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# Comparison of *in vitro* antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa*, *Garcinia lucida* and *Hymenocardia lyrata*

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ABSTRACT

**Objective:** To investigate the *in vitro* antiradical and antioxidant properties of methanol extracts of *Garcinia lucida*, *Hymenocardia lyrata (H. lyrata)* and *Acalypha racemosa*.
**Methods:** We determined the *in vitro* antioxidant activity and the total phenolic content, and performed the phytochemical screening of the extracts from fruits, barks, leaves, roots and stems of these plants using standard procedures.
**Results:** The results of the antioxidant properties showed that more than 60% of DPPH·, NO· and HO· radicals were inhibited by the extracts. The radical scavenging activity, the total antioxidant capacity as well as the reducing power increased with the concentration of the extracts. The 50% inhibitory concentration ($IC_{50}$) varied from 1.46 to 21.65 μg/mL depending of the type of extract and the antioxidant tests. The results indicated that all the extracts exhibited antioxidant properties and the roots of *H. lyrata* showed the best antioxidant activity [(217.17±9.45) mg Equivalent catechin/g of extract] while the highest total phenol content was found in its barks [(169.782±3.025) mg Eq cat/g of extract].
**Conclusions:** The methanol extract of *H. lyrata* possess the most antioxidant property among the three extracts.

## 1. Introduction

The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs. Products of higher plant origin have been shown to be effective sources of chemotherapeutic agents without underlining side effect[1,2]. Several culinary herbs and spices have been evaluated for their biologic activities in the management of chronic diseases, including Alzheimer disease[3]. Reactive oxygen species (ROS)/free radicals have been implicated in causation of more than 100 diseases including diabetes, inflammation, cancer, neurodegenerative disorders, atherosclerosis, liver cirrhosis, nephrotoxicity *etc*[4-6]. ROS are various forms of activated oxygen such as superoxide anion radicals ($O_2^-$) and hydroxyl radicals (OH·), as well as non-free radicals species ($H_2O_2$) and singlet oxygen ($^1O_2$)[7]. Experimental, clinical, and epidemiological studies have provided evidence in support of the role of ROS in the etiology of cancer[8]. All aerobic organisms including humans have antioxidant defense mechanisms that protect against oxidative damage. However, the natural antioxidant defense mechanisms can be insufficient and hence dietary intake of antioxidant components is important and recommended[2]. It has been suggested that fruits, vegetables and plants are the main source of antioxidant in the diet. Natural antioxidants may have free-radical scavengers, reducing agents, complexes of pro-oxidant metals, quenchers of singlet oxygen *etc*.

Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal products to replace synthetic antioxidants, which are being restricted due to their adverse reaction such as carcinogenicity. Plants therefore constitute the main source of natural antioxidant molecules which have the capacity to eliminate or neutralize the deleterious ROS[9]. The

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importance of medicinal plants to prevent or control diseases has been attributed to the antioxidant properties of their constituents, commonly associated with a large number of molecules such as phenols and flavonoids[10,11]. Polyphenolic compounds are believed to have chemopreventive and suppressive activities against cancer cells by inhibiting many metabolic enzymes involved in the activation of potential carcinogens or arresting the cell cycle[12]. Several studies demonstrated the antimicrobial activity of polyphenol and flavonoids[13-15]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases such as cancer, heart diseases and stroke, and increase the antioxidant capacity of the plasma as well as lipid oxidative rancidity in foods[16,17]. The secondary metabolites compounds from higher plants like phenolics and flavonoids have been reported to be a potent free radical scavenger. The antioxidant capacity of phenolic compounds is mainly attributed to their redox properties, which allow them to react as a reducing agent, an electron donor, an oxygen quencher singular or chelat metal[18]. Study on medicinal plants and a vegetable strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems[19]. In developing countries, particularly in Cameroon, a large number of the population still rely on folk medicine to treat serious diseases including infections, cancers and different types of inflammations. On continuation of our experimental work for the search of antioxidant activity of medicinal plants, we studied extracts of three medicinal plants.

*Garcinia lucida* Vesque (*G. lucida*) is well-known in South Cameroun as ESSOK (Boulou). The seed, fruit and bark are the most commonly used parts in traditional medicine and food. It also represents an important economic potential for local population. The fresh bark and sometimes the seeds are used in fermentation of traditional alcohol from palm tree or raffia[20]. The bark and the seeds dried or fresh, are widely used for medicinal purposes for the treatment of gastric and gynecological infections, diarrheas, and as antidote against poison as well as to cure snake bites. It is believed to possess some aphrodisiac properties and to hunt ghost[21]. Studies demonstrated the cytotoxicity activity, the antimicrobial property and the inhibitory effect of β-lactamase of *G. lucida*[22-24]. In the partial chemical investigation of *G. lucida*, the terpenoids and xanthones products such as 1,2-dihydroxy-xanthone, 1-hydroxy-2-methoxyxanthone, putranjivic acid, methyl putranjivate and friedelin were isolated in the $CH_2Cl_2$ fraction[25].

*Acalypha racemosa* (*A. racemosa*) is considered as an indigenous source of medicine exhibiting antibacterial activity[26]. Also, traditional healers use the decoction of *A. racemosa* leaves to treat liver disorders and other disease conditions which result in jaundice. Flavonoids and phenolics compounds have been detected in the aqueous and methanolic extracts of the leaves of *A. racemosa*[27]. The plant leaves of *A. racemosa* are used in traditional medicine for treatment of sores and skin diseases among children[26]. The aqueous extract of *A. racemosa* has demonstrated hepatoprotective and antibacterial properties[26,27]. Study on the nutritional composition of the leaves of *A. racemosa* demonstrated the presence of total ash (13.14%); crude fat (6.3%), crude protein (16.9%), crude fiber (7.2%), carbohydrates (45.26%) and moisture content (11.91%)[27].

*Hymenocardia lyrata* (*H. lyrata*) is used as ornamental plants and for mood disorders[28]. Very few researches have been carried out extensive biological study on this plant.

The objective of this report is to determine the anti-radicals and the antioxidant properties, and screen the phytochemical constituents of methanol extracts of *G. lucida*, *A. racemosa* and *H. lyrata*. The radical activities were assessed using several test including 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, nitric radical scavenging activity assay and hydroxyl radical scavenging activity assay, whereas the antioxidant property was evaluated by 3 tests [reducing power assay; total phenols content (TPC) assay and total antioxidant activity assay].

## 2. Materials and methods

### 2.1. Collection of plant material and identification

*G. lucida* (Clusiaceae), *H. lyrata* (Euphorbiaceae) and *A. racemosa* (Euphorbiaceae), used in this study were harvested in the east and the south regions of Cameroon during the period of May and June 2010. They were identified in the Cameroon National Herbarium where each voucher specimen was deposited. These plants were referenced under the following numbers: 32301/HNC, 53354/HNC and 62835/HNC respectively for *H. lyrata*, *G. lucida* and *A. racemosa*.

### 2.2. Extraction and extract preparation

The plant materials (fruits and barks for *G. lucida*, roots and barks for *H. lyrata*, leaves and stems for *A. racemosa*) were air-dried in shed at room temperature (26 °C) for 3 weeks, after which they were grinded to a uniform powder. The methanol extracts were prepared by macerating 50 g each of the dry powder in 500 mL of methanol and keep at room temperature for 72 h. The solution obtained was filtered through a Whatmann filter paper No. 1. The filtrate was concentrated using a rotary evaporator (Heidolph WB 2000). The filtrate was evaporated to dryness in a water bath at 60 °C. Then the crude extract of each plant material was stored at 4 °C. A serial of dilutions (640, 320, 160, 80, 40, 20, 10 μg/mL) were prepared for each extract for the determination of the test. Catechin used as control was prepared in the same condition.

### 2.3. Phytochemical screening of the methanol extracts

Qualitative phytochemical tests were carried out to identify some bioactive components of the extracts. The main bioactive groups (alkaloids, tannins, saponins, flavonoids,

cardiac glycosides and polyphenols) were identified in each extract using different standard methods[29].

Test for alkaloids: 0.5 g of the sample was stirred with 5 mL of 1% aqueous HCl on a steam bath and then filtered. A total of 1 mL of the filtrate was treated with a few drops of Mayer's reagent and a second 1 mL portion was treated similarly with Dragendorff reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids in the extract.

Test for tannins: 0.5 g of dried extract was stirred with 5 mL of distilled water. This was filtered and ferric chloric ($FeCl_3$) reagent was added to the filtrate. A blue-black precipitate was taken as evidence for the presence of tannins.

Test for saponins: 0.5 g of plant extract was shaken with water in a test tube. Persisted frothing on warming was taken as evidence for the presence of saponins.

Test of flavonoids: In 2 mL extract solution was added 2 mL of concentrated HCl and a stiff of pink magnesium. The appearance of the tomato red color indicated the presence of flavonoids.

Test for cardiac glycosides: 1 mL glacial acetic acid, 1 mL of $FeCl_3$ and 1 mL of concentrated $H_2SO_4$ was respectively added into 2 mL of the extract solution. A green-blue color indicated the presence of cardiac glycosides.

Test for polyphenols: 2 mL of plant extract was heated for 30 min in a water bath. About 1 mL of 1% $FeCl_3$ was added to the mixture then followed by the addition of 1 mL of 1% potassium ferrocyanide. The mixture was filtered and the formation of a green-blue colour indicated the presence of polyphenols.

## 2.4. Determination of antioxidant property

### 2.4.1. Scavenging activity of DPPH radical

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the investigated extract. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple color typical for free DPPH radical decays and the absorbance change is measured at $\lambda$=517 nm. The antiradical activity of the plant extract was examined based on the scavenging effect of the stable DPPH free radical activity[30].

Briefly, in 3 mL of each diluted extract, 1 mL of methanol solution of DPPH (0.1 mmol/L) was added. The mixture was kept in the dark at room temperature for 30 min and the absorbance was measured at 517 nm against a blank. The following equation was used to determine the percentage of the radical scavenging activity of each extract.

Percentage of radical scavenging activity=[(OD control-OD sample)/OD control]×100

Where OD is the optical density.

The $IC_{50}$ value (μg/mL) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from linear regression analysis.

### 2.4.2. Nitric radical scavenging activity

At physiological pH, nitric oxide generated from aqueous sodium nitroprusside solution interacts with oxygen to produce nitrite ions, which may be quantified by the Griess Ilosvoy reaction[31]. Each diluted extract (1 mL) was added in 2 mL of sodium nitroprusside (10 mmol/L) solution (in phosphate buffer pH 7.4; 50 mmol/L). After mixing and incubating at room temperature for 150 min, 1 mL of sulfanilic acid (0.33% in 20% of glacial acetic acid) was added to 0.5 mL of the mixture. After 5 min of complete diazotization, 1 mL of Naphthylethylenediamine dihydrochloride (0.1%) was added and incubated at room temperature for 30 min. The pink chromophore formed in the scattered light (in diffused light) was read at 540 nm against the blank. The following equation was used to determine the percentage of the nitric radical scavenging activity of each extract.

Percentage of nitric radical scavenging activity=[(OD control-OD sample)/OD control]×100

Where OD is the optical density.

The extract concentration providing 50% inhibition ($IC_{50}$) was calculated and obtained by interpolation from linear regression analysis.

### 2.4.3. Hydroxyl radical scavenging activity

The scavenging activity of the extract on hydroxyl radical was measured according to a previously described method[32]. In 1.5 mL of each diluted extract, 60 μL of $FeCl_3$ (1 mmol/L), 90 μL of 1,10-phenanthroline (1 mmol/L), 2.4 mL of 0.2 mol/L phosphate buffer, pH 7.8 and 150 μL of $H_2O_2$ (0.17 mol/L) were added respectively. The mixture was then homogenized and incubated at room temperature for 5 min. The absorbance was read at 560 nm against the blank. The percentage of the hydroxyl radical scavenging activity of each extract was calculated from the equation below:

Percentage of hydroxyl radical scavenging activity=[(OD control-OD sample)/OD control]×100

Where OD is the optical density.

The extract concentration providing 50% inhibition ($IC_{50}$) was calculated and obtained by interpolation from linear regression analysis.

### 2.4.4. Reducing power assay

One milliliter of different concentration of extract (640, 320, 160, 80, 40, 20, 10 μg/mL) diluted in distilled water was mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of potassium ferrocyanide (1%). The mixture was incubated at 50 °C for 20 min. Aliquot (2.5 mL) of trichloroacetic acid (10%) was added into the mixture and centrifuged at 3000 r/min for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%). The increased absorbance measured at 700 nm against the blank indicates the increasing of the reducing power[33].

### 2.4.5. Determination of TPC

TPC was recorded by Folin Ciocalteu reagent[34]. A dilute extract of each plant extract (30 μL) was mixed with Folin Ciocalteu reagent (1 mL, 1:10 diluted with distilled water) and aqueous $Na_2CO_3$ (4 mL, 1 mol/L). After 10 min the absorbance of the mixture was measured at 765 nm.

### 2.4.6. Total antioxidant activity by ferric reducing antioxidant power assay (FRAP)

The FRAP method was used to determine the total antioxidant activity which measures the reduction of ferric ion to the ferrous form in the presence of antioxidant compounds[35]. The fresh FRAP reagent consists of 500 mL of acetate buffer (300 mmol/L pH 3.6), 50 mL of 2,4,6-Tris (2-pyridyl)-s-triazin (10 mmol/L), and 50 mL of $FeCl_3 \cdot 6H_2O$ (50 mmol/L). For the assay, 75 μL of each extract were mixed with 2 mL of FRAP reagent and the optical density was read after 2 min at 593 nm against the blank.

### 2.5. Statistical analysis

Each test was performed in triplicate and the results were expressed as mean±standard deviation. The Kruskal-Wallis non parametric test followed by a *post hoc* Dunnet $T_3$ ($P<0.05$) was used to analyze the antioxidant capacity, total phenols content as well as the radical scavenging activity of each extract. The correlation between antioxidant capacity and total phenols content were established using the Pearson product moment correlation. The $IC_{50}$ was determined with the multiple regression analysis. The software SPSS version 10.1 for Windows was used for statistical analysis.

## 3. Results

### 3.1. Phytochemical screening of the plant extracts

The phytochemical screening showed that the different methanol plant extracts yielded the alkaloids, tannins, glycosides and flavonoids and polyphenols. The steroids were absent in the extracts of *H. lyrata* (barks and roots) and *A. racemosa* (stems). However terpenoids were detected in those extracts (Table 1).

**Table 1**

The phytochemical profile of the extracts.

| Plant extracts | P | T | St | S | Ta | F | Al | Gl |
|---|---|---|---|---|---|---|---|---|
| *G. lucida* (fruits) | + | − | + | + | + | + | + | + |
| *G. lucida* (barks) | + | − | + | − | + | + | + | + |
| *H. lyrata* (roots) | + | + | − | + | + | + | + | + |
| *H. lyrata* (barks) | + | + | − | + | + | + | + | + |
| *A. racemosa* (leaves) | + | − | + | + | + | + | + | + |
| *A. racemosa* (stems) | + | + | − | + | + | + | + | + |

The sign (+) indicates the presence of the compounds and (−) the absence.

P: Polyphenols; T: Terpenoids; St: Steroids; S: Saponins; Ta: Tannin; F: Flavonoids; Al: Alkaloids; Gl: Glycosides.

### 3.2. DPPH radical scavenging activity

The antiradical activity of the extract was measured by the ability to scavenge DPPH free radicals and was compared with the standard catechin. It was observed that all the extract tested have higher scavenging activity. At the concentration of 160 μg/mL, the scavenging activity of all the extracts tested reached 80% (Figure 1). Most of the extracts demonstrated higher scavenging activity at the concentration between 10 and 320 μg/mL.

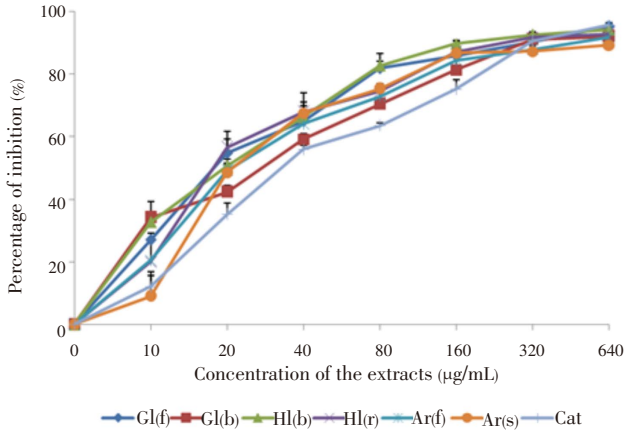


**Figure 1.** Scavenging activities of the extracts by DPPH assay.

The results are expressed as means±SD ($n$=3). Gl(f): *G. lucida* (fruits); Gl(b): *G. lucida* (barks); Hl(b): *H. lyrata* (barks); Hl(r): *H. lyrata* (roots); Ar(l): *A. racemosa* (leaves); Ar(s): *A. racemosa* (stems); Cat: Catechin.

### 3.3. Nitric oxide radical scavenging

As shown in the Figure 2, the nitrite oxide radical scavenging activity of *H. lyrata* (barks) and *A. racemosa* (leaves) extracts increased up to 70% at the concentration of 160 μg/mL. The nitrite oxide radical scavenging activity of these two extracts was higher compared to the standard. Other extracts exhibited moderated or weak scavenging activity.

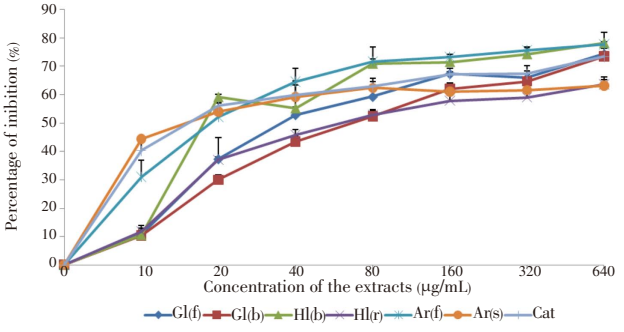


**Figure 2.** Nitric radical scavenging activity of the extracts.

The results are expressed as means±SD ($n$=3). Gl(f): *G. lucida* (fruits); Gl(b): *G. lucida* (barks); Hl(b): *H. lyrata* (barks); Hl(r): *H. lyrata* (roots); Ar(l): *A. racemosa* (leaves); Ar(s): *A. racemosa* (stems); Cat: Catechin.

### 3.4. Hydroxyl radical scavenging

The hydroxyl scavenging activity of the extracts expressed

as percentage of inhibition increased proportionally with the concentration of the extracts (Figure 3). About 87% of the hydroxyl scavenging activity was observed for all the extracts at 160 μg/mL. At the mentioned concentration, the hydroxyl scavenging activity of all the extracts was lower than the standard.

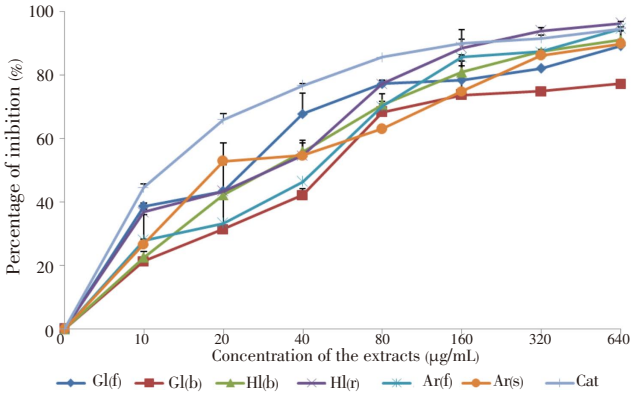


**Figure 3.** Hydroxyl radical scavenging activity of the extracts.

The results are expressed as means±SD ($n$=3). Gl(f): *G. lucida* (fruits); Gl(b): *G. lucida* (barks); Hl(b): *H. lyrata* (barks); Hl(r): *H. lyrata* (roots); Ar(l): *A. racemosa* (leaves); Ar(s): *A. racemosa* (stems); Cat: Catechin.

### 3.5. Total polyphenols content

TPC of the extracts is expressed as milligrams of catechin equivalent per gram. The values of TPC ranged between 54.58 and 165.81 milligrams of catechin equivalent per gram extract depending on the extract and part of plant used (Figure 4). At the highest concentration of the extract, the highest TPC was observed with the extract of *H. lyrata* (barks) followed by *H. lyrata* (roots) and *A. racemosa* (leaves).

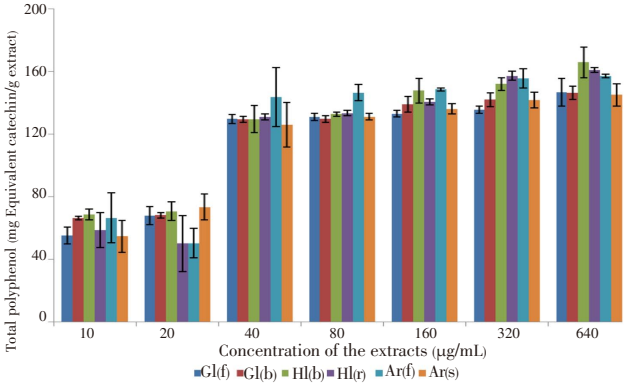


**Figure 4.** Total polyphenol content of the extracts

The results are expressed as means±SD ($n$=3). Gl(f): *G. lucida* (fruits); Gl(b): *G. lucida* (barks); Hl(b): *H. lyrata* (barks); Hl(r): *H. lyrata* (roots); Ar(l): *A. racemosa* (leaves) ; Ar(s): *A. racemosa* (stems).

### 3.6. Reducing power activity

The reducing power activity of the extract increased with the concentration of the extract. At the concentration higher than 40 μg/mL, a significant variation of the reducing power property of the extracts was noted compared to the standard (Figure 5). All the extracts tested exhibited the lower power reducing activity compared to catechin. The extracts of *H. lyrata* (barks), *H. lyrata* (roots) and *A. racemosa* (leaves) demonstrated the highest power reducing property at these concentrations.

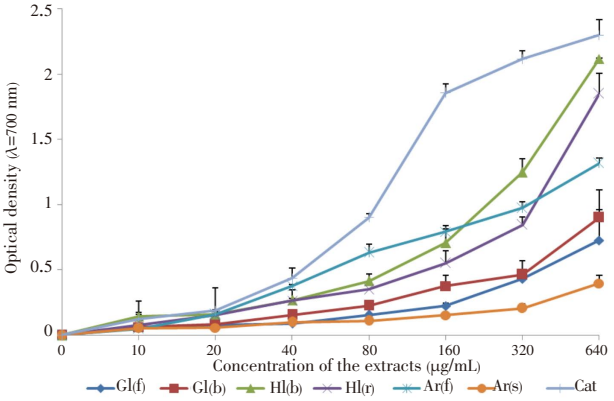


**Figure 5.** Reducing power activity of the extracts.

The results are expressed as means±SD ($n$=3). Gl(f): *G. lucida* (fruits); Gl(b): *G. lucida* (barks); Hl(b): *H. lyrata* (barks); Hl(r): *H. lyrata* (roots); Ar(l): *A. racemosa* (leaves); Ar(s): *A. racemosa* (stems); Cat: Catechin.

### 3.7. Total antioxidant activity

Figure 6 showed the antioxidant activity of the extracts using the FRAP method. The total antioxidant activity of the extracts increased with the concentration of the extract. *H. lyrata* (roots) extract showed the highest total antioxidant capacity among the extract between 40 and 640 μg/mL. A significant difference ($P$<0.005) was also noted between antioxidant potential of the extracts at different concentrations. *A. racemosa* showed the lowest total antioxidant capacity at all concentrations.

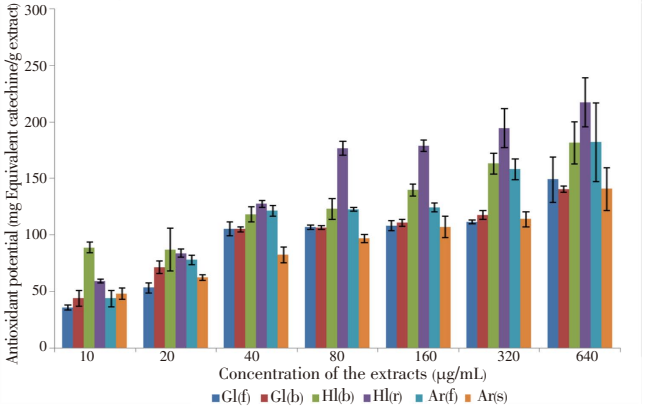


**Figure 6.** Total antioxidant capacity of the extracts.

The Results are expressed as means±SD ($n$=3). Gl(f): *G. lucida* (fruits); Gl(b): *G. lucida* (barks); Hl(b): *H. lyrata* (barks); Hl(r): *H. lyrata* (roots); Ar(l): *A. racemosa* (leaves); Ar(s): *A. racemosa* (stems); Cat: Catechin.

### 3.8. $IC_{50}$ of different radical scavenging tests (DPPH˙, NO˙, HO˙)

Table 2 below presents the $IC_{50}$ (50% inhibitory

concentration) of the different extracts tested. The results showed that the $IC_{50}$ varied between 1.46 μg/mL and 10.04 μg/mL depending on the type of the plant extracts and radicals. All the extracts tested for the DPPH radical showed the lowest $IC_{50}$ compare to the catechin. Similar results were observed with extracts of *A. racemosa* (stems) concerning the nitrite oxide radical. The extract of *G. lucida* (fruits) has the lowest $IC_{50}$ for the hydroxyl radical, but this value remains higher than the standard.

**Table 2**

Fifty percent inhibitory concentration ($IC_{50}$) of different plants extracts.

| | $IC_{50}$ (μg/mL) | | |
|---|---|---|---|
| Extracts | DPPH· | NO· | HO· |
| *G. lucida* (fruits) | 1.83±1.61 | 3.12±0.31 | 1.99±0.09 |
| *G. lucida* (barks) | 2.35±2.14 | 3.59±1.36 | 2.01±1.03 |
| *H. lyrata* (roots) | 1.96±1.84 | 3.82±2.36 | 2,43±0.74 |
| *H. lyrata* (barks) | 1.74±0.56 | 2.46±0.54 | 3.30±2.06 |
| *A. racemosa* (leaves) | 2.11±1.13 | 1.92±1.46 | 2.12±1.03 |
| *A. racemosa* (stems) | 2.28±0.98 | 1.46±0.58 | 10.04±0.48 |
| Catechin | 2.61±1.02 | 1.66±2.51 | 1.70±1.03 |

The results are expressed as means±SD ($n$=3).

## 4. Discussion

In this study, six extracts from three plants were extracted and evaluated for the antioxidant activity and TPC. The antioxidant activity of the extracts was firstly evaluated based on their ability to trap DPPH radical. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts[36,37]. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of those diseases. DPPH is a stable free radical and can accept an electron or hydrogen radical to become a stable diamagnetic molecule. All the plant extracts tested inhibited the DPPH radical but, in different manners. This result proved that the extracts are capable of donating an electron or hydrogen which could react with DPPH radical. The variation observed between the scavenging activities of the same extract depends on the part of the plant used for the study. This difference could be attributed to an unequal distribution of the antioxidant molecules such as polyphenol, flavonoids identified in the different parts of the plant. The extract of *H. lyrata* (barks) had the lowest $IC_{50}$ (1.74 μg/mL) followed by the fruits extract of *G. lucida* (1.83 μg/mL) and the roots extract of *H. lyrata* (1.96 μg/mL), demonstrating that *H. Lyrata* (barks) has a greatest scavenging activity. All the extracts tested exhibited the lower $IC_{50}$ than standard (2.61 μg/mL). Extracts are made of a mixture of several scavenging compounds which could act in a synergetic manner to enhance the antiradical activity. Moreover, the antiradical activity of the extracts to trap DPPH radical depends on the availability and the ability of these extracts to give hydrogen or electron atom[38]. The therapeutic potential of natural medicinal plants as an antioxidant in reducing free radical induced tissue injury suggests that many plants have antioxidant activities that can be therapeutically useful[39]. It is well known that nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse[40]. All the extracts demonstrated an ability to inhibit nitrite radical. The $IC_{50}$ of the extract of *A. racemosa* (stems) scavenging nitrite oxide radical was lower (1.46 μg/mL) than the standard (1.66 μg/mL). This result indicates that the extracts could either serve as a free radical inhibitors or scavengers using their proton-donating ability or could act as the primary antioxidants[41]. The extract inhibits the nitrite radical formation by directly competing with oxygen in the reaction which could lead to the inhibition of the nitrite formation[42]. The accumulation or the non inhibition of the nitrite radical formed in the living cells could be toxic and induce mutagenic reactions with superoxide radical, forming the highly reactive peroxynitrite anion ($ONOO^-$) [43]. The present study proved that the extracts have potent nitric oxide scavenging activity and can be useful for the management of diseases in which radical nitrite is directly involved.

The formation of OH radical by the Fenton reaction is representative of the events that occur *in vivo* in iron-rich tissues like liver where it contributes to the initiation of lipid peroxidation. Besides, its high reactivity can lead to DNA mutagenesis and inactivation of various proteins[44,45]. The oxy-radicals are involved in the initiation of the inflammation, carcinomas and toxicity of the tissues. Hydroxyl radicals ($HO^·$) formed in the biological systems are among the most reactive species and have been recognized as extremely damaging[46]. Therefore, the scavenging activity of this radical can be considered as one of the best indicators of the antioxidant potential of a compound. All the extracts have inhibited significantly the hydroxyl radicals. The results in this study indicated a dose-dependent increase in the capacity to quench hydroxyl radicals for all extracts, especially *G. lucida* (fruits and barks) and *A. racemosa* (leaves) with comparable $IC_{50}$ values [(1.99±0.09), (2.01±1.03) and (2.12±1.03) μg/mL respectively]. The difference observed in the scavenging activity between the extracts is either due to the type of the free radical or to the presence of several bioactive compounds in the extracts such as phenols and flavonoids which are capable of yielding proton or hydrogen atoms to stabilize free radicals[47].

Since the reducing power activity of the compounds could serve as a significant indicator of the antioxidant potential, we assessed this property by measuring the ability of the extracts to transform $Fe^{3+}$ to $Fe^{2+}$ and to donate an electron[48]. At 640 μg/mL, the extract of *H. lyrata* (barks) showed the highest reducing capacity (optical density=2.317 ±0.105) which remained lower than the standard (optical density=2.499±0.117). The ability of the extracts to reduce $Fe^{3+}$ could be attributed either to the reducing agents such as phenol groups and the number or/and the position of the hydroxyl molecule on these groups[22]. Antioxidant

activity increased proportionally to the polyphenol content. The phenols contain hydroxyls that are responsible for the radical scavenging effects mainly due to the redox properties[49]. A regression analysis showed a positive and significant ($P<0.01$) correlation between the antioxidant capacity and the TPC of the extracts. Several researches showed a strong correlation between antioxidant activity and TPC, confirming the importance of polyphenols as a potential antioxidant biomolecules[48-50]. According to recent reports, a highly positive relationship between total phenol and antioxidant activity appears to be the trend in many plant species[51]. Phenolic and flavonoid compounds seem to have an important role in stabilizing lipid oxidation, associated with antioxidant activity[6]. In this study, it appeared that the higher TPC of the plant extracts resulted in higher antioxidant activity. A significant variation of antioxidant activity could be attributed to the redox properties of phenol content which acts as a reducing agents, hydrogen donors, free radical scavenger, singlet oxygen quenchers and metal chelators[52]. A significant and linear relationship existed between the antioxidant activity and phenolic content of the extracts, thus indicating that phenolic compounds are major contributors to antioxidant activity.

In recent years, the search for phytochemicals possessing antioxidant properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases. The phytochemical screening of the extracts revealed the presence of alkaloids, tannins, glycosides and flavonoids and polyphenols. The steroids were absent in the extracts of *H. lyrata* (barks and roots) and *A. racemosa* (stems). Several researchers showed that most of these compounds (phenols and flavonoids) have antioxidant properties[49]. The finding of these molecules in the plants extracts could furthermore explain or confirm their therapeutic use. The results obtained demonstrated that the extracts of *A. racemosa*, *G. lucida* and *H. lyrata* have higher TPC and antioxidant activity. There was a good correlation between TPC and antioxidant activity (DPHH, TPC and FRAP assay), supporting the idea of phenols as contributor of the antioxidant power of plants extracts. However, extensive investigations need to be done either to isolate the antioxidant compounds or to determine the *in vitro* or *in vivo* biological activity of these extracts.

## Conflict of interest statement

The authors declare that they have no competing interests.

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