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In vitro Antioxidant, Lipoxygenase and Xanthine Oxidase inhibitory activity of fractions and macerate from *Pandiaka angustifolia* (vahl) Hepper

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ABSTRACT

Pandiaka angustifolia (Vahl) Hepper, a species belonging to Amaranthaceae's family is used in traditional medicine of Burkina Faso for the treatment of several diseases. The aim of this study was to evaluate the phenolics and flavonoids content, the antioxidant capacity, the lipoxygenase and xanthine oxidase inhibition potentials of *Pandiaka angustifolia* hydroacetonic macerate and fractions. Assay revealed that the Dichloromethane Fraction (DCMF) possess the best inhibitory activity of lipoxygenase, a key enzyme involved in inflammatory process with 83.84±1.89% of inhibition, as regards Xanthine Oxidase (XO) inhibiting percentage, n-hexane fraction (n-HF) exhibited the highest percentage of inhibition with 76.22 ± 4.78% at a concentration of 100µg/ml. Phenolics and flavonoids content in fractions and hydroacetonic macerate evaluation showed that hydroacetonic macerate possess the highest rate in total phenolic and the Dichloromethane fraction (DCMF) the highest rate in flavonoids content. This study showed that the apolar fractions of *P. angustifolia* is a potential natural source for the treatment of oxidative stress and inflammatory related diseases.

INTRODUCTION

Plants have provided humanity with all its need in term of shelter, clothing, food, flavors and fragrances as not least, medicines. Plants have formed the basis of sophisticated traditional medicine systems like Ayurvedic, Unani, and Chinese medicine (Gurib-Fakim, 2006). African traditional medicine is still not well known but abounds in knowledge and practices and remain the main supplier of medical care in different parts of the continent. One of the plant families commonly used in the treatment of illness is amaranthaceae. *Pandiaka angustifolia* a member of this family is traditionally used in Burkina Faso as a tonic drink for parturient, as a spasmolytic, in the treatment of, blennorrhoea, annexite salpingitis, in the treatment of women genital apparatus inflammation, in the treatment of malaria (Nacoulma, 1996). Despite the widespread use of species belonging to the amaranthaceae's family, scientific literature provide little data on the biological potential of the extract/fraction of *pandiaka angustifolia*. In the vision to bring our modest contribution to Burkina Faso's traditional medicine, the aim of the present study was to evaluate and compare phenolic content, the antioxidant activities, the lipoxygenase and xanthine oxidase inhibition potentials of five fractions and the hydroacetonic macerate of *P. angustifolia*.

MATERIALS AND METHODS

Plants materials

The whole plant of *pandiaka angustifolia* (Vahl) Hepper was collected in august 2012 in Yaagma, 15 km in the northern vicinity of Ouagadougou, capital of Burkina Faso and authenticated by Professor Jeanne Millogo-Rasolodimby from botanical

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department of the University. A voucher specimen ($SD_{-te\ 001}$) was deposited in the herbarium of the University of Ouagadougou. Whole plant were dried at room temperature and subsequently pulverized and stored in airtight bag before utilization.

Reagents and solvents

To carry out our different activities, we used solvents, enzymes and various classic reagents. All reagents were analytical grade. Folin-Ciocalteu reagent, Dragendorff's reagent, sodium carbonate (Na_2CO_3), sodium hydroxide, gallic acid, quercetin, aluminium trichloride ($AlCl_3$), ferric trichloride, sodium phosphate dibasic, monobasic potassium phosphate, hydrogen peroxide solution, thiobarbuturic acid, Xanthine oxidase from bovine milk (EC 1.1.3.22) and xanthine (2.6-dihydroxypurinol), allopurinol, hydrochloric acid, magnesium chloride, bovine serum albumine (BSA), 15-lipoxygenase (EC 1.13.11.12), linoleic acid and Boric acid were purchased from Sigma Aldrich chemie (Steinheim, Germany), ammonium ferric citrate, potassium persulfate, DPPH (2,2'-diphenyl-1- picrylhydrazyl) and trichoroacetic acid were supplied by Fluka chemie(Buchs, Switzerland); sulfuric acid, acetic anhydride, ferric trichlorure, hexane, chloroform, ethyl acetate, acetone, butanol, ethanol, methanol, sodium tetraborate, and potassium hexacyanoferrate [$K_3Fe(CN)_6$] were sourced from Prolabo (Paris, France) ; ascorbic acid and tannic acid were supplied by Labosi (Paris, France).

Extraction

Twenty five grams (25 g) of powdered plant were extracted with acetone (80%) for 24 h using an electric mixer. The macerate obtained was filtered (through Whatman N°1 paper), concentrated using a rotary evaporator and lyophilized. A portion of lyophilized material was fractionated by successive liquid-liquid partitioning with n-hexane, dichloromethane, ethyl acetate and n-butanol.

Phytochemical investigation

Total phenolics content

The total polyphenols were estimated by the method of singleton *et al*. (1999). The method assesses all the phenolic compounds using phosphomolybdotungstic reducing reagent or Folin-Ciocalteu Reagent (FCR). A volume of 105 µL of FCR (0.2 N in distilled water) was mixed with 25 µL of plant macerate/fraction (0.1 mg/mL in distilled water) in a 96-well plate. After 5 min, 100 µL of Na_2CO_3 (75g /L) was added to the preceding mixture. The mixture was then left to stand in dark for 2 hours. The absorbance was subsequently read at 760 nm using a BioteckEpoch spectrophotometer. A standard calibration curve (y =0.005X+0.00968; R^2 =0.99) was plotted using gallic acid (0-100 mg/L). Experiment were carried out in triplicate and the result was expressed as milligrams of gallic acid equivalent to 100 mg of extract (mgGAE/100 mg).

Total flavonoids content

The total flavonoids were estimated according to Dowd method as adapted by Arvouet-Grand *et al.* (1994). A volume of 75 µL of $AlCl_3$ prepared in methanol is mixed with 75 µL of macerate/fraction at the concentration of 100µg/mL prepared in methanol. Absorbance were subsequently read at 425 nm after 10 min of incubation against a blank (mixture of 75 µL of macerate/fraction and 75 µL of methanol) using a BioteckEpoch spectrophotometer.

A standard calibration curve (y= 0,0289X + 0,0036; R^2 = 0.99) was plotted using quercetin (0-100 mg/L). the amount of flavonoids in plant extract/fraction were expressed as milligram of quercetin equivalent (QE)/100 mg of extract.

Antioxidant activities

DPPH radical method

Radical scavenging activity of plant macerate/ fraction against stable DPPH (20mg/mL) (2,2- diphenyl-1picrylhydrazyl) was determined using UV/ visible light spectrophotometer (BioteckEpoch) at 517 nm by using the method described by Kadalm et *al.* (2010)**.** In each 100µL of successively half dilution of methanolic extract/fraction was added 200µL of DPPH solution. The solution absorbance was read after 15 min of dark incubation against a blank. DPPH reduction percentage of each dilution was obtained according the following formula.

$$\text{AAR}(\%) = \frac{Abs\ (blk) - Abs\ (ext)}{Abs\ (blk)} \times 100$$

Abs (blk) : absorbance without extract ; **Abs(ext)** : absorbance with extract

Each test was repeated three times, and the value of IC_{50} (the minimum concentration that inhibits 50 % of control) was determined graphically using a plot obtained from different concentration of each extract. A low IC_{50} value indicates strong antioxidant activity.

Iron(III) Reduction Activity (FRAP)

The FRAP (Ferric Reducing Antioxidant Power) assay was performed according Lamien-Meda et *al.* (2008) method with minor modifications. 0,1 mL of each extract (1 mg/mL) was mixed with 0.25 mL of phosphate buffer (0.2M, pH 6.6) and 0.25 mL of aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution (1%). After 30 min of incubation at 50 °C, 0.25 mL of trichloroacétic acid (10%) was added and the mixture was centrifuged at 2000 g during 10 min then the upper floating solution (125 µL) was mixed with water(125 µL) and a freshly prepared $FeCl_3$ solution (25 µL, 0.1%). A blank is prepared in the same conditions but extracts are replaced with solvent. Absorbance were read at 700 nm on a spectrophotometer and ascorbic acid was used to plot a calibration curve (R^2= 0.99). The iron (III) reducing activity of extracts was performed in triplicate and expressed in mmol Ascorbic Acid Equivalent per gram of extract.

Lipid peroxidation inhibition in rat liver homogenate

The ability to inhibit lipid peroxidation in rat liver homogenate of *P. angustifolia* macerate/fraction was determined by measuring malondialdehyde formation according to the thiobarbituric acid method as described by Su et *al.* (2009) $FeCl_2$ was used to induce the rat liver (Wistar rats, 155-201g) homogenate peroxidation. For this assay 0.2 mL of each fraction (1.5 mg/mL in Tris-HCl buffer 20 mM ; pH 7.4) was mixed with 1 mL of 1% liver homogenate (in Tris-HCl buffer 20 mM ; pH 7.4) ; 50µL of $FeCl_3$ (0.5mM) and H_2O_2(0.5mM) was then added. The mixture was incubated at 37°C for 60 minutes, and then 1 mL of trichloroacetic acid (15%) and thiobarbituric acid (0.67%) was added. Subsequently the previous mixtures were incubated in boiled water for 15 min. Each sample was then subject to centrifugation (2000 rpm for 5 min), and the absorbance of the supernatant was recorded at 532 nm. Ascorbic acid was used as positive control. The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = [1- (A_1-A_2)/A_0]\text{x } 100$$

A0 : control (without extract) absorbance
A1 : absorbance of sample containing extract/ fraction
A2 : absorbance of sample without liver homogenate

Enzyme inhibition Assay

Xanthine oxidase (XO) inhibition assay

The xanthine oxidase inhibition activity was assayed on a spectrophometer as described by Owen and Timothy (1999) with minors modifications. The assay mixture consisted of 150 µL of phosphate buffer (0.066M ; pH,7.5), 50µL of extract solution (1 mg/mL in phosphate buffer), and 50 µL of enzyme solution (0.28U/mL). After pre-incubation at room temperature (25°C) for 3 minutes, the reaction was initiated by addition of 250µL of substrate solution (Xanthine, 0.15 M in the same buffer). A blank without enzyme solution was also prepared. The reaction was monitored for 3 minutes at 295 nm and velocity (Vo) was recorded. Phosphate buffer was used as negative control (activity of the enzyme without extract solution). Allopurinol was used as positive control. The percentage of xanthine oxidase inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = [(Vo_{\text{control}} - Vo_{\text{sample}}) \text{ X } 100]/Vo_{\text{control}}$$

Vo_{control} = activity of enzyme without macerate/fraction
Vo_{sample} = enzyme activity in presence of macerate/fraction or allopurinol

Lipoxygenase (LOX) inhibition assay

The lipoxygenase inhibiting activity was essayed spectrophotometrically as described by Lycklander and Malterud (1992) with minor modifications. Briefly 100 µL of the enzyme solution (at the final concentration of 200 U/mL) was prepared in boric acid buffer (0.2 M; pH, 9) and mixed with 25 µL of extract solution (1 mg/mL in boric acid buffer) and then incubated at room temperature for 3 minutes. Reaction was subsequently initiated by the addition of substrate solution (linoleic acid, 250 µM), and the velocity was recorded for 2 min at 234 nm. Negative control was prepared and contained 1 % methanol solution without fraction solution. Quercetin was used as positive control. The percentage of lipoxygenase inhibition was calculated according to the following equation:

$$\text{Inhibition (\%)} = [(Vo_{\text{control}} - Vo_{\text{sample}}) \text{ x } 100]/Vo_{\text{control}}$$

Vo_{control} is the activity of enzyme in absence of extract solution, and Vo_{sample} is the activity of the enzyme in the presence of extract or quercetin.

STATISTICAL ANALYSIS

All the reactions were performed in triplicate, and data are presented as mean standard deviation. Data were analysed by one way analysis of variance followed by Tukey multiple-comparison test. Analysis were done using XLSTAT7.1 software. A P value less than 0.05 was used as criterion for statistical significance.

RESULTS AND DISCUSSION

Enzyme inhibition assay

XO and LOX inhibition potential of fractions/macerate

Lipoxygenase and Xanthine Oxidase are key enzymes implicated in many mediated inflammation diseases such as cancers, arthrosclerosis, hypertension and diabetes (Osher et *al*., 2006). Their inhibition could be a way for finding new compound with antiinflammatory ability. *Pandiaka angustifolia* fractions/ macerate effects on these enzymes was evaluated trought the percentage of inhibition and results were consigned in table 1. The amount of lipoxygenase inhibited varied from 83.84±1.89 to 22.31±0.53. The highest percentage of inhibition was obtained with DCMF and the lowest with WF.

Xanthine oxidase inhibition percentage ranged from 77.61±0.98 to 1.68±0.06. The highest inhibition percentage of inhibition was obtained with n-HF and the lowest with HAF.

Regarding the results we notice that the best inhibiting percentage of both LOX and XO were exhibited by apolar fractions comparatively to the polar fractions and the hydoacetonic macerate. These results could be explained by the abundance of aglyconic phenolic compounds with high potential in these fractions.

Table. 1: LOX and X.O inhibitions activities.

Extract/fraction	Lipoxygenase ……………………………(%)…………………….	Xanthine oxidase
n-HF	81.21±0.4 ^{a,b}	77.61±0.98^{a}
DCMF	83.84±1.89^{a}	71.48±1.89^{b}
EAF	45.49±3.14^{c}	61.48±2.03^{c}
n-BF	49.17±0.25^{c}	10.09±1.53^{d}
WF	22.31±0.53^{d}	5.41±0.53^{e}
HAF	78.13±0.41^{b}	1.68±0.06^{e}
Quercetin	52.74±0.78	75.11±4.53
Allopurinol	ND	77.13±0.41
Ibuprofen	79.15±0.31	ND

Data are Mean ± SEM (n=3), HAE : Hydroacetonic extract, EAF: Ethyl acetate fraction, DCMF : Dichloromethane fraction, WF : water fraction ;n-BF : n-butanol fraction , n-HF : n-hexane fraction. Values showing the same letter are not significantly different (p>0.05) from one to another in the same columns.

Biological activities

Antioxidant capacities

Recent advances in many diseases etiology revealed the preponderant role of oxidants. Compound with antioxidant ability from herbal medicine could be so beneficial in the prevention and the treatment of major diseases such as inflammation, chronic diseases such as arteriosclerosis, cancer, asthma, diabetes, neurodegenerative diseases…(Reuter et *al.*, 2013 ;Oliver Sorg ,2004). The antiradical effects and the iron III reducing abilities of *Pandiaka angustifolia*'s fraction and hydroacetonic macerate were presented in table 2. Assay revealed that; the apolar fractions exhibit the highest capacity of inhibition using FRAP as anti-LPO methods.

Table. 2: Antioxidant and anti-LPO activities.

Antioxidant activity	FRAP(mmol AAE/g)	DPPH (IC_{50} ; µg/mL)	Anti-LPO (%)
n-HF	2.37 ± 0.045^{b}	----------	76.22 ±4.78^{a}
DCMF	3.75 ±0.082^{a}	----------	46.35 ±1.03^{b}
EAF	1.75 ±0.029^{d}	8.30±1.72	35.90 ±3.63^{c}
n-BF	0.19 ±0.0041^{e}	----------	9.35 ± 0.23^{e}
WF	0.087 ± 0.015^{e}	----------	1.59 ±0.39^{f}
HAE	2.23 ±0.02^{d}	4.35± 0.23	24.73 ±1.29^{c}
Quercetin	ND	3.60±0.09	28.00±2.90
Ascorbic acid	5.70±0.15		48.75±1.28
Gallic acid	ND		ND

Data are Mean ± SEM (n=3), HAE : Hydroacetonic extract, EAF: Ethyl acetate fraction, DCMF : Dichloromethane fraction, WF : water fraction; n-BF: n-butanol fraction , n-HF : n-hexane fraction. Values showing the same letter are not significantly different (p>0.05) from one to another in the same columns.

Phytochemical investigation

Total phenolic contents

Total phenolic content of fractions and hydroacetonic macerate of *pandiaka angustifolia* were measured (Table 1). The hydroacetonic macerate presented the highest total phenolic content (127.73± 4.23 mg of quercetin equivalents/100 mg of fraction/extract), followed by ethyl acetate fraction, n-hexan fraction, Dichloromethane fraction, Butanolic fraction and water fraction. The total phenolic content of hydroacetonic macerate, ethyl acetate fraction, n-hexane fraction and Dichloromethanolic fraction was significantly different from each other (P< 0.05). However, significant differences in total phenolic content were not found among butanolic and water fractions (p<0.05). Comparing the phenolic content in different fractions and macerate, its clearly appears that *pandiaka angustifolia* contains different type of phenolic compound (from apolar to polar). Phenolic compound are well known to be effective in radical scavenging activities, enzyme inhibiting (Hua Li et *al.*, 2008, Vermerris and Nicolson, 2006). So then their presence in these fractions can justifies their antioxidant activities. The LOX and XO inhibition could also be attributed to the antioxidant activities of the fractions. And this is strengthened by a good correlation between antioxidant activity and anti-inflammatory drug activities.

Total flavonoids contents

Flavonoids constitute a class of phenolic compounds produced by plants to adapt their environmental conditions (hichri et *al.*,2010). Human being use these properties in the treatment of diseases and as dyeing substances (Maria and francesca, 2009). Total flavonoids contents of five fractions and the hydroacetonic macerate of *pandiaka angustifolia* are presented in table 1. Analysis revealed that the Dichloromethane fraction had the highest total flavonoid content (11.31 ± 0.21 mg of quercetin equivalents/100 mg of fraction/extract), followed by n-hexan fraction. There was significant difference (p<0.05) in total flavonoids content between Dichloromethane fraction, n-butanol fraction , n-HF : n-hexane and Ethyl acetate fraction. however, significant differences in total flavonoids content were observed between Ethyl acetate fraction and hydoacetonic macerate, n-butanol fraction and water fraction (p<0.05). in this study there was 95-fold difference in total flavonoids content between the highest and lowest ranked fractions, Dichloromethane fraction and water fraction.

Table. 3: Totals phenolic and totals flavonoïds content.

Extract/fractions	Total phenolics (mg EQ/100mg of extract)	Total flavonoids (mg EQ/100mg of extract)
n-HF	44.40 ± 2.06^{c}	09.77 ± 0.48^{b}
DCMF	36.40 ± 2.11^{d}	11.31 ± 0.21^{a}
EAF	60.84 ± 0.25^{b}	02.03 ±0.07^{c}
n-BF	08.64 ± 1.37^{e}	00.65 ±0.03^{d}
WF	04.63±0.12^{e}	00.12±0.01^{d}
HAE	127.73± 4.23^{a}	01.73 ±0.05^{c}

Data are Mean ± SEM (n=3), HAE : Hydroacetonic extract, EAF: Ethyl acetate fraction, DCMF : Dichloromethane fraction, WF : water fraction ;n-BF : n-butanol fraction , n-HF : n-hexane fraction. Values showing the same letter are not significantly different (p>0.05) from one to another in the same columns.

CONCLUSION

In this study ; antioxidant activities and capacities to inhibit the 5-lipoxygenase, the xanthine oxidase as well as the phenolic content of five fractions and hydroacetonic macerate of *Pandiaka angustifolia* were evaluated. Results obtained concerning enzyme inhibitions effets and antioxidant capacity of samples analyzed revealed that for both tests the apolar fractions are more effective than the polar ones. *Pandiaka angustifolia* is commonly used in aqueous decoction as a tonic drink for new parturient, as a spasmolytic, in the treatment of women genital apparatus inflammation, in the treatment of malaria in Burkina Faso, particularly because water is the most available and attainable solvent for the populations. The use of a species during many centuries attests its efficiency for treating a disease. Regarding our results, the aqueous fraction of *pandiaka angustifolia* seems to be less efficient than non-polar ones. Thus it will be necessary to do further studies in the aim of isolation and elucidation of compound present in the more active fractions, all thing that may help in the improvement of traditional herbal medicine in Burkina Faso.

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