 294sh, 346
Dvidi7	Luteolin	36.142	253, 267sh, 291sh, 347
Dlic 8	Chrysoeriol-6,8-di-C-glucoside	41,633 ± 0,066	253sh, 267, 343
Dlic9	Scutellarein-6,4´-dimethylether	41.217	274, 332
Dlic10	Acacetin-7-O-(rhamnosyl(1-2)glucoside)	29.579 ± 0,052	267, 333
Evido11	Neocarlinoside	30.569	256sh, 271, 348
Evidi12	Acacetin-7-O-(rhamnosyl(1-2)glucoside)	31,845 ± 0,146	271, 332
Evidi 13	Isoorientin	32.153	245, 268sh, 290sh, 348
Evidi14	Orientin	33,114 ± 0,093	254, 268sh, 290sh, 350
Evidi15	Luteolin-7-O-rutinoside	36.201	254, 267sh, 348
Evidi 16	Luteolin-7-O-(glucuronosyl(1-2)glucuronide)	36.274	255, 267sh, 345
Evidi 17	Chlorogenic acid	39.09	245sh, 296sh, 324
Elar18	Dof 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	dof Luteolin-7,3´-di-O-glucuronide	36,795 ± 0,923	250, 278, 337
Elar22	Rosmarinic acid	36.201	250sh, 290sh, 328
Alar23	Vitexin	29.674	271, 331
Alar24	Acacetin-7-O-(rhamnosyl(1-2)glucoside)	30,54 ± 1,12	271, 332
Alic25	Scutellarein-6,4´-dimethylether	53.17	274, 332
Avidi26	Luteolin	47,949 ± 0,062	252sh, 266sh, 290sh, 348
Avidi27	Luteolin-7-O-glucoside	36,89 ± 0,06	254, 266sh, 348

D: decoction, E: ethanol-water, A: acetone, LAR: *L. rhodesiensis*, LIC: *L. chevalieri*, VIDI: *V. diversifolia*, VIDO: *V. doniana*.

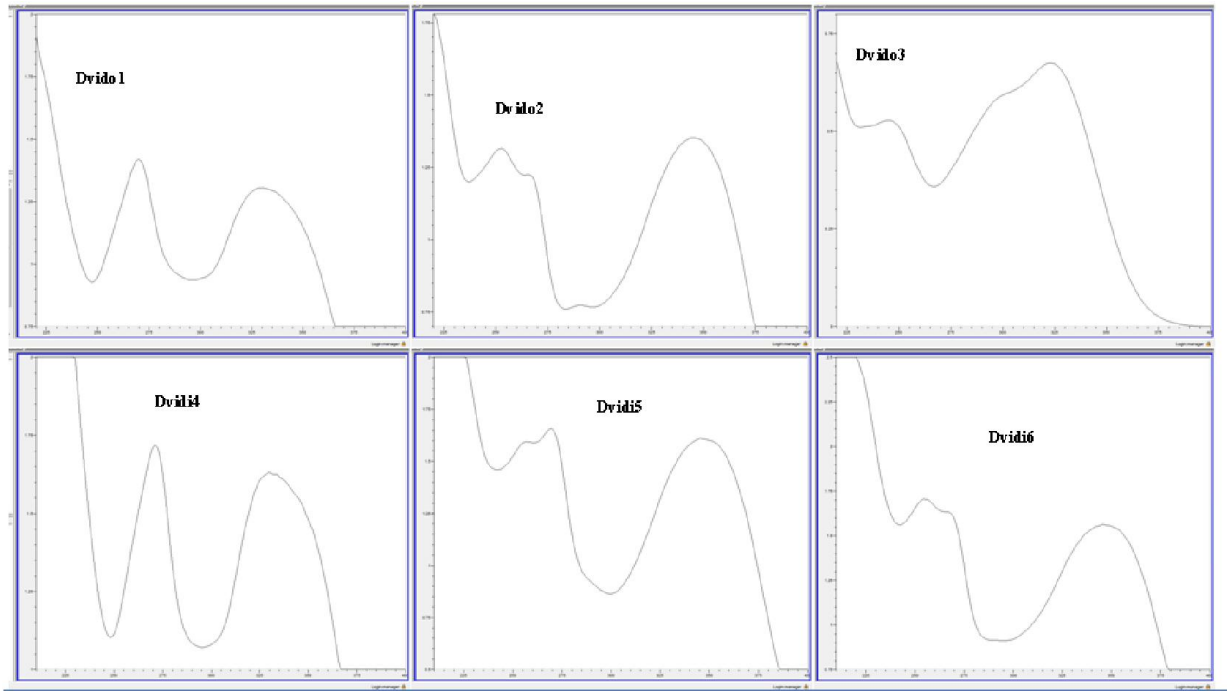

Figure 1: UV spectra of 41 phenols acids flavonoids found in the four edible plants (1/7). Dvido: decoction extract of *Vitex doniana*, Dvidi: decoction extract of *Vitex diversifolia*.

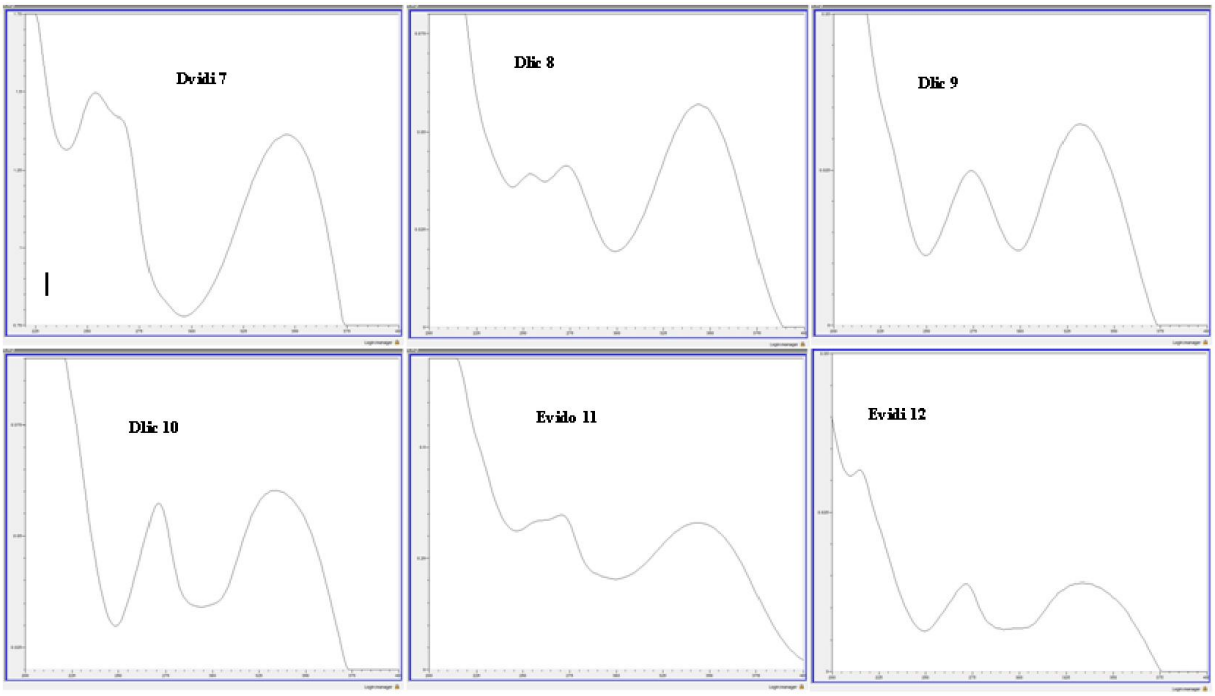

Figure 1: UV spectra of 41 phenols acids flavonoids found in the four edible plants (2/7). Dvidi: decoction extract of *Vitex diversifolia*, Dlic: decoction extract of *Lippia chevalieri*, Dvido: decoction extract of *Vitex doniana*.

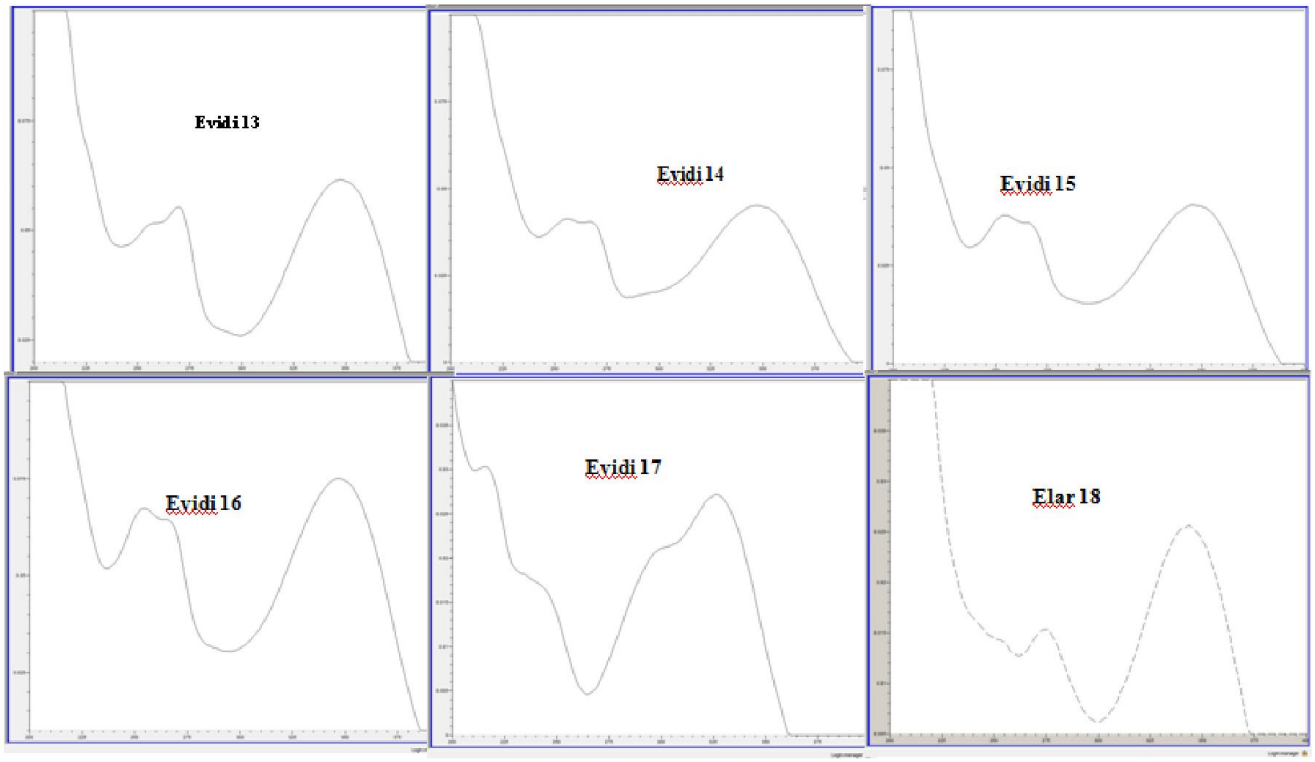

Figure 1: UV spectra of 41 phenols acids flavonoids found in the four edible plants (3/7). Evidi: ethanol-water extract of *Vitex diversifolia*, Elar: ethanol-water extract of *Lantana rhodesiensis*.

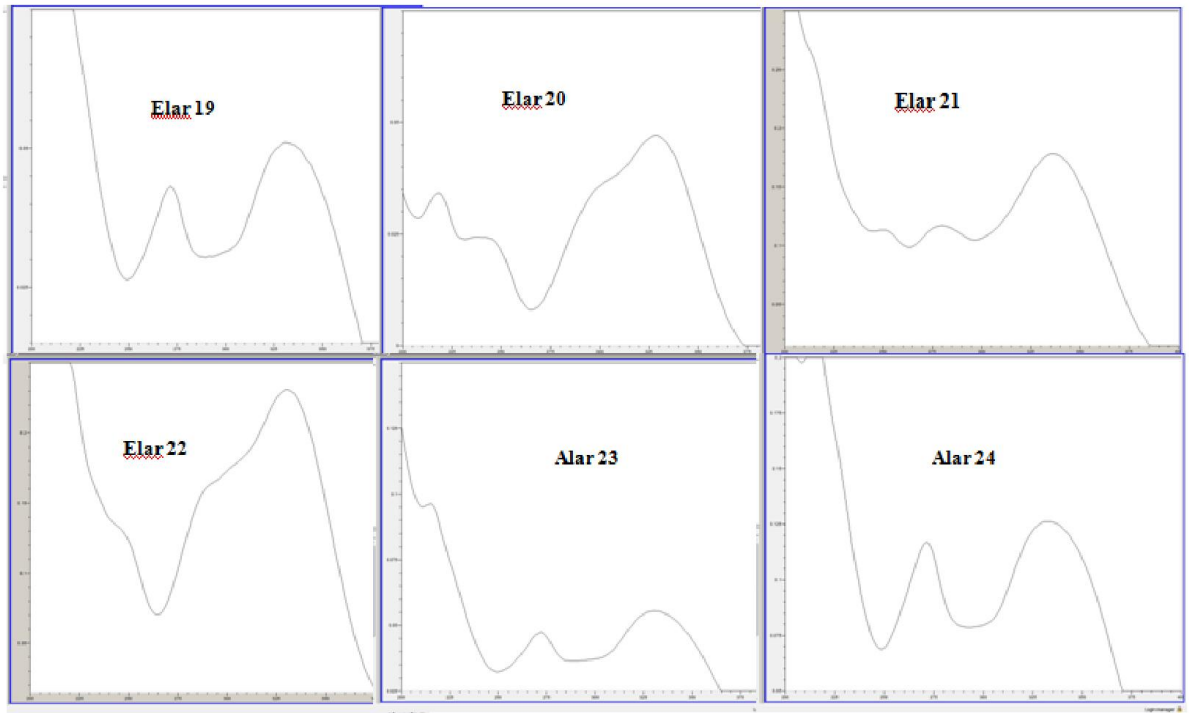

Figure 1: UV spectra of 41 phenols acids flavonoids found in the four edible plants (4/7). Elar: ethanol-water extract of *Lantana rhodesiensis*, Alar: acetone extract of *Lantana rhodesiensis*.

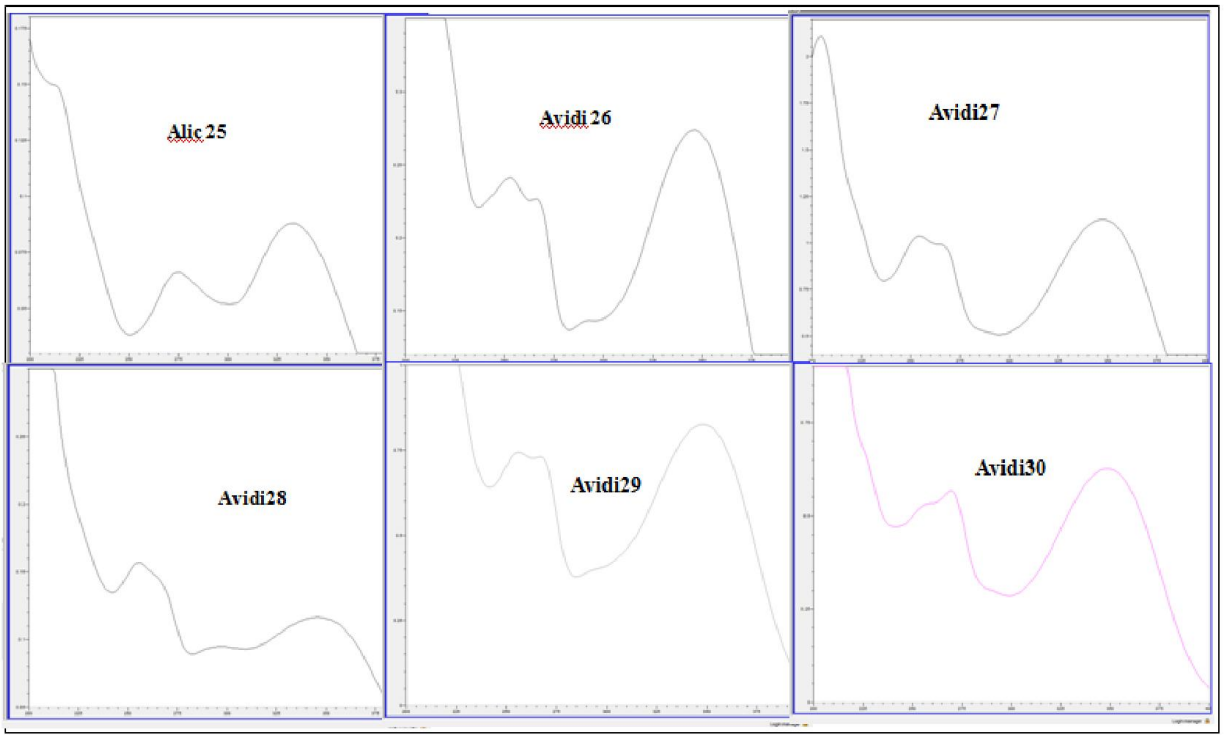

Figure 1: UV spectra of 41 phenols acids flavonoids found in the four edible plants (5/7). Alic: ethanol-water extract of *Lippia chevalieri*, Avidi: acetone extract of *Vitex diversifolia*.

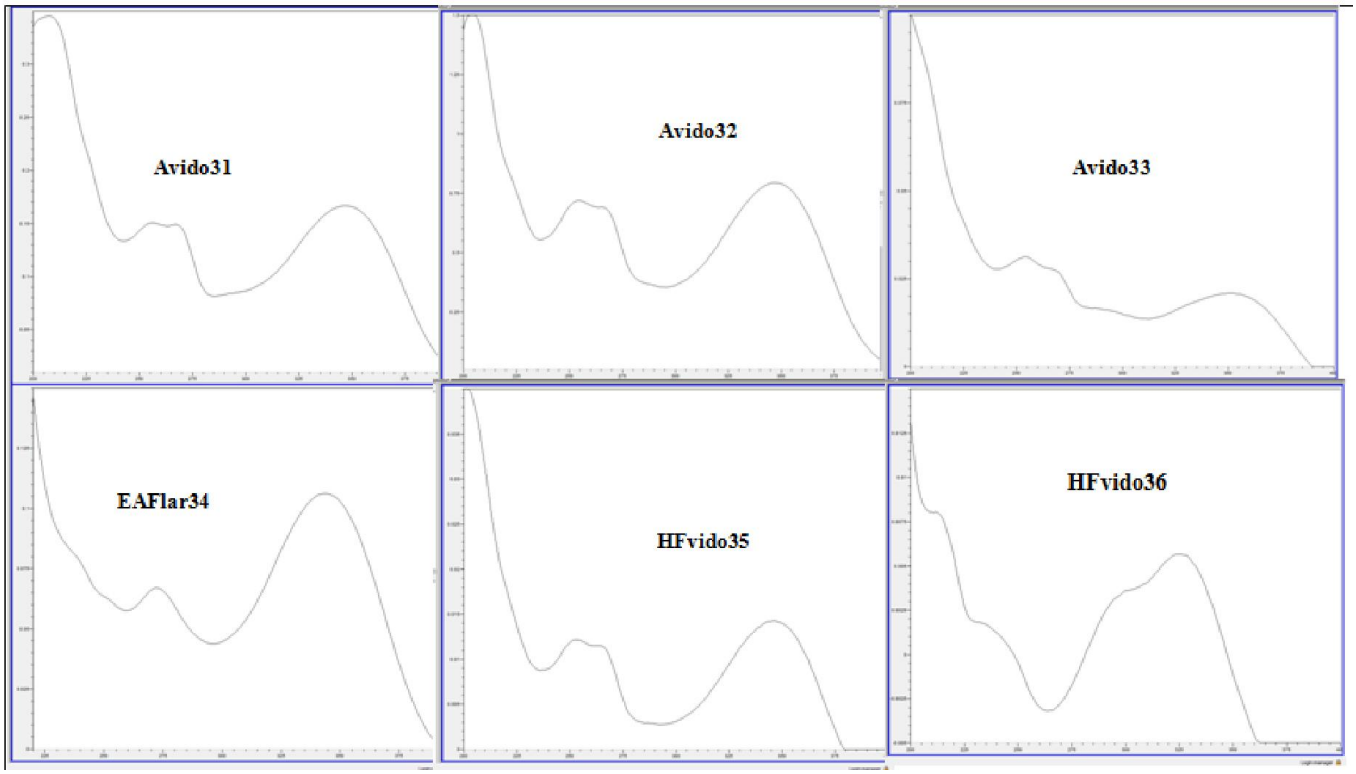

Figure 1: UV spectra of 41 phenols acids flavonoids found in the four edible plants (6/7).). Avido: acetone extract of *Vitex doniana*, HFvido: hexane fraction of *Vitex doniana*, EAFlar: ethyl acetate fraction of *Lantana rhodesiensis*,

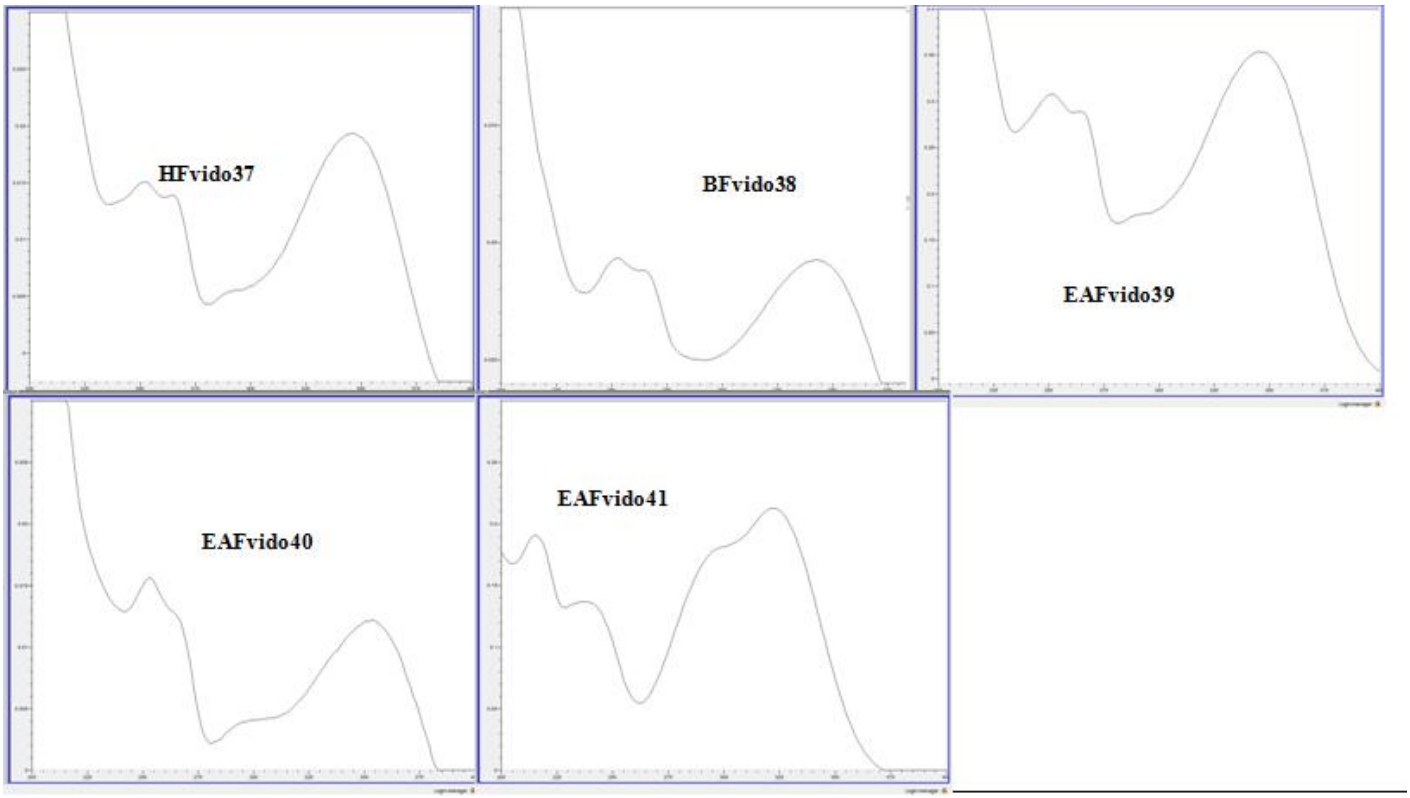

Figure 1: UV spectra of 41 phenols acids flavonoids found in the four edible plants (7/7). HFvido: hexane fraction of *Vitex doniana*, BFvido: butanolic fraction of *Vitex doniana*, EAFvido: ethyl acetate fraction of *Vitex doniana*

Relationship between Antioxidants Activities And Polyphenolic Compounds Estimated

The interest of the relation investigation is sought to understand the real implication of each group of compounds implied in the observed activities. It emerges from ours investigations that total phenolic are implied in the antioxidant activities (Table 2). In fact, 16.10 mg EAG/g dry extract (decoction extract) of *L. chevalieri* produced the best total antioxidant activity (0.850 mg EAA/mL) against 4.78% of flavonoids extract contribution. However one expected that activity is held by *L. rhodesiensis* (ethanol-water extract) with 69.48 mg EAG/g dry extract. If the activities were related to the flavonoids contribution, then the best activity in this case would have been that of *V. diversifolia* of acetone extract (47.58% of contribution). That is not the case. It is the same for iron reducing power (contribution of total flavonoids in the best activity is 4.78%) and free radical scavenging activity (6.82% of total flavonoids contribution). It's thus difficult to establish a clear correlation between biological activity and polyphenolic compounds contents. It generally arises this controversy as for the implication of flavonoids in the antioxidant activities [9]. These results corroborate with those of Meda et al. [40]. Former research established that this controversy wouldbe due to several possibilities: (i) either the compounds existing inthe extracts have large molecular weights or are heterosidic[9],[33], (ii) orthe majority of the flavonoids are not antioxidants[9],[31], (iii) or there isunder estimate of the flavonoids by the method of $AlCl_3$ [30],[34], [40].

The flavonoids inactive and stabilize the free radicals thanks to their hydroxyl grouping (C3-OH) strongly reactive. Indeed, the glycosylation of the 3-OH group of quercetin (case of rutin) or its suppression (case of the luteolin) decreases antioxidant activity[8]. That could explain the weak activity of *V. diversifolia* extracts where we highlighted a derivative of quercetin (quercetin-3-O-arabinoside). The spectral study enabled us to characterize some compounds (Table 4). With through the literature we know that 6-hydroxyflavones are much more stable than their 8-isomers [41]. According to these authors [41], flavonols like acacetin, genkwanin, chrysoeriol and diosmetin cannot be separate well on HPLC, but can be distinguished by their different colors reactions in UV light. According to the same authors, an additional effect existed between 6-hydroxyl group and apigenin which make the compound more polar and shorten the retention times [41]. That could explain in the same order the difference between retention times that we obtained (Table 4) and those of the method used [20]. Thus, hydroxylation decrease the retention time and methylation increase it [41],[42]. Concerning the antioxidant effect of these compounds, it's well known that the polyhydroxy substituted flavonoids

commonly found in most plants present the highest antioxidant activity. Specifically the C-ring plays an important role in this activity ([8],[42]).Traditional medicine being a science of natural substances synergy, we will be able to justify these activities observed by this synergy of plant compounds.

What happens exactly of antioxidants mechanism in the cellular metabolism? According to several authors, much time oxygen oxidizing take place in mitochondria, cytochrome P450, peroxisomes with some enzymes like NADPH oxidase and xanthine oxidase [43],44],[45],[46]. Reactive oxygen species (ROS) include free radical of oxygen: superoxide anion, hydroxide radical and some derivative non radical form like hydrogen peroxide [25]. Figure 2 give some detail of oxygen share take origin probably in the mitochondria. Seifried et al. [45], tried to establish opposition between excess of oxidants and excess of antioxidants, and then the consequences on human body (Figure 3). It resort in this case, that excess of both of them have negative effect. The best situation is to establish balance in our cells [40]. But like any things, excess harms. The same authors were demonstrated that the excess of antioxidants (enzymatic antioxidant and non-enzymatic antioxidant) can involve the loss of their property [43],[45], [46],[47], [48],[49], [50]. In general the role of primary enzymes (superoxide dismutase, catalase and glutathione peroxide), help to the detoxication of xenobiotics compounds [51]. The superoxide dismutases are the first defensive lines against the ROS, while the catalases prevent the oxidative damages by ensuring the passage of H_2O_2 into H_2O and in O_2 [25]. In the case of cells proliferation of cancer, the conjugaison of glutathione (GSH) to the electrophilic compound permit to protect the element. It is well-known the great relationship between glutathione-s-transferase (GST) and glutathione. Indeed, all high concentration of glutathione present in the intracellular middle is always occupied, and it's a powerful detoxicant of xenobiotics compounds and free radicals [25]. And, the glutathione-s-transferases are implied in the resistance of tumorals cells [52],[53].

In a normal healthy human body, the generation of pro-oxidants in the form of ROS is effectively kept in check by the various levels of antioxidant defense [54]. Human body exposure to adverse physicochemical, environmental or pathological agents such as pollutions and overnutrition etc… maintained balance shifted in favor of pro-oxidants resulting in "oxidative stress" [54]. Natural compounds like dietary sources (fruits and leaves of *L. chevalieri*, *L. rhodesiensis*, *V. doniana*) provide a large number of antioxidants, which can prevent many human diseases [8],[54]. Some beverages such as tea (*L. chevalieri*) are also rich sources of antioxidants [8],[29], [54],[55], [56],[57]. According to Tilak et al. [54], a growing body of evidence suggests that moderate consumption of tea and dietary may protect against several forms of cancer, Alzheimer's disease, cardiovascular diseases, and formation of kidney stones, bacterial infections, and dental cavities.

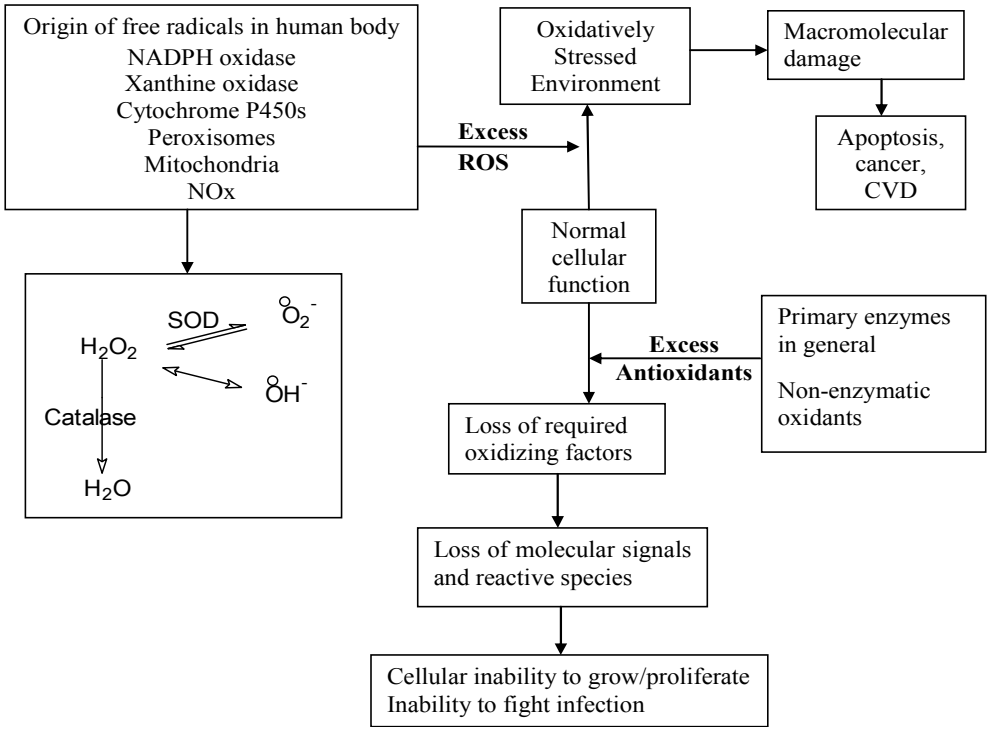

Figure 2: cellular oxidative interactions [45]

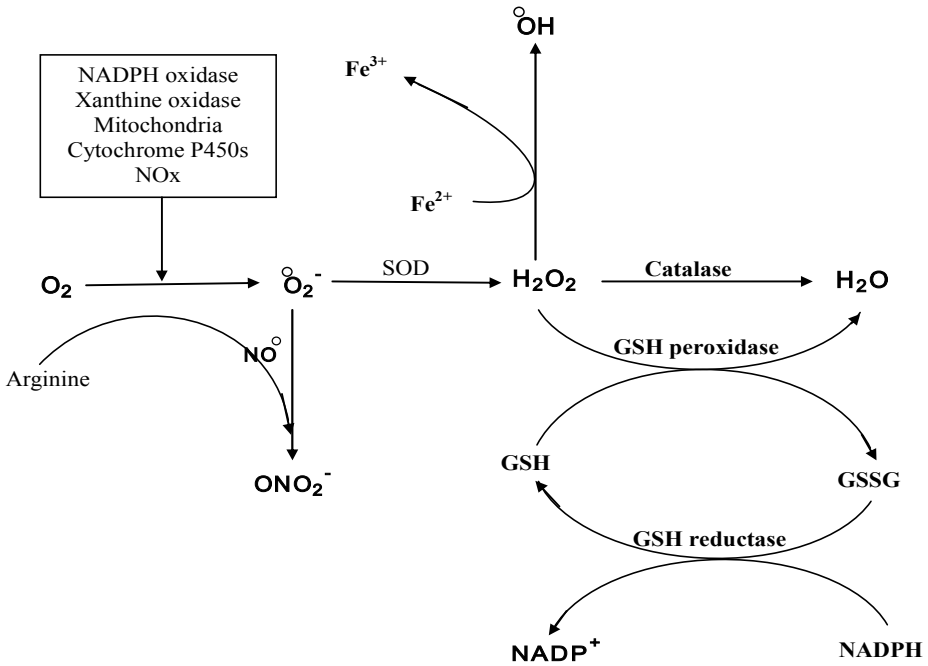

Figure 3: defence mecanism against ROS [44]. **GSH**: glutathione reduced, **GSSG**: oxidized glutathione,**SOD**: superoxide dismutase, **NADPH**: nicotinamide adenine dinucleotide phosphate.

Conclusion

Antioxidant activity was investigated together with polyphenolic compounds, using acetone extracts, decoction extracts and ethanol-water extracts. The strongest totals phenolic and flavonoids contents were obtained with ethanol-water extracts. Four types of fractions of ethanol-water were evaluated for polyphenolic quantification. Among the four edible species study, *L. rhodesiensis* and *L. chevalieri* presented the best activities on total antioxidant activity, anti-DPPH*, and iron reducing power respectively. 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 thus 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 glutathion in the human organism.

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