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Phenolic Content and Antioxidant Activity of Six Acanthaceae from Burkina Faso

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Abstract: In this study the total phenolic and flavonoid content as well as the antioxidant activity of six Acanthaceae namely *Blepharis linearifolia* PERS, *Dicliptera verticillata* (FORRSK.) C. CHRISTENS, *Dyschoriste perrottetii* (NEES) O. KTZE, *Hygrophila auriculata* (SCHUMACH.) HEINE, *Lepidagathis anobrya* NEES, *Nelsonia canescens* (LAM) SPRENG were evaluated. The total phenolic and flavonoid of their aqueous acetone extract were assessed by Folin-ciocalteu and AlCl₃ method, respectively, whereas the antioxidant activities were determined by the DPPH method. *Lepidagathis anobrya*, *Hygrophila auriculata* and *Nelsonia canescens* which had the highest phenolic content, were found to possess the best antioxidant activities. The results suggest that these plants are good sources of antioxidants and support their use in cardiovascular and antiinflammatory diseases.

Key words: Antioxidant activity, total phenolic content, total flavonoid content, Acanthaceae, Burkina Faso

INTRODUCTION

It is now well known that free radicals play a fundamental role in several diseases. The biochemical damage that they cause to cells and tissues, lead to the development of diseases such as arteriosclerosis, hypertension, aging, cancer, diabetes mellitus, inflammation, renal failure, liver disease, AIDS etc. (Allard *et al.*, 1998; Cheng *et al.*, 2003; Ellnain-Wojtaszek, 2003; Govindarajan *et al.*, 2005; Tiwari, 2004). Several epidemiological studies have shown that compounds that can scavenge free radicals are effective in ameliorating the progress of these related diseases. Of these, phenolic substances present in foods and plants possess strong antioxidant properties, and thus, are being increasingly investigated.

Blepharis linearifolia, *Dicliptera verticillata*, *Dyschoriste perrottetii*, *Hygrophila auriculata*, *Lepidagathis anobrya*, *Nelsonia canescens* are plants that belong to the Acanthaceae family used in traditional medicine in Burkina Faso for the treatment of several diseases (Table 1) (Nacoulma, 1996)

Pharmacological investigations have shown that the aqueous extracts of *Hygrophila auriculata* possess significant hepatoprotective and antioxidant activities (Shanmugasundaram and Venkataraman, 2005). The

ethanolic extracts of the leaves of *Nelsonia canescens* have been shown to possess analgesic and anti-inflammatory activities (Oweyele *et al.*, 2005). The efficiency of *Dicliptera verticillata* in combination with *Aloe buettneri*, *Justicia insularis*, and *Hibiscus macranthus* to induce *in vitro* the production of oestradiol have been shown (Telefo *et al.*, 2004).

However, little is known on the pharmacology of *Blepharis linearifolia*, *Dyschoriste perrottetii*, *Lepidagathis anobrya*, and scientific information on the phenolic content and the antioxidant potential of Acanthaceae of Burkina Faso are still rather scarce.

The objective of the present study was to evaluate the antioxidant activity and quantify the total phenolic and flavonoid contents of plants of the Acanthaceae family.

MATERIALS AND METHODS

This study was carried out during the year 2005 at Laboratoire de Biochimie et Chimie Appliquées, UFR/SVT, University of Ouagadougou, Burkina Faso.

Chemicals: The Folin Ciocalteu reagent, gallic acid and quercetin were purchased from Sigma-Aldrich Chemie, Steinheim, Germany; sodium carbonate, ascorbic acid and

Table 1: Medicinal uses of the six investigated plants

Name of plants	Part used	Medicinal uses
<i>Blepharis linearifolia</i> PERS	Stems, leaves	Malaria, measles, whitlow, sexually transmitted infections, fever, inflammation.
<i>Dicliptera verticillata</i> (FORRSK.) C. CHRISTENS	Stems, leaves	Fever, diarrhea, inflammation
<i>Dyschoriste perrottetii</i> (NEES) O. KITZE	Stems, leaves	Malaria, Fever, diarrhea,
<i>Hygrophila auriculata</i> (SCHUMACH.) HEINE	Stems, leaves, roots	Snake and scorpion bites, fever, inflammation, gonorrhoea
<i>Lepidagathis anobrya</i> NEES	Stems, leaves, inflorescence	Migraine, malaria, hyperpyretic convulsions, snake bites, delay of children's locomotion
<i>Nelsonia canescens</i> (LAM) SPRENG	Stems, leaves, roots	Diarrhea, fever, smallpox, vomiting, hypertension, pregnancy nausea, inflammation

aluminium trichloride (AlCl₃) were from Labosi, Paris, France; 2,2-diphenyl-picrylhydrazyl (DPPH) and solvents used were from Fluka Chemie, Switzerland. All chemicals used were of analytical grade.

Plant material: Stems with leaves of *Dicliptera verticillata* were collected in August 2004 in the botanical garden of the Research institute in Health Science of Ouagadougou (Burkina Faso) and voucher specimen was deposited in herbarium of University of Ouagadougou. Stems with leaves of *Blepharis linearifolia*, *Dyschoriste perrottetii*, *Hygrophila auriculata*, *Lepidagathis anobrya*, *Nelsonia canescens* were bought from practitioner of traditional medicine in "Nabi Yaar" market of Ouagadougou in January 2005. All these plants were identified by Prof. Millogo, a botanist from university of Ouagadougou.

Preparation of plant extracts: The dried plants were pulverised into fine powder using a grinder. For each plant, 25 g of powder were extracted with 80% aqueous acetone (250 mL) for 48 h under agitation using a mechanical shaker (SM 25, Edmund BÜHLER, Germany). After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI Rotavapor R-200, Switzerland) and the remained aqueous solution lyophilised using a freeze drying system (Cryodos 50, TELSTAR, Spain).

Determination of total phenolic content: The Singleton *et al.* (1999) method, using Folin-Ciocalteu reagent, was used to determine the total phenolic content. Each plant extract was prepared at a concentration of 1 mg mL⁻¹. The absorbances of all samples were measured at 760 nm against a methanol blank using a spectrophotometer (CECIL CE 2041, CECIL Instruments, England). The standard calibration curve was plotted using gallic acid. The mean of three readings was used

and the results expressed as g of Gallic Acid Equivalents (GAE) per 100 g of lyophilised extract.

Determination of total flavonoid content: The total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand *et al.* (1994). Briefly, 2 mL of plant extract (2 mg mL⁻¹) were mixed with 2 mL of aluminium trichloride (AlCl₃) in methanol (2%). The absorbance readings at 415 nm were taken after 10 min against a blank consisted in 2 mL of plant extract and 2 mL of methanol without AlCl₃. Quercetin was used as a reference compound to produce the standard curve. The mean of three readings was used and expressed as g of quercetin equivalents per 100 g of lyophilised extract.

Determination of antioxidant activity: The free radical scavenging activity of plants extract for DPPH (2,2-diphenyl-picrylhydrazyl), was determined as described by Velazquez *et al.* (2003) with some modifications. Extract solutions were prepared by dissolving 100 mg of each extract in 10 mL of methanol. The solution of DPPH in methanol (20 mg L⁻¹) was prepared daily, before absorbances measurements. 1.5 mL of this solution were mixed with 0.75 mL of various concentration of each extract (3.9-500 µg mL⁻¹) except for *Dicliptera verticillata* where higher concentrations were used. Methanol was used as blank sample. The mixtures were kept in the dark for 15 min at room temperature and then the decrease in absorbance was measured at 517 nm. Quercetin (0-50 µg mL⁻¹) and ascorbic acid (0-40 µg mL⁻¹) were used as positive controls. The radical scavenging activity was calculated as follows as (Motalleb *et al.*, 2005).

% Inhibition = [(A_B - A_A)/A_B] × 100, where: A_B is the blank absorbance and A_A the sample absorbance. The mean of three IC₅₀ (concentration causing 50% inhibition) value of each extract was determined graphically.

Correlation coefficients between phenolic content and antioxidant activity were calculated using the Sigma

Table 2: Total phenolic, total flavonoid contents and radical scavenging activity of the 6 plants studied

Plant	Total phenolic content (g GAE/100 g)	Total flavonoids content (g QE/100 g)	IC ₅₀ (µg mL ⁻¹) for DPPH scavenging activity
<i>Blepharis linerifolia</i> PERS	15.38±1.48	1.29±0.13	44±4
<i>Dicliptera verticillata</i> (FORRSK.) C. CHRISTENS	2.82±0.20	2.33±0.33	785.67± 6.03
<i>Dyschoriste perrottetii</i> (NEES) O. KTZE	11.17±0.63	2.40±0.57	45.83±3.82
<i>Hygrophila auriculata</i> (SCHUMACH.) HEINE	17.75±0.30	0.033±0.002	20.33±1.04
<i>Lepidagathis anobrya</i> NEES	23.67±0.85	0.37±0.07	16.33±1.04
<i>Nelsonia canescens</i> (LAM) SPRENG	15.48±0.55	1.85±0.07	24.33±2.52

Stat 2.0 Jandel Scientific software (Person product moment correlation function).

RESULTS

Phenolic and flavonoid content: The amount of phenolic compounds was determined from regression equation of calibration curve ($Y = 89.59X$, $R^2 = 0.99$). Values were expressed in gallic acid equivalents (GAE) and varied from 2.82 to 23.67g GAE/100 g of lyophilised extract (Table 2).

The highest phenolic content was found in the extract of *Lepidagathis anobrya* (23.67±0.85 g GAE/100 g).

The flavonoid content expressed in quercetin equivalents (QE)/100 g of lyophilised extract, were determined from regression equation of calibration curve $Y = 40.55X$, $R^2 = 0.99$. The values varied from 0.03 to 2.4 g QE/100 g of lyophilised extract (Table 2).

The highest amounts of flavonoids were found in extracts of *Dyschoriste perrottetii* (2.4±0.57 g QE/100 g) and *Dicliptera verticillata* (2.33±0.33) g QE/100 g.

Antioxidant activity: This investigation was based on the measurement of the relative inhibitory effect of extract tested at different concentration. Table 2 shows capacity of each plant extract to scavenge the DPPH radical. Values of the 50 % inhibition concentration (IC₅₀) varied between 16.33 and 785.67 µg mL⁻¹. The IC₅₀ of quercetin and ascorbic acid were 0.87±0.06 and 1.8±0.43 µg mL⁻¹, respectively.

Lepidagathis anobrya extract have shown the best scavenging activity with IC₅₀ value of 16.33 µg mL⁻¹. *Dicliptera verticillata* is one of the six Acanthaceae that possess a weak antioxidant activity with an IC₅₀ value of 785.67±6.03 µg mL⁻¹.

The correlation between total phenolic and 1/IC₅₀ was 0.95 (p<0.005). No significant correlation was found between total flavonoid and 1/IC₅₀.

DISCUSSION

Among the six investigated plants, only five have presented a remarkable radical scavenging activity with

IC₅₀ value ranging from 16.33 to 45.82 µg mL⁻¹. The chemical composition of these plants indicates the presence of phenolic compounds including tannins and flavonoids (Nacoulma, 1996) which are known to possess antioxidant activities (Aderogba *et al.*, 2005; Badami *et al.*, 2003; Motalleb *et al.*, 2005). Present study shows that the extract with an amount of phenolic compounds higher than 10 g/100 g present good antioxidant activity. *Dicliptera verticillata* which was found to have a weak antioxidant activity possess the lowest phenolic content of the six investigated Acanthaceae.

The scavenging activity of all samples on the DPPH radical was found to be strongly dependent on the extract concentration. It has been shown that the scavenging effects on the DPPH radical increase sharply with increasing concentration of the samples and standards to a certain extent (Motalleb *et al.*, 2005). Some authors found a correlation between the phenolic content and the antioxidant activity, while others found no such relationship. Velioglu *et al.* (1998) have reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products. Javanmardi *et al.* (2003) have found a significant correlation ($R^2 = 0.71$) between the total antioxidant activity and total phenolic contents of Iranian *Ocimum* accessions. In contrast, Kahkonen *et al.* (1999) do not find such kind of correlation between antioxidant activity and phenolic content in plant extracts. In this study, we have found a significant correlation between antioxidant activity and total phenolic content. In contrast, such a kind of relationship was not observed with the flavonoids contents. *Hygrophila auriculata*, with a very low concentration of flavonoids (0.033 g QE/100 g) presents an antioxidant activity higher than *Dyschoriste perrottetii* which has the highest flavonoid content (2.4 g QE/100 g). These are in agreement with the findings of Miliauskas *et al.* (2004) and Garcia-Alonso *et al.* (2004) who found a weak correlation between antioxidant activity and flavonoid content in fruits.

These results indicate that the studied plants among Acanthaceae family, especially *Lepidagathis anobrya*,

Hygrophila auriculata and *Nelsonia canescens* could possess therapeutical effects arising from their antioxidant activity, in area such as inflammatory diseases and cardiovascular protection. Such kind of anti-inflammatory effects (Oweyele *et al.*, 2005) and hepatoprotective effects (Shanmugasundaram and Venkataraman, 2005) have already been shown for *Nelsonia canescens* and *Hygrophila auriculata*, respectively.

These findings give a scientific basis to the traditional uses of the investigated plants.

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