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Full Length Research Paper

Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso

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Polyphenols from four medicinal plants of Burkina Faso, *Combretum micranthum, Khaya senegalensis, Pterocarpus erinaceus* and *Sida acuta*, were screened for their antioxidant and antimicrobial activities against pathogenic bacteria. The medicinal plants displayed different polyphenols contents and antioxidant activities. The bark of *P. erinaceus* had the highest antioxidant activity. Some microorganisms were susceptible to polyphenol extracts with minimal bactericidal concentration values between 20 and 2000 µg/ml while other microorganisms appeared to be resistant to the extracts. Microbicide and microbiostatic activities of the extracts were dependent on the type of strains. Results suggest that these plants are not only interesting sources for antimicrobial activities but also potential sources of phenolic antioxidants.

Key words: Antioxidants, polyphenols, antimicrobials, medicinal plants.

INTRODUCTION

The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants (Newman et al., 2000). However, plants used in traditional medicine are still understudied, particularly in clinical microbiology (Kirby, 1996). It is reported that most antibiotics derived from microorganisms, and one to three antibiotics are launched every year (Clark, 1996). In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethnomedicinal plants may still be needed. It is obvious that these phytochemicals will find their way in the arsenal of antimicrobial drugs prescribed by physicians (Cowan, 1999). Any antibiotic has a limited effective life and the public is becoming increasingly aware of problems with the over prescription of these antibiotics. In addition, many people, principally in the developed countries are interested in having more autonomy over their medical care, so self-medication is commonplace (Eisenberg et al., 1993).

In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population use medicinal plants as remedies (Kirby, 1996; Hostettmann and Marston, 2002). In our previous screening for antimalarial compounds in some medicinal plants from Burkina Faso, we found that antimalarial activities were due to alkaloid compounds (Karou et al.,

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Abbreviations: ABTS, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 1,1-diphenyl-2-picrylhydrazyl; ORAC, oxygen radical absorbance capacity; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; Cbm, *Combretum micranthum*; Ksb, *Khaya senegalensis* bark; Ksl, *Khaya senegalensis* leaves; Peb, *Pterocarpus erinaceus* bark; PMA, phosphomolybdenum assay; Pel, *Pterocarpus erinaceus* leaves; Sac, *Sida acuta*.

2003, Sanon et al., 2003a,b). Therefore, it is a new challenge to seek for the *in vitro* antimicrobial activity of natural compounds such as polyphenols from these ethnomedicinal plants on pathogenic bacteria. Polyphenols are a group of highly hydroxylated phenolic compounds present in the extractive fraction of several plant materials. Polyphenols in plants include hydroxycoumarins, hydroxycinnamate derivatives, flavanols, flavonols, flavanones, flavones, anthocyanins, proanthocyanidins (tannins), hydroxystilbenes, aurones, etc. Polyphenols are well documented to have microbicide activities against a huge number of pathogenic bacteria (Scalbert, 1991; Cowan, 1999). Oxidized polyphenols also have inhibitory activity against bacterial growth (Field and Lettinga, 1992; Cowan, 1999). The mechanism of polyphenols toxicity against microbes may be related to inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesins, cell envelope transport proteins, non specific interactions with carbohydrates, etc (Cowan, 1999).

The evaluation of the antioxidant activities of polyphenols from ethnomedicinal plants may also be necessary because they are among desired medicinal properties of plants due to their nutraceutical effects (Zhu et al., 2004). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides. Antioxidant activities of polyphenols have been suggested to exert beneficial pharmacological effects on neurological disorders on the basis of *in vitro* observations (Moosmann et al., 1999; Parr and Bolwell, 2000). Polyphenolic compounds in plant, including the catechins, exert anticarcinogenic, antimutagenic and cardioprotective effects linked to their free radical scavenging (Parr and Bolwell, 2000; Santos-Buelga and Scalbert, 2000). They are reported to be chemopreventive agents by lowering cholesterol and roughly limit cell damage (Ferriera and Slade, 2002). In addition to their individual effects, antioxidants interact in synergistic ways and have sparing effect in which one may protect another against oxidative destruction. These justify the overwhelming interest in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Parr and Bolwell, 2000).

METHODS

Plant materials

Plants were chosen following leads supplied by local healers. Indeed a survey made with the healers revealed that the plants were used to treat either diarrhea or malaria and many other diseases. The following plant materials were used:

- Leaves of *Combretum micranthum* G. Don (Combretaceae).
- Leaves and bark of *Khaya senegalensis* (Desr) *A. Juss* (Meliaceae).
- Leaves and bark of *Pterocarpus erinaceus* Poir (Fabaceae).
- Whole plant of *Sida acuta Burm.f* (Malvaceae).

All samples were harvested in the area of Ouagadougou (Burkina Faso) between July and August 2002. They were botanically authenticated at the department of Plant Biology and Ecology UFR/SVT of the University of Ouagadougou. Voucher specimens were deposited at Laboratoire de Pharmacologie et de Biochimie clinique: CRSBAN. Their code numbers are: Bc-cm01, Bc-ks01, Bc-pe01 and Bc-sa01 for *C. micranthum*, *K. senegalensis*, *P. erinaceus* and *S. acuta,* respectively.

Reagents

2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid, were obtained from Sigma. Ascorbic acid was from Merck. Ammonium molybdate was from Fisher. Potassium persulfate was from Labosi. All the solvents were of analytical grade.

Polyphenols extraction

The harvested plant materials were dried in the laboratory at room temperature (20-25°C), afterwards samples were ground to pass a sieve of 0.3 mm. Polyphenols were extracted with aqueous acetone (70%, v/v) essentially as described by Yu and Dahlgren (2000). The extracts were then washed with hexane to remove chlorophyll and other low molecular weight compounds. Acetone was evaporated and the extracts were lyophilized and stored at –22°C prior to biological tests. For the biological tests, lyophilized samples were dissolved in water at the desired concentration. This is referred to us as freshly prepared extracts.

Determination of total phenolic compounds

Total phenolic compounds from lyophilized samples were quantified using Folin-Ciocalteu's method (Singleton et al., 1999) adapted to a 96 well-plate (Dicko et al., 2002). 25 µl of Folin-Ciocalteu's reagent (50%, v/v) was added to 10 µl of 1 mg/ml (w/v) of lyophilized plant extract dissolved in water. After 5 min incubation at room temperature, 25 µl of 20 % (w/v) sodium carbonate and water were added to a final volume of 200 µl per well. Blanks were prepared by replacing the reagent by water to correct for interfering compounds. After 30 min of incubation, the absorbencies were read at 760 nm using a multiwell plate reader (*µQuant* Bio-Tek Instrument, Inc) on line interfaced with a computer. Raw absorbencies were automatically recorded using KC junior software version 1.31.5 (Bio-Tek instrument, Inc., USA). All the assays were carried out at least in duplicate. Gallic acid was used as standard and results were expressed as gallic acid equivalent per gram of lyophilized sample.

Determination of antioxidant activity

The lyophilized samples were dissolved in water to obtain a concentration of 100 µg/ml (w/v). The 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay (Re et al., 1999) was performed by incubation of 250 µL of chemically generated

$ABTS^{\bullet+}$ radical in potassium persulfate (Re et al., 1999) with 10 µL of total phenolic extract. The $ABTS^{\bullet+}$ radical concentration was determined using a molar extinction coefficient of 12867 $M^{-1}cm^{-1}$ at 734 nm. At that wavelength, a linear absorbance as a function of the $ABTS^{\bullet+}$ radical concentration was obtained up to an absorbance of two. The decrease in absorbance at 734 nm was monitored at 1 min intervals for 30 min. The absorbances of control and sample were monitored simultaneously in a 96-well plate. The amount of $ABTS^{\bullet+}$ radical scavenged was calculated from the difference in absorbance between the control and the total phenolic extract containing sample, using the extinction coefficient (ε_{734}= 12 867 M^{-1} cm^{-1}) of the radical (Re et al., 1999). The phosphomolybdenum assay (Prieto et al., 1998) was performed by incubating 50 µL of total phenolic extract with 500 µL of the reagent (0.6 M sulfuric acid, 28 mM Na_2HPO_4 and 4 mM $(NH_4)_6Mo_7O_{24}.4H_2O$ in an 1.5 mL Eppendorf tube at 95°C, for 90 min. The blank was prepared by replacing the total phenolic extract with the acidified methanol. After cooling, 200 µL of the formed green phosphate/Mo^{V} complex was put in a microtiter plate, and the absorbances were read at the maximum absorption (820 nm). Calibration curve was made using Trolox as standard. Trolox antioxidant equivalents were expressed on weight basis (µmol/g of lyophilized extract, dry matter).

Bacterial strains

Microorganisms used in this study included reference strains and fresh clinical strains isolated from fecal specimens at Centre Hospitalier National Yalgado Ouédraogo of Ouagadougou. The following microorganisms, all identified by the conventional methods were tested: *Shigella dysenteriae, Shigella boydii, Shigella flexneri, Salmonella thyphi, Salmonella parathyphi* B, *Salmonella parathyphi* C, *Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella ozenae, Escherichia coli and Staphylococcus aureus*. Three reference strains were tested: *Shigella dysenteriae* CIP 504051, *Escherichia coli* CIP 105182 and *Staphylococcus aureus* ATCC 25923.

Susceptibility tests

The susceptibility tests were performed by the Mueller Hinton agar-well diffusion method (Perez et al., 1990). 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 Mac Farland standards (10^8 cfu/ml). The suspension was used to inoculate 90 mm diameter Petri dishes with a sterile non-toxic cotton swab on a wooden applicator. Wells (6 mm diameter) were punched in the agar and filled with 50 µl of 5000 µg/ml extract. Plates were incubated in air at 37°C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters. After each test, the extracts were stored in an open air at 4°C. This is referred to us as stored extracts.

Determination of MIC and MBC values

MICs and MBCs were determined using the Mueller Hinton broth microdilution in 96 well-plates according to National Committee for Clinical Laboratory (2000). The same 0.5 Mac Farland suspensions were diluted with Mueller Hinton broth to inoculate 96 well-plates containing 2-fold serial dilutions of extracts. Drug concentrations ranged from 10 to 2000 µg/ml. The final volume in wells was 200 µl. The final inocula as determined by colony counts from the growth control wells were approximately 10^5cfu per well. Plates were incubated in air 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.

RESULTS AND DISCUSSION

The total phenolic compound contents in the plant extracts are shown in Table 1. It appeared that the bark of *K. senegalensis* had the highest content of phenolic compounds and *S. acuta* had the lowest content.

Free radical scavenging of phenolic compounds is an important property underlying their various biological and pharmacological activities. The phosphomolybdenum assay is based on the reduction of Mo^{VI} to Mo^{V} by antioxidant compounds and a formation of a green phosphate/Mo^{V} complex with a maximal absorption at 820 nm. The assay has been successful in the quantification of vitamin E antioxidant activity (Prieto et al., 1999) and it was efficient to extend its application to plants polyphenols (Lu and Foo, 2001).

The decolorization of $ABTS^{\bullet+}$ cation radical is an unambiguous way to measure the antioxidant activity of phenolic compounds. Recently, Awika et al. (2003) found positive correlations between the determination of phenolic antioxidant using the oxygen radical absorbance capacity (ORAC), ABTS and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. Thus monitoring the antioxidant activity of phenolic compounds by their ability to scavenge $ABTS^{\bullet+}$ radical was demonstrated to give good prediction of their ORAC. Results revealed that polyphenols from bark of *K. senegalensis, C. micranthum* and *P. erinaceus* had high antioxidant activities (Table 1). The total phenolic compounds contents were also highly correlated with the antioxidant activities ($r=0.91$ and $r=0.94$ with the $ABTS^{\bullet+}$ decolorization and the phosphomolybdenum method, respectively). A good correlation ($r>0.9$) was found between polyphenol content and antioxidant activities in all screened plants showing that antioxidants activities present in the extracts were essentially due to polyphenols.

C. micranthum, K. senegalensis, P. erinaceus and *S. acuta* are ethnomedicinal plants widely used in West Africa to treat many diseases. A previous study conducted on these plants revealed that *S. acuta* has a good antimalarial activity related to its alkaloid contents (Karou et al., 2003). As the same plants are used to treat diarrhea in the same area, we were prompted to investigate for their antimicrobial activity on pathogenic bacteria that are responsible for the major cases of diarrhea in this region. It was reported that most of children gastro-intestinal infections in Burkina Faso were due to *Enterobacteriaceae*, principally *E. coli, Salmonella* spp and *Shigella* spp (Bonfiglio et al., 2002).

Table 1. Polyphenols content and antioxidant activities of medicinal plants from Burkina Faso.

Plant extracts	Polyphenols in the lyophilized extract (%)[a]	Antioxidant activities	
		PMA[b] (µmol Trolox/µg)	ABTS assay[c] (µmol Trolox/µg)
Leaves of *C. micranthum*	37.80 ± 1.04	2.08 ± 0.02	16.37 ± 0.01
Bark of *K. senegalensis*	47.19 ± 0.13	2.21 ± 0.04	21.97 ± 0.46
Leaves of *K. senegalensis*	34.91 ± 1.88	1.50 ± 0.03	15.47 ± 2.65
Bark of *P. erinaceus*	40.80 ± 1.75	1.89 ± 0.08	22.20 ± 0.29
Leaves of *P. erinaceus*	28.42 ± 0.75	1.88 ± 0.03	8.08 ± 0.06
Whole plant of *S. acuta*	10.11 ± 0.29	1.20 ± 0.04	6.12 ± 1.18

[a]Gallic acid equivalents.
[b]Antioxidant activities monitored with the phosphomolybdenum assay.
[c]Antioxidant activities monitored by scavenging $ABTS^{\bullet+}$ cation radicals.

Table 2. Antimicrobial activities expressed as inhibition zone diameters (mm).

Microorganisms	Cbm		Ksb		Ksl		Peb		Pel		Sac	
	t_0	t_{21}	t_0	t_{21}	t_0	t_{21}	t_0	t_{21}	t_0	t_{21}	t_0	t_{21}
S. dysenteriae	25	21	13	15	10	-	-	-	-	-	15	25
S. dysenteriae CIP 504051	-	-	-	-	-	-	-	-	-	-	-	-
S. flexneri	20	14	15	11	12	11	-	-	-	-	-	-
S. boydii	17	-	-	-	-	-	-	-	-	-	-	-
S. thyphi	15	13	11	-	-	-	-	-	-	-	-	-
S. parathyphi B	12	29	11	22	-	16	-	22	-	12	-	16
S. parathyphi C	12	11	-	-	-	-	-	-	-	-	-	-
K. pneumoniae	15	-	11	-	-	-	-	-	-	-	-	-
K. ozenae	20	12	-	15	-	15	-	11	-	-	-	18
K .oxytoca	-	-	-	-	-	-	-	-	-	-	-	-
S. aureus	15	22	12	18	-	18	-	-	-	-	-	15
S. aureus ATCC 25923	12	15	12	16	-	15	-	12	-	-	-	-
E. coli	12	-	-	-	-	-	-	-	-	-	-	-
E. coli CIP 105182	-	-	-	-	-	-	-	-	-	-	-	-

t_0: Freshly prepared extracts at time zero.
t_{21}: Stored extracts for 21 days.
(-): Resistant strains.

The results of susceptibility tests showed that freshly prepared extracts displayed different antimicrobial activities compared to stored extracts (Table 2). From the time zero of polyphenols extraction, tests were made twice a week. The results showed that after three weeks, the inhibition zone diameters did not vary considerably. Table 2 shows that *S. dysenteriae* CIP 504051, *K. oxytoca* and *E. coli* CIP 105182 were not inhibited by any extract at 5000 µg/ml. In addition, the freshly prepared extract of leaves of *P. erinaceus* failed to inhibit all the tested microorganisms. These results also revealed that *S. parathyphi* B, *S. aureus* and *S. aureus* ATCC 25923 were susceptible to stored extracts, while *S. flexneri* was susceptible to freshly prepared extracts. In the other hand, *S. dysenteriae* and *K. ozenae* were susceptible to both extracts. However, Arias et al. (2003) did not find any difference in antibacterial activities of stored and freshly prepared extracts of *Acacia aroma*.

Since the activities were quite dynamic, MIC and MBC were determined in extracts optimal activity conditions. Table 3 provides MBC and MIC values. Results showed different values of MBCs and MICs, suggesting a

Table 3. Bactericidal activities expressed as MBCs[a] and MICs[b].

Microorganisms	Cbm		Ksb		Ksl		Peb		Pel		Sac	
	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
S. dysenteriae	600	600	-	1000	1000	1000	-	-	-	-	20	20
S. dysenteriae CIP 504051	-[c]	-	-	-	-	-	-	-	-	-	-	-
S. flexneri	-	1000	-	-	-	-	-	-	-	-	-	-
S. boydii	-	1000	-	-	-	-	-	-	-	-	-	-
S. thyphi	-	1000	-	-	-	-	-	-	-	-	-	-
S. parathyphi B	80	80	80	80	1200	400	400	80	-	-	2000	2000
S. parathyphi C	-	-	-	-	-	-	-	-	-	-	-	-
K. pneumoniae	-	1000	-	-	-	-	-	-	-	-	-	-
K. ozenae	1000	1000	-	-	-	1000	-	-	-	-	-	1000
K .oxytoca	-	-	-	-	-	-	-	-	-	-	-	-
S. aureus	50	20	600	600	240	240	-	-	-	-	2000	2000
S. aureus ATCC 25923	-	400	600	600	1200	1200	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	-	-	-	-
E. coli CIP 105182	-	-	-	-	-	-	-	-	-	-	-	-

[a]Minimal bactericidal concentration (µg/ml of extract).
[b]Minimal inhibitory concentration (µg/ml of extract).
[c](-) Values greater than 2000µg/ml.

Table 4. Summary of the microbicide and microbiostatitic activities of plants polyphenolic extracts on specific strains.

Plant material	Microbicide activity on specific strains	Microbiostatic activity on specific strains
Leaves of *C. micranthum*	*S. dysenteriae, S., parathyphi B, K. ozenae*	*S. flexneri, S. boydii, S. thyphi, K. pneumoniae, S. aureus, S. aureus ATCC 25923*
Bark of *K. senegalensis*	*S. parathyphi B, S. aureus, S. aureus ATCC 25923*	*S. dysenteriae*
Leaves of *K. senegalensis*	*S. dysenteriae, S. aureus and S. aureus ATCC 25923*	*S. parathyphi B, K. ozenae*
Bark of *P. erinaceus*	nd*	*S. parathyphi B*
Leaves of *P. erinaceus*	nd	nd
Whole plant of *Sida acuta*	*S. dysenteriae, S., parathyphi B, S. aureus*	*K. ozenae*

*No detected antibacterial activity.

selective activity of the extracts. In order to elucidate whether the observed antimicrobial effects were microbicide or microbiostatic, MBC/MIC ratios were calculated. Extracts with ratios greater than 1 were considered as microbiostatic, while the other extracts are microbicide. Results on antibacterial activities of the extracts are illustrated in Table 3. From these data, it can concluded that some extracts have either microbicide or microbiostatic activities on specific strains (Table 4).

Polyphenols have been reported to exhibit antibacterial activities with distinguished characteristics in their reactivity with proteins related polyamides polymers (Haslam, 1996). The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bounding with vital proteins such as microbial enzymes (Scalbert, 1991). Phenolic compounds notably proanthocyanidins (often called condensed tannins) are vulnerable to polymerization in air through oxidization reactions. Therefore, an important factor governing their toxicity is their polymerization size. Oxidized condensation of phenols may result in the toxification of microorganisms. On the other hand, polymerization can result in the detoxification of phenols (Scalbert, 1991; Fiel and Lettinga, 1992). These supports the fact polyphenols may be responsible for the antimicrobial activities of the extracts of the screened plants.

Freshly prepared active extracts may be more beneficent in the case of traditional uses, since most of plants are traditionally used as decoction and this is often freshly prepared and is not stored. Sometimes, plants materials, principally the barks are ground and the powders are stored to be dissolved in water or in local beer “dolo.” In this way, this study shows that oxidization reactions may influence their pharmacological activities.

Results from this investigation show the rationale behind the use of these plants in traditional medicine. These plants are not only interesting sources for antimicrobial activities but also potential sources of phenolic antioxidants.

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