African Journal of Biotechnology Vol. 9(33), pp. 5407-5411, 16 August, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.611
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Antioxidant and antibacterial activities of *Piliostigma reticulatum* (DC.) Hochst extracts

Alexandre Zerbo[1], Jean Koudou[2*], Noufou Ouédraogo[1], Rasmata Ouédraogo[3] and Innocent P. Guissou[1]

[1]Laboratoire de Pharmacologie-Toxicologie (UFR/SDS), Université de Ouagadougou, 03BP7021 Ouagadougou Burkina Faso.
[2]Laboratoire de chimie de substances naturelles, Faculté des Sciences, Université de Bangui, BP908 Bangui Centrafrique.
[3]Laboratoire de bactériologie-Virologie (UFR/SDS), Université de Ouagadougou, 03BP7021 Ouagadougou Burkina Faso.

Accepted 7 August, 2009

The methanol and aqueous extracts of *Piliostigma reticulatum* barks were investigated for its antioxidant and antibacterial activities. The antioxidant test using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method demonstrated important radical scavenging activity for the methanol extract with IC_{50} = 0,37 ± 0,04 µgmL^{-1}. The 2 extracts were then examined for antibacterial activity by using the broth micro dilution method. The extracts possessed the antibacterial effect against the almost germs tested; the aqueous extract exhibited a better activity than methanol extract. These results indicate that methanol and aqueous extracts of *P. reticulatum* could be used as a source of antioxidant and antibacterial ingredients in the food industry.

Key words: *Piliostigma reticulatum*, Ceasalpiniaceae, bark extracts, antioxidant, antibacterial.

INTRODUCTION

Recently, there is a growing interest in substances exhibiting antioxidant and antimicrobial properties that are supplied to human organisms as food components or as specific pharmaceutics. The role of free radicals is becoming increasingly recognized in the pathogenesis of many human diseases, including inflammation, cancer, diabetes, renal failure, atherosclerosis and hypertension (Aderogba et al., 2005). Antioxidants retard oxidation and arrest the adverse effect of free radicals and are sometimes added to meat and poultry products to prevent or slow oxidative rancidity of fats that cause browning and deterioration. However, antioxidant compounds from natural sources are receiving consideration due to the side effect of the synthetic ones (Ito et al., 1983). It has been known that plant extracts which which contain phenolic and flavonoid compounds have antoxidant and antibacterial effects (Bruneton, 1999; Mendes da Silva et al., 2006; Majhenic et al., 2007; Pereira et al., 2007). Therefore, research in the determination of nature source of antioxidants and the antibacterial potential of plants is important.

In the literature, there are only 2 papers dealing with the antioxidant and antimicrobial activities of leaf extract of *Piliostigma reticulatum* (Aderogba et al., 2005; Olalekan et al., 2008), but the properties of bark extracts have not been reported. *P. reticulatum* (DC.) Hochst (Ceasalpiniaceae) is a tree or shrub which is usually 1 to 10 m and occurs often in the form of bushes (Arbonnier, 2002). This plant Sudano Sahelian is traditionally used in the treatment of many diseases such as dysentery, diarrhea, inflammation, infections, neuralgia, smallpox, malaria, rheumatism. In addition the leaves and barks of *P. reticulatum* are used in food for the preparation of local alimentary paste (Yelemou et al., 2007). Barks are stimulants digestion.

*Corresponding author. E-mail: jean_koudou@yahoo.fr. Tel: +226.78.87.03.38. Fax: +226.50.36.85.73.

To our literature survey, no study concerning antioxidant and antibacterial properties of barks of this species has been done before. The present work reports results of *in vitro* antioxidant and antibacterial activities of methanol and aqueous extracts of *P. reticulatum* barks with the aim to contributing to the search for beneficial uses of this plant.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade. Na_2CO_3, DPPH, gallic acid, Quercetin and 2.0 M Folin-Ciocalteu phenol reagent, Neu reagent was purchased from Sigma –Aldrich (Steinheim, Germany). Methanol, ethyl acetate, chloroform, acetic acid, n-hexan, NaOH, HCl, H_2SO_4, $FeCl_3$, Mg, NH_4OH, $AlCl_3$ and Drag gendorff from Merck (Germany). galangin, chrysin, acacetin, genestein, apigenin, lutealin, quercitrin, cinnamic acid, rutin, ferulic acid, gallic acid, hydrocinnamic acid, cafeic acid and vanillic acid were supplied by Fluka (Switzerland).

Plant material

Barks of *P. reticulatum* were collected in March, 2006 in the region of Ouaga dougou (Burkina, Faso). The plant was authenticated by Dr. Thombiano of Botany and Ecology Section, Faculty of Sciences, University of Ouagadougou and voucher specimen was deposited.

Preparation of extracts

Methanol extraction

The dried and powdered barks (100 g) were macerated with 200 mL in methanol for 48 h at room temperature. The extract was filtered using Whatman filter paper n°1 and then concentrated in vacuo at 40°C using a rotary evaporator Büchi. The residue obtained was stored at 4°C in a refrigerator.

Aqueous extraction

50 g of dried and powdered barks were extracted with 200 mL of boiling water for 4 h. The aqueous extract was filtered using Whatman filter paper n°1 and its lyophilization was performed on a Cryodos 50 (Telstar, Spain).

Analysis

The phytochemical analysis of *P. reticulatum* extracts was carried out in using stan dard qualitative methods (Ciulei, 1982) for the determination of different chemical groups: tannins and phenolic compounds, flavonoïds, saponins, triterpenoids, steroids, coumarins, alkaloids, anthocyanins. The extracts were analyzed to determine phenolic compounds and flavonoids in using 2 methods according to Wagner (1996) and Males and Medic-Saric (2001) on thin layer chromatography with silicagel plates F_{254}, using 2 systems of solvents: S1: hexan/ethyl acetate/acetic acid (31/14/5; v/v/v) and S2: ethyl acetate/formic acid/acetic acid/water (100/11/11/27; v/v/v/v). The components were revealed with the Neu reagent at 254 and 365 nm. The chemical references used are: galangin, chrysin, acacetin, genestein, apigenin, lutealin, quercitrin, cinnamic acid, quercetin, rutin, ferulic acid, gallic acid, hydrocinnamic acid, cafeic acid and vanillic acid.

Amount of total phenolic compounds

So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts. Total phenolic compounds were determined with the Folin-Ciocalteu reagent according to the method of Singleton et al. (1999). Methanol and aqueous extracts were prepared at concentration of 1 mg/mL in water and absorbance measured at 760 nm against a methanol blank using spectrophotometer (CECIL CE 2041, CECIL Instruments England). An amount of 1 mL aliquot of the prepared samples were mixed with 1 mL Folin-Ciocalteu reagent (previously diluted with water 1:1 (v/v)) and 2 mL of satured sodium carbonate (Na_2CO_3) solution and 10 mL of deionized water. The mixtures were intensively shaken for 2 h at room temperature. The standard calibration curve was plotted using gallic acid (0 – 200 mgL^{-1}). All tests were performed in triplicate and the concentration of total phenolic compounds was expressed as mg of gallic acid equivalents (GAE)/100 mg of extract.

Amount of total flavonoids

The determination of total flavonoids was conducted with alumine trichloride ($AlCl_3$) according to the method of Dowd adapted by Arvouet-Grand et al. (1994). Quercetin was used as a standard. For each extract, 2 mL of methanolic solution (100 $\mu g.mL^{-1}$) was mixed with 2 mL of $AlCl_3$ 2% in methanol. After 10 min of contact, the absorbance was read at 415 nm against a blank sample consisting of a 2 mL of methanol and 2 mL of of plant extract without $AlCl_3$. All tests were performed in triplicate and the results expressed as mg of quercetin equivalents (QE)/100 mg of extract.

Evaluation of antioxidant activity

Antioxidant activity of plant extracts was determined according to the DPPH radical (2% in MeOH, w/v) method as recommended by Velazquez et al. (2003). 1.5 mL solution of DPPH was added to 0.75 mL of various concentrations of each sample solution (1, 5, 10, 15, 20, 25 and 30 μgmL^{-1}). The solution of DPPH in methanol was prepared daily, before UV measurements.The mixtures were kept in the dark for 15 min at room temperature and the decrease in absorbance was measured at 517 nm against a blank consisting of a 1.5 mL of methanol and 0.75 mL of extract solution. Quercitin and gallic acid were used as positive controls. These were converted to percent DPPH radical scavenging which is calculated with the equation:

$$\% \text{ inhibition} = [(A_{blank} - A_{sample})/ A_{blank}] x\ 100$$

Where A_{blank} is the blank absorbance and A_{sample} the sample absorbance (tested extract solution) The IC_{50} value of each extract was determined graphically and all tests were performed in triplicate. A lower IC_{50} value indicates greater antioxidant activity.

Determination of antibacterial activity

Microorganisms

The microorganisms used in this study consisted of collection/serotyped strains. The following serotyped strains used are: Gram-positive bacteria: *Bacillus cereus* LMG 13569 BHI;

Table 1. Total phenolic, total flavonoid contents and radical scavenging activity of methanol and decoction extracts of *P. reticulatum* (D.C.) HOSCHT.

Extract	Total phenolic content (mg GAE/100 mL extract)	Total flavonoid content (mg QE/100 mL extract)	IC_{50} (μgmL^{-1}) for DPPH scavenging activity
Methanol	52.96 ± 1.55	0.18 ± 0.02	0.37 ± 0.04
Decoction	6.76 ± 0.22	0.28 ± 0.03	15.16 ±0.15
Quercetin	-	-	Quercetin
Gallic acid	-	-	0.60± 0.01

Staphylococcus aureus ATCC 25923; *Enterococcus faecalis* CIP 103907 BHI; *Stophylococcus camorum* LMG 13567 BHI and *Listeria innocua* LMG 13568 BHI. Gram-negative bacteria: *Escherichia coli* ATCC25922; *Salmonella typhimurium* ATCC 14028; *Shigella sonnei* ATCC 25931 and *Proteus mirabilis* CIP 104588 BHI. They were obtained from Laboratory of bacteriology-Virology, (UFR/SDS), University of Ouagadougou. Before testing, pure cultures were realized with all strains in Mueller Hinton Agar and Tryptic Soy Broth. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 McFarland standard.

Antibacterial activity assay

MICs and MBCs were determined using the Mueller Hinton broth micro dilution in 96 well-plates according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001, 2004).The broth from methanolic extract was only supplemented with DMSO at a concentration of 10% in order to enhance solubility (Coulidiati et al., 2009). The bacterial strains grown on nutrient agar at 37°C for 18 to 20 h were suspended in a saline solution (0.85%, w/v) to a turbidity of 0.5 Mc Farland standards (10^8 cfu/mL). The suspensions were diluted with Mueller Hinton broth to inoculate 96 well-plates containing 2-fold serial dilutions of extracts. Drug concentrations ranged from 1 to 60 mg/mL. The final volume in wells was 160 µL. The final inocula as determined by colony counts for the growth control wells were approximately 10^5 cfu per well. Plates were incubated at 37°C for 24 h. MIC was recorded as lowest extract concentration demonstrating no visible growth in the broth. MBC was recorded as a lowest extract concentration killing 99.9% of bacterial inocula. MBC values were determined by removing 100 µL of bacterial suspension from subculture demonstrating no visible growth and inoculating nutrient agar plates. Plates were incubated at 37°C for 48 h.

Statistical analysis

Data were expressed as mean ± SEM. A one way variance was used to analyse data. $P < 0.01$ represented significant difference between means (Duncan's multiple range test).

RESULTS AND DISCUSSION

Phytochemical screening

Chemical study and TLC revealed the presence of tannins, phenols acids, phenolic compounds, anthocyanins, saponins and triterpenoids in the both extracts, particulary quercitrin and rutin were detected with Neu reagent at 365 nm. Tannins, phenolic compounds and anthocyanins could contribute to the observed antioxidant activity (Coulidiati et al., 2009; Pereira et al., 2007) and the presence of saponins among the first ones could reinforce the antibacterial effect (Bruneton, 1999).

Total phenolic compounds and flavonoids contents

The total phenolic compounds and flavonoids contents in the bark extracts are shown in Table 1. The content of phenolic compounds in methanolic extract was determined from regression equation of calibration curve ($y = 0.0095x$, $R^2 = 0.9966$) and expressed in gallic acid equivalents (GAE). The highest amount of phenolic compounds was found in the methanol extract. The content of flavonoids in quercetin equivalents determined from $y = 0.0249x$, $R^2 = 0.9943$, varied between 0.18 and 0.28. The aqueous extract had the highest amount of flavonoids than the methanol extract, this suggests that there were most non-glycosides compounds in the methanol extract than in water sample.

Antioxidant activity

The IC_{50} values of quercetin and gallic acid were 0.88 ± 0.11 µg/mL and 0.60 ± 0.14 µg/mL. From the quantitative determination, the methanolic extract demonstrated a high antioxidant activity with good ability of scavenging DPPH free radicals which was correlated with the high quantity of total phenolic contents (Karou et al., 2005) and had the lowest IC_{50} value than quercetin and gallic acid employed as standard agent. The IC_{50} value of aqueous extract was larger, this suggests that the aqueous extract may be weak as antioxidant because of its lowest amount of total phenolic compounds contents. Significantly minor total phenolic compounds presence in the aqueous sample could be explained by the less solubility of these compounds in water, and minor compounds as total flavonoids might cause also the antioxidant activity exhibited (Bruneton, 1999; Oliveira et al., 2008). The high antioxidant effect of methanolic sample could be due by the presence of a high amount of phenolic compounds. Possible synergetic effects of these compounds in the two extracts should be taken into consideration. However, antioxidant activity of non-

Table 2. Antibacterial activity of methanol and decoction extracts from *P. reticulatum* (D.C.) HOSCHT.

Bacteria	Methanol extract			Decoction extract		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
Escherichia coli	6.25	12.50	2	25	50	2
Salmonella typhimurium	3.12	6.25	2	25	50	2
Shigella sonnei	6.25	25	4	12.50	25	2
Staphylococcus aureus	3.12	6.25	2	3.12	6.25	2
Staphylococcus camorum	3.12	12.50	4	3.12	12.50	4
Proteus mirabilis	3.12	12.50	4	12.50	25	2
Enterococcus faecalis	1.51	3.12	2	25	25	1
Listeria innocua	3.12	12.50	4	12.50	25	2
Bacillus cereus	3.12	6.25	2	12.50	25	2

MIC: Minimum inhibitory concentration ($mg.mL^{-1}$).
MBC: Minimum bactericidal concentration ($mg.mL^{-1}$).

phenolic compounds as triterpenoids should be also taken into account.

Antibacterial activity

The results showed that all most of bacterial strains were sensitive to the methanol and aqueous extracts (Table 2). MIC and MBC values were different and suggested a selective activity of the extracts. In order to elucidate the antibacterial effect, MBC/MIC ratios were calculated. When the ratio value was lower than 2 the extract exhibited a bactericidal effect. The aqueous extract was bactericidal for *E. coli* ATCC25922, *Salmonella typhimirium* ATCC 14028, *S. sonnei* ATCC 25931, *S. aureus* ATCC 25923, *P. mirabilis* CIP 104588 BHI, *E. faecalis* CIP 103907 BHI, *L. innocua* LMG.

13568 BHI et *B. cereus* LMG 13569 BHI, while the methanol extract was bactericidal for *E. coli* ATCC25922, *S. typhimirium* ATCC 14028, *S. aureus* ATCC 25923, *E. faecalis* CIP 103907 BHI and *B. cereus* LMG 13569 BHI. The most sensitive strain was *E. faecalis* CIP 103907 BHI for aqueous sample. The better MICs were observed with methanol extract and antibacterial activity could be due to presence of chemical compounds such as tannins, polyphenols, saponins and triterpenes which possess antibacterial properties (Bruneton, 1999; Nacoulma, 1996). The results showed that aqueous extract had the best antibacterial effect than methanol extract and its spectrum was very large with gram-positive and gram-negative strains.

Conclusion

Methanolic extract exhibits the strongest radical scavenging activity than aqueous extract and the lowest bactericidal effect. While, the aqueous extract possess a weak antioxidant activity than the one and demonstrates a large spectrum of bactericidal effect. Because of its higher antioxidant activity, the methanolic preparation is more useful than the aqueous one in medical approach, particularly in case when high activity of preparation is desired during anti-cancer therapy or other degenerative diseases. Unfortunately, high concentration polyphenols and phenolic compounds in methanolic solution often cause undesirable gastric disorders. The solution of this problem is found by the traditional healers who used to prescribe decoctions to the public. Furthermore decoctions and the use of *P. reticulatum* barks may help to prevent oxidative damages and infections such as diarrhoea and dysentery in the human body and may contribute to food preservation. These results show that the bark of *P. reticulatum* could be used as a potential natural antioxidant and antibacterial agent. Further investigations will be performed by i) the isolation and identification of pure compounds in the extracts, ii) testing these compounds against pathogenic bacteria and determining their antioxidant activity, and ii) the comparison of the antibacterial activities of extracts with those of polyphenols of reference

REFERENCES

Aderogba MA, Okoh EK, Idowu TO (2005). Evaluation of the antioxidant activity of the secondary metabolites from *Piliostigma reticulatum* (DC) Hochst. J. Bio. Sci. 5(2): 239-242.

Arbonnier M (2002). Arbre, arbustes et lianes des zones sèches d'Afrique de l'Ouest. Muséum d'Histoire Naturelle, 2è édition, CIRAD.

Arvouet-Grand A, Vennat B,Pourrat A, Legret P (1994). Standardisation d'un extrait de propolis et identification des principaux constituants. J. Pharm. Belg. 49 : 462-468.

Bruneton J (1999). Pharmacognosie, phytochimie, plantes médicinales. Paris, 3è édition technique et documentation Lavoisier.

Coulidiati TH, Millogo-Koné H, Lamien-Méda A, Lamien CE, Lompo M, Kiendrébéogo M, Bakasso S,Yougbaré-Ziébrou M, Millogo-Rasolodimby J, Nacoulma OG (2009). Antioxidant and antibacterial activities of *Combretum nioroense* Aubrév.Ex Keay (Combretaceae). 12(3): 264-269.

Ciulei I (1982). Practical manuals on the industrial utilization of chemical and aromatic plants. Methodology for analysis of vegetable drugs. 1st Edn. Ministry of chemical Industry, Bucharest.

Ito N, Fukushima S, Hassegawa A, Shibata M, Ogiso T (1983).

Carcinogenecity of butylate hydroanisole in F 344 rats. J. Natl. cancer. Inst. 70: 343-347.
Karou D, Dicko MH, Simpore J, Traore AS (2005). Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. Afri. J. Biotechnol. 4(8): 823-828.
Majhenic L, Skerget M, Knez Z (2007). Antioxidant and antimicrobial activity of guarana seed extracts. Food Chem. 104(3):1258-1268.
Males Z, Medic-Saric M (2001). Optimisation of TLC analysis of flavonoids and polyphenolic acids of *Helleborus atrorubens* Waldst. Et Kit. J. Pharm. Biomed. Anal. 24: 535-359.
Mendes da Silva JF, De Souza MC, Matta SR, De Andrade MR, Vidal FVN (2006). Correlation analysis between phenolic levels of brazilian propolis extracts and their antimicrobial and antioxidant activities. 99(3):431-435.
Nacoulma OG (1996). Plantes medicinales et pratiques medicinales traditionnelles au Burkina Faso: cas du plateau central T1&T2. Thèse de Doctorat es Sciences Naturelles, Université de Ouagadougou pp: 242-285.
NCCLS-National Committee for Clinical Laboratory Standards (2001). Performance standards for antimicrobial susceptibility testing eleventh informational supplement. Document M100-S11. National Committee for Clinical Laboratory Standards Wayne, PA, USA.
NCCLS-National Committee for Clinical Laboratory Standards (2004). Performance standards for antimicrobial susceptibility testing. Fourteenth informational supplemented. Document M100-S14. Wayne, PA, USA.
Oliveira I, Sousa A, Ferreira ICFR, Bento A, Estevinho L, Pereira JA (2008). Total phenols, antioxidant potential and antimicrobial activity of Walnut (*Jugians regia* L.) green husks. Food chem. toxicol. 46: 2326-2331.
Pereira JA, Oliveira I, Sousa A, Valentao P, Andrade PB, Ferreira ICFR, Ferreres F, Bento A, Seabra R, Estevinho L (2007). Walnut (*Juglans regia* L) leaves: phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. Food Chem. Toxicol. 45(11): 2287-2295.
Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and oxidization substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 152-177.
Velazquez E, Yournier HA, Mordujovitchole BP, Saavedra G, Scinella GR (2003). Antioxidant activity of Paraguyan plant extracts. Fitoterapia. 74: 91-97.
Yelemou B, Bationo BA, Yameogo G, Millogo-Rasolodimby J (2007). Gestion tradition nelle et usage de *Piliostigma reticulatum* sur le plateau central du Burkina Faso. Bois et forêts des tropiques, n° 291, Paris, édition Lavoisier.
Wagner H, Bladt S (1996). Plant drug analysis: A thin layer chromatography. Atlas, 2è edition Springer p. 384.