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Antioxidant and anticancer activities of polyphenolic compounds from three Acanthaceae medicinal species from Burkina Faso

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Abstract

Phytochemical screening and biological activities of methanol extracts from three Acanthaceae species namely *Hygrophila auriculata* (*H. auriculata*), *Nelsonia canescens* (*N. canescens*) and *Peristrophe bicalyculata* (*P. bicalyculata*), widely used in local ethnomedicine, were carried out. Phenolic content quantification indicated that *H. auriculata* extract possessed the highest polyphenolics content followed by *N. canescens* and *P. bicalyculata*. Biological activities assessment showed that H. *auriculata* extract has the best antioxidant (through DPPH, FRAP and ABTS methods) capacity. Acetylcholinesterase and Lipoxygenase inhibition tests revealed interesting activities from *P. bicalyculata*. Testing cancer cells antiproliferation, it was showed that *H. auriculata and N. canescens) were* of good interest (27.00±1.55 and 29.70±3.11 µg/mL).
These results showed that the phenolic compounds of these plants could justify their local traditional use to treat inflammatory and tumoral diseases.

Keywords: Acanthaceae, phenolics, Antioxidant, anti-acetylcholinesterase, anti-lipoxygenase, Antitumoral.

Introduction

Acanthaceae botanical family consists of about 250 genus and more than 2000 species which are mainly tropical or subtropical [1]. In the central region of Burkina Faso, these plants are especially used in the treatments of pathologies of childhood [2]. *Hygrophila auriculata*, *Nelsonia canescens* and *Peristrophe bicalyculata* are also traditionally used for malaria, cancer, gout, cardiovascular and inflammatory diseases treatments [3-6].
Previous biological investigations have demonstrated that *H. auriculata* possess antioxidant property (using ferric thiocyanate and thiobarbituric acid methods), hepatoprotector capacity (against CCl_4-induced liver toxicity in rats) [7]; anti-inflammatory and antipyretic activities [8]. His ethanolic extract showed an *in vivo* reduction of rats' serum creatinine and serum urea which are considered as the most important manifestation of kidney severe tubular necrosis [9]. Leaves ethanolic extract of *N. canescens* has an *in vivo* analgesic and anti-inflammatory activities using respectively formalin-induced paw licking and carrageenan-induced inflammation [10]. Ethanolic extract of *P. bicalyculata* have an *in vivo* anti-inflammatory and analgesic activities using respectively carrageenan-induced inflammation and cotton pellet induced granuloma [12].
In the best of our knowledge, there is no scientific information concerning the in vitro antiproliferative and the anti-inflammatory activities in relation with polyphenolic contents of these medicinal plants.
The aim of this study was to evaluate: (a) the total polyphenolic contents, (b) the antiradical scavenging capacities and ferric reduction ability; (c) Acetylcholinesterase and Lipoxygenase inhibition and antiproliferative capacity of plants extracts.

Material

Chemicals

All the chemicals used were analytical grade. Folin-Ciocalteu reagent, Sodium carbonate (Na_2CO_3), gallic acid, quercetin, aluminium trichloride ($AlCl_{3)}$, linoleic acid, tannic acid, boric acid, sodium tetraborate, hydrogenophosphate sodium, Tris/HCl, acethylcholine iodide (ATCI) were purchased from Sigma-Aldrich chemie (Steinheim, Germany); ammonium ferric citrate, ammoniac, potassium persulfate, DPPH (2, 2'-diphenyl-1-picrylhydrazyl), ABTS 2,2'- azinobis (3-ethylbenzothiazoline-6-sulphonate), methanol, ethanol and trichloroacetic acid were supplied by Fluka chemie (Buchs, Switzerland); potassium hexacyanoferrate [$K_3Fe(CN)_6$] was sourced from Prolabo (Paris, France); ascorbic acid and ion trichloride were supplied by labosi (Paris, France); 3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide assay (MTT) was supplied from Sigma–Aldrich, Belgium.

Biological material

U373 cell line (ATCC, Manassas, VA, USA) was used in this investigation. All media and complement were supplied form GIBCO-Invitrogen (Merelbecke, Belgium). 15-Lipoxygenase (EC 1.13.11.12) type I-B (Soybean) was supplied from Sigma (St. Louis, MO) and Acetylcholinesterase was supplied from Sigma-Aldrich chemie (Steinheim, Germany).

Plants Material

Whole plants of Acanthaceae species was collected in September 2008 in Lombila situated at 25 Km north of Ouagadougou, capital of Burkina Faso. The plants were identified by Prof. Millogo-Rasolodimby botanist. Voucher specimens with accession numbers ID 10259, ID 10152 and ID 10320 respectively for *Hygrophila auriculata*, *Nelsonia canescens* and *Peristrophe bicalyculata* were deposited at the Herbarium of the Laboratory of plants biology and ecology of the University of Ouagadougou. The collected samples were washed then dried in the shade under laboratory (37ºC) before pulverization.

Methods

Extracts Preparation

For each species 25 g of powdered plant material were extracted with methanol, using a Soxhlet system, for six hours. The methanol extract was then dry-evaporated (Büchi rotavapor R-200, Switzerland). Extracts thus obtained were used for the dosage and biological activities investigation.

Polyphenols Determination

Total Phenolics Contents

The total phenolics were estimated by the Singleton method as adapted by Lamien-Meda & *al.* [13]. Thus, the total phenolic content is determined by extrapolation on a standard calibration curve (y = 0.0095x, R^2 = 0.99) of the Gallic Acid (0-200 mg/L). In each test tube were added, 0.125 mL of the sample and 0.625 mL of Folin Ciocalteu Reagent FCR (0.2 N in distilled water).After 5 min, 0.5 mL of sodium carbonate (75 g/L) were added. After 2 h of incubation in the dark at room temperature, absorbencies were measured at 760 nm against a blank (0.5 mL Folin-Ciocalteu reagent + 1 mL Na_2CO_3) on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England). The experiments were carried out in triplicate. The results were expressed as mg of Gallic Acid Equivalents (GAE)/g of extract.

Total Flavonoids Contents

The total flavonoids were estimated according to Dowd method as adapted by Lamien-Meda & *al.* [13] method. 0.75 mL of methanolic $AlCl_3$ (2%, w/v) were mixed with 0.75 mL of methanolic extract solution (0.1 mg/mL). After 10 min, the absorbencies were measured at 415 nm against a blank (mixture of 0.5 mL methanolic extract solution and 0.5 mL methanol) on a UV/visible light spectrophotometer and compared to Quercetin calibration curve (Y= 0.0249x ; R^2 = 0.99). The data obtained were the means of three determinations. The amounts of flavonoids in plant extracts were expressed as mg of Quercetin Equivalents (QE)/g of extract.

Total Flavonols Contents

The contents of flavonols were determined by using the method described by Abarca & *al.* [14]. Aliquots were prepared by mixing of 0.75 mL ethanolic extract solutions (0.1 mg/mL) and 0.75 mL aqueous $AlCl_3$ (20% w/v). The absorptions were read at 425 nm after 10 min of incubation against a blank (mixture of 0.75 mL ethanolic extract solutions and 0.75 mL ethanol) on a UV/visible light spectrophotometer. All determinations were carried out in triplicate. A standard calibration curve was plotted using Quercetin (y= 0.0353x + 0.0016 ; R^2= 0.99). The results were expressed as mg of Quercetin Equivalents (QE)/g of extract.

Total Tannins Contents

Total tannins contents were determined as described by European community and adapted by Sombié et *al.* [15], using tannic acid as a standard. In test tube, 0.2 mL of aqueous extract, 1 mL of distilled water, 0.2 mL of ammonium ferric citrate (3.5 g/L) and 0.2 mL of ammoniac (20%) were mixed. After 10 min, the absorbencies of samples were measured at 525 nm against a blank (0.2 mL aqueous extract + 1.2 mL distilled water) on a UV/visible light spectrophotometer. The data obtained was the mean of three determinations. The results were expressed as mg of Tannic Acid Equivalents (TAE) per g of extract (mg TAE/g extracts).

Measurement of Antioxidant Activity

DPPH Radical Scavenging Activity

The ability of the extract to scavenge the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical was evaluated. In the presence of an antioxidant which can donate an electron to DPPH, the purple color which is typical for DPPH free radical decays and the change in absorbency at 517 nm is followed spectrophotometrically. The method of Velazquez was used as adapted by Lamien-Meda & *al.* [13]. Briefly, 1 mL of a freshly prepared methanolic solution of DPPH (20 mg/L) was mixed with 0.5 mL of extract solutions (0.003-1 mg/mL). After 15 mn of incubation in the dark, at room temperature, absorbencies were read at 517 nm against a blank sample consisting of a 1.5 mL of methanol and 0.75 mL of extract solution. All determinations were performed in triplicate. DPPH radical inhibition percentage was calculated according to the following formula: inhibition (%) = $[(A_B\ A_A)/A_B]$ 100, where A_A is the sample (tested extract solution) absorbance and A_B is the blank absorbance. The extract concentration providing 50% of radicals scavenging activity (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration.

ABTS.+ scavenging Assay

Antioxidant activity was measured using an improved ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] method as described by Re and adapted by Lamien-Meda & *al.* [13] . The ABTS radical cation ($ABTS^{\cdot+}$) solution was prepared through the reaction of 7 mM of ABTS with 2.45 mM of potassium persulfate, after incubation at room temperature in the dark for 12 h. The $ABTS^{\cdot+}$ solution was then diluted with 95% ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Each sample (2 mL) was added to 2 mL of $ABTS^{\cdot+}$ solution and mixed vigorously. The reaction mixture was allowed to stand at room temperature for 6 min and the absorbance at 734 nm was immediately recorded. A standard curve (y= -0.0352x + 0.6438 ; R^2 = 0.99) was obtained using ascorbic acid standard solution at various concentrations in 95% ethanol. The capacity of free radical scavenging was expressed as µmol Ascorbic Acid Equivalent (AAE) per g of dry extract

Iron Reduction assay

The FRAP (Ferric Reducing Antioxidant Power) method is based on the reduction of the ferric ion (Fe^{3+}) in ferrous ion (Fe^{2+}). The method of Hinnebourg modified by Lamien-Meda & *al.* [13] was used. 0.5 mL of each extract (0.1 $mg.mL^{-1}$) was mixed with 1.25 mL of phosphate buffer (0.2M, pH 6.6) and 1.25 mL of aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution (1%). After 30 min of incubation at 50 C, 1.25 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 2000 g for 10 min. Then, the upper layer solution (0.625 mL) was mixed with distilled water (0.625 mL) and a freshly prepared $FeCl_3$ solution (0.125 mL, 1%). Absorbencies were read at 700 nm on a UV/visible light spectrophotometer and Ascorbic acid was used to produce the calibration curve (y= 0.0079x ; R^2 = 0.99). The iron (III) reducing activity determination was performed in triplicate and expressed in mmol Ascorbic Acid Equivalent (AAE) per g of extract.

Lipoxygenase (LOX) Inhibition Assay

Lipoxygenase inhibiting activity of plant extracts with linoleic acid as a substrate was measured with a UV/visible light spectrophotometer as described by Malterud and adapted by Compaoré & *al.* [17]. Extracts were screened for Lipoxygenase inhibitory activity at a final concentration of 50µg/mL. The mixture assay consisted of 150 µL of phosphate borate buffer (1/15 M, pH 7.5), 50 µL of extract solution (1mg/mL) and 50 µL of enzyme solution (0.28 U/mL in phosphate borate). The reaction was initiated by adding 250 µL of substrate solution (0.15 mM in water). Enzymatic kinetic was recorded at 234 nm for 02 min. Negative control was prepared and contained 1% methanol solution without extract solution. All experiments were performed in triplicate. Lipoxygenase inhibitory activity was expressed as the percentage inhibition of Lipoxygenase, calculated as (%) inhibition following equation: (%) inhibition = (A - B/A) 100, where A is the change in absorbance of the assay without the plants extracts and B is the change in absorbance of the assay with the plants extracts.

Acetylcholinesterase (AChE) inhibition assay

The AChE inhibitory assay was conducted according to the protocol described by Lopez as adapted by Kiendrebeogo & *al.* [16]. The experiment assay consisted to mix 200µL of Tris- HCl buffer (50 mM, pH8), 0.1% BSA, 100µL of extract (final concentration of 1mg/mL dissolved in buffer- MeOH 10%) and 100µL of AChE (0.22 U/mL). The mixture was incubated at room temperature for 2min before the addition of 500µL of DTNB (3mM) and 100µL of substrate (ATCI, 15Mm). The developing yellow color was measured at 405nm after 4min on a UV/visible light spectrophotometer. Galanthamine was used as a positive control at a final concentration of 0.2µg/mL in the assay mixture. AChE inhibitory activity was expressed as percent inhibition of AChE, calculated as (A-B/A) x 100, where A is a change in absorbance of the assay without the plant extract and B is the change in absorbance of the assay with the plant extract.

Cancer cell lines antiproliferative Assay

Cell culture conditions

All the mediums and complements of cellular culture come from GIBCO-Invitrogen (Merelbecke, Belgium). The cells are cultivated in cellular culture bottles (T25 or T75) containing the culture medium MEM supplemented with beef fetal serum (5%), glutamine (0.6mg/ml), gentamycin (0.1mg/ml) and penicillin-streptomycin (200 UI/ml).The culture is done in incubators with saturated atmosphere (CO_2 5% at 37 C). The medium was renewed every 72 hours.The cells are treated with trypsin and suspended then in medium in order to obtain a density allowing a specific cellular sowing for each biological analysis.

U373 cell proliferation inhibition test

The *in vitro* efficiency of plant extracts on the inhibition of human cancer cells growth was investigated [18] using the colorimetric 3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide assay (MTT). The extracts dissolved in DMSO and the culture medium, are added for concentrations going from 100µg/ml to 0.01µg/mL then left in contact with the cells during 72h.The inhibition of cancerous cell proliferation based on enzymatic reduction of 3-[4,5-diméthylthiazol-2yl]-diphényl tétrazolium to formazan cristal of which absorbance measured to 570 nm is proportional to the number of alive cells was used. The extent of the reduction of MTT was quantified by measurement of the absorbance at 570 nm. Concentration of sample which inhibited 50% (IC_{50}) of cell proliferation was determined graphically.

Statistical Analysis

The data were expressed as Mean±Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p<0.05$ and linear regression) was carried out with XLSTAT 7.1.

Results and discussion

Polyphenols contents

The phenolic contents of the methanolic extracts of these species are given in Table 1. Different plant extracts presented similar phenolics contents. But in flavonoid and flavonol contents, the quantities decreased in the following order: *P. bicalyculata*> *N. canescens*> *H. auriculata*. In contrast, *H. auriculata* extract has showed the best content. In previous studies, phenolic and flavonoid contents were evaluated in *H. auriculata* and *N. canescens* aqueous acetone extracts [19]. Actually, it is well demonstrated that plant polyphenol and consequently, flavonoids have several beneficial action on human health [20, 21].

Table 1: Polyphenols contents and biological activities of plant extracts

Species	*H. auriculata*	*N. canescens*	*P. bicalyculata*
Polyphenolic contents			
Total phenolic (mg GAE/g extract)	171.5±1.5 ^{a}	164.5±4.8 ^{a}	150.5±4.1 ^{b}
Total flavonoid (mg QE/g extract)	05±0.2 ^{c}	19.1±2.4 ^{b}	46±2.6 ^{a}
Total flavonol (mg QE/g extract)	0.2±00 ^{c}	2.6±0.2 ^{b}	9.8±1.0 ^{a}
Total tannin (mg TAE/g extract)	156.7±2.9 ^{a}	91.7±10.4 ^{b}	29±1.0 ^{c}
Antioxidant activities effects			
DPPH IC_{50} (µg/mL)	05.1 ±0.29^{b}	07.03±0.25^{b}	31.83 ±2.57^{a}
ABTS (µmol AAE/g extract)	73.37±4.15^{a}	60.90±1.21^{b}	66.80±1.73^{a} ^{b}
FRAP (mmol AAE/g extract)	01.46±0.02^{a}	01.53±0.01^{a}	0.59±0.09^{b}
Enzyme and cell Proliferation inhibition effects			
AChE Inhibition (%)	61.39±1.75^{a}	13.71±2.50^{b}	01.96±0.67^{c}
LOX Inhibition (%)	28.20±1.91^{c}	36.04±1.13^{b}	42.86±0.56^{a}
Cell Proliferation Inhibition IC_{50} (µg/mL)	29.70±3.11^{a}	27.00±1.55^{a}	not active

Notes : GAE, equivalent Gallic acid ; QE, equivalent Quercetin; TAE, equivalent Tannic acid; AAE, equivalent Ascorbic Acid; IC_{50}, concentration inhibiting 50%
Values are Mean ±SD (n=3). Results within each line with different letters (a-c) differ significantly (P<0.05) for our different extracts.

Antioxidant Activity

The results, indicated in table 1 showed that the plant extracts are able to scavenge radicals and to reduce iron (III). *H. auriculata* extract has demonstrated the best anti-DPPH (05.1±0.29), anti-ABTS (73.37±4.15) activities and a best reducing abilities (1.46±0.02); suggesting his wealth in antioxidant compounds. The active compounds could be a polyphenolic one because this extract has presented best phenolic, flavonoid and tannin contents. Especially since, polyphenol compounds were endowed with antioxidant powers [13]. In fact, antioxidant compounds roles were to prevent the radical toxicity by eliminating their development. Then, plants compounds with antioxidant properties are well promising more especially as the link oxidative stress with onset and/or development of number human diseases, such as inflammatory and cardiovascular diseases is well demonstrated [22, 23]. In previous studies, *H. auriculata* and *N. canescens* scavenging activity have been demonstrated [19]. For *H. auriculata*, this antioxidant property could justify its *in vivo* antioxidant activities [7].

In vitro Enzymes and cell proliferation Inhibitory activities

The results are showed in table 1. *H. auriculata* extract showed the best AChE inhibition (61%) while LOX was most sensitive to *P. bicalyculata* extract (42%). When exposed to plant extracts, U373 proliferation was significantly inhibited, mainly *H. auriculata* and *N. canescens* extracts which presented 29.70±3.11 and 27.00±1.55 µg/mL as IC_{50} respectively. However, U373 cells proliferation was not affected *by P. bicalyculata* extract. These results were demonstrated that *H. auriculata* extract was able to inhibit cell proliferation as LOX and AChE activities. Biological activities of LOX and AChE were connected to number disorder including bronchial asthma, inflammatory diseases, cancer, neurodegenerative and cardiovascular diseases [24, 25] and then an inhibition of these enzymes was a promising ways to search antitumoral compound.
Remarkably, *H. auriculata* and *N. canescens* extracts have presented similar antioxidant and cell inhibition abilities and polyphenol contents. Interestingly, *H. auriculata* extract that has presented the best AChE inhibition and a highest cell proliferation activity demonstrated a possible contribution of AChE inhibitor compounds to cell proliferation activity, contrary to *N. canescens* and *P. bicalyculata* extracts. The extracts anti-proliferation activities could be due to their capacities to break tumor cell communication by inhibiting AChE and LOX enzymes expression as demonstrated in previous biological investigations [26, 27].
According to the American National Cancer Institute (NCI) recommends, a plant extract having antitumor values of IC_{50} lower than 30 µg/mL was interesting for antitumor compound investigation [28]. *N. canescens* and . *auriculata* could be regarded as good candidate for the isolation of antitumor bioactive molecules.

Conclusion

In the present study *H. auriculata*, *N. canescens* and *P. bicalyculata* methanolic extracts contained appreciably levels of

total phenolic contents, flavonoids, flavonols and tannins. All the sample extracts from these species also exhibited high antioxidant activity depending to the antioxidant method used. The results of the present study suggest that plant extracts of the three Acanthaceae species can be substantial source of multi-purpose bioactive principles, which act as natural antioxidants, acetylcholinesterase and/or lipoxygenase inhibitors and also as antitumoral. Already now, these scientific data allow justifying the traditional use of the three plants species. Further molecular elucidation investigations are necessary in order to identify the bioactive structures responsible to these interesting activities.

Authors' contributions

All authors have made substantial contributions and final approval of the conceptions, drafting and final version.

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