

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Antioxidant and Antibacterial Activities of *Combretum niroense* Aubrév. Ex Keay (Combretaceae)

<sup>1</sup>T.H. Couliadiati, <sup>2</sup>H. Millogo-Koné, <sup>3</sup>A. Lamien-Méda, <sup>4</sup>C.E. Lamien, <sup>2</sup>M. Lompo, <sup>1</sup>M. Kiendrébéogo,  
<sup>1</sup>S. Bakasso, <sup>2</sup>M. Yougbaré-Ziébrou, <sup>5</sup>J. Millogo-Rasolodimby and <sup>1</sup>O.G. Nacoulma

<sup>1</sup>Laboratoire de Biochimie et Chimie Appliquées, UFR/SVT, Université de Ouagadougou,  
09 BP 848 Ouagadougou 09, Burkina Faso

<sup>2</sup>Institut de Recherche en Sciences de la Santé,  
Centre National de la Recherche Scientifique et Technologique, 03 B.P. 7192,  
Ouagadougou 03, Burkina Faso

<sup>3</sup>Institut for Applied Botany and Pharmacognosy, University of Veterinary Medicine Vienna,  
Veterinärplatz 1, A-1210 Vienna, Austria

<sup>4</sup>Animal Production Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, IAEA, Vienna, Austria

<sup>5</sup>Laboratoire de Biologie et d'Ecologie Végétales, UFR/SVT,  
Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso

**Abstract:** In this study, the antioxidant and antibacterial activities of acetone extract, ethyl acetate, n-butanol and n-hexane fractions of acetone extract from leaves of *Combretum niroense* Aubrév. ex Keay were investigated. The total phenolics and total flavonoids contents in the fractions and acetone extract were determined by spectrophotometric methods using Folin-Ciocalteu and AlCl<sub>3</sub>, respectively. Two methods were adopted to assess the antioxidant activities: the Ferric Reducing Antioxidant Power (FRAP) and the radical scavenging activity of 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS). The Minimum Inhibitory Concentrations (MICs) of the extract and fractions against pathogenic bacteria (4) and serotyped bacteria (4) from American Type Culture Collection (ATCC) were also determined using the agar-well diffusion method. The results showed that the butanol fraction, with the highest phenolic content, exhibited the best antioxidant and antibacterial activities as compared to the ethyl acetate fraction which contains more flavonoids.

**Key words:** *Combretum niroense*, total phenolics, total flavonoids, antioxidant, antibacterial

### INTRODUCTION

Infectious diseases caused by bacteria, viruses, fungi and other parasites are major causes of death in humans in spite of the enormous progress recorded by the modern medicine. The World Health Organization (2002) and UNAIDS (2007) reported that between 14 and 17 million people die each year due to infectious diseases. In developing countries, infectious diseases cause 43% mortality as against 1% in developed countries. In traditional medicine, *Combretum* species are used for many medicinal purposes such as diarrhea, pneumonia, gonorrhoea, syphilis, malaria, hypertension and even cancer (Nacoulma, 1996; Fyhrquist *et al.*, 2002).

Combretaceae is a large family with at least 475 species in the world, 244 species in Tropical Africa and 20 species in Burkina Faso (Thiombiano, 2005). Some

species of the Combretaceae family have been investigated and have revealed to possess a large number of bioactive compounds and to exert strong antioxidant and antimicrobial activities. Among those plants are *Combretum micranthum*, *Combretum erytrophillum*, *Terminalia catappa* (Martini and Eloff, 1998; Karou *et al.*, 2005; Kloucek *et al.*, 2005). *Combretum niroense*, also a species of this family, is less known; literature relates very few about its chemical structures and biological activities. In Burkina Faso, *Combretum niroense* is localized in the eastern part of the country. It is used to fight against many diseases associated with microorganisms and the decoction of leaflets is used as drink for new born babies (Thiombiano, 2005).

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents

(Salvat *et al.*, 2001; Ordoñez *et al.*, 2003; Arias *et al.*, 2004). Among the biological potential of medicinal plants, the antioxidant activity has gained an increase interest these last years because of the role they play in the prevention of chronic ailments such as heart disease, cancer, diabetes, hypertension, stroke and Alzheimer's disease by combating oxidative stress (Liu, 2003; Riboli and Norat, 2003; Cole *et al.*, 2005).

The aim of the present study was to evaluate the antioxidant and the antibacterial activities of acetone extract, n-hexane, ethyl acetate and n-butanol fractions of acetone extract of the leaves of *Combretum niroense*.

## MATERIALS AND METHODS

**Chemicals:** All reagents were of analytical grade: Folin Ciocalteu-reagent,  $\text{NaH}_2\text{PO}_4$ ,  $\text{Na}_2\text{HPO}_4$ , sodium carbonate, aluminium trichloride ( $\text{AlCl}_3$ ), gallic acid and quercetin were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonate) ABTS; trichloroacetic acid, potassium persulfate, acetone, methanol, n-hexane, ethyl acetate and n-butanol were supplied by Fluka Chemie (Buchs, Switzerland). Potassium hexacyanoferrate [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] was from Prolabo (Paris, France); ascorbic acid and iron trichloride were supplied by Labosi (Paris, France).

**Plant material:** The leaves of *Combretum niroense* aubrev ex keay were collected in the Namounou region of Burkina Faso in June 2008 and identified by Prof. Millogo-Rasolodimby, a botanist from the University of Ouagadougou. Voucher specimen (CRV 01) was deposited in the Herbarium of Laboratoire de Biologie et d'Ecologie végétales UFR/SVT, University of Ouagadougou.

**Preparation of plant extracts:** Air-dried and powdered leaves (50 g) of *Combretum niroense* were extracted by maceration at room temperature with 500 mL of acetone, during 48 h. The extract was subsequently filtered and concentrated to dryness at 40°C under vacuum. Extract yield with respect to the dried plant was 6.5%. The dry residue (270 mg) was subjected to successive liquid-liquid fractionation (Eloff, 1998). Three fractions were obtained: the n-hexane fraction, the ethyl acetate fraction, the butanol fraction. Solvents have been eliminated and the different residues obtained were used for different biological activities.

**Phytochemical screening:** The phytochemical screening was conducted with the acetone extract for alkaloids,

tannins, flavonoids, saponins, triterpenoids, steroids and coumarins using the method described by Ciulei (1982).

### Determination of total phenolics and total flavonoids

**contents:** The total phenolics of plant extract were determined by the Folin-Ciocalteu method (Lamien-Meda *et al.*, 2008). The diluted aqueous solution of each extract (0.5 mL) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 mL). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution ( $75 \text{ g L}^{-1}$  in water, 2 mL) was added. After 2 h incubation, the absorbencies were measured at 760 nm against water blank. A standard calibration curve was plotted using gallic acid ( $0-200 \text{ mg L}^{-1}$ ). The results were expressed as mg of gallic acid equivalents GAE  $\text{g}^{-1}$  of extract or fractions.

The total flavonoids were estimated according to the Dowd method as adapted by Lamien-Meda *et al.* (2008). A diluted methanolic solution (2 mL) of each extract was mixed with 2 mL of aluminium trichloride ( $\text{AlCl}_3$ ) in methanol (2 %). The absorbance was read at 415 nm after 10 min against a blank consisting of 2 mL of methanol and 2 mL of plant extract (without  $\text{AlCl}_3$ ). Quercetin was used as reference to produce the standard curve and the results were expressed as mg of quercetin equivalents (QE)  $\text{g}^{-1}$  of extract or fractions.

### Antioxidant activity

**Iron (III) to iron (II) reduction activity (FRAP):** The total antioxidant capacity of the plant extract was determined using the iron (III) reduction method (Hinneburg *et al.*, 2006). One milliliter of the diluted aqueous solution of plant extract, at a concentration of  $100 \mu\text{g mL}^{-1}$ , was mixed with phosphate buffer (0.2 M, pH 6.6, 2.5 mL) and 1% aqueous potassium hexacyanoferrate [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] solution (2.5 mL). After 30 min incubation at 50°C, 2.5 mL of a trichloroacetic acid 10 %, was added and the mixture was centrifuged at 3000 rpm for 10 min. Then, the upper layer solution (2.5 mL) was mixed with water (2.5 mL) and aqueous  $\text{FeCl}_3$  (0.1 %) solution (0.5 mL). The absorbance was read at 700 nm and ascorbic acid was used to produce the calibration curve. The iron (III) reducing activity determination was expressed in mmol ascorbic acid equivalents per gram of extract or fractions.

**ABTS radical cation decolorization assay:** The radical scavenging capacity of antioxidants for the ABTS (2, 2'-azinobis-3-ethyl-benzothiazoline-6-sulphonate) radical cation was determined as described by Lamien-Meda *et al.* (2008).  $\text{ABTS}^{\bullet+}$  was generated by mixing a 7 mM aqueous solution of ABTS with 2.5 mM potassium persulfate (final concentration) followed by

storage in the dark at room temperature for 12 h before use. The mixture was diluted with ethanol to give an absorbance of  $0.70 \pm 0.02$  units at 734 nm using spectrophotometer.

The diluted methanol solution of the extract (10  $\mu$ L) was allowed to react with fresh ABTS<sup>+</sup> solution (990  $\mu$ L) and then the absorbance was measured 6 min after initial mixing. Ascorbic acid was used as a standard and the capacity of free radical scavenging was expressed as mmol ascorbic acid equivalents  $g^{-1}$  of extract or fractions. Quercetin and gallic acid were used as positive controls.

### Antibacterial study

**Microorganisms:** The microorganisms used in this study consisted of clinical isolates and collection/serotyped strains. The clinical isolates were obtained from biomedical laboratories. They were: *Escherichia coli*, *Salmonella* sp., *Klebsiella pneumoniae*, *Staphylococcus aureus*. The following serotyped strains used in this study are: *Escherichia coli* ATCC 25922; *Salmonella typhimurium* ATCC 13311; *Staphylococcus aureus* ATCC 6538 and *Proteus mirabilis* ATCC 35659. Before testing, pure cultures were realized with all the 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 Mc Ferland standard.

**Antibacterial tests:** The agar-well diffusion method (Ojala *et al.*, 2000) was used to evaluate the antibacterial activity. Minimum Inhibitory Concentrations (MICs) of the extract and fractions of *Combretum niroense* were determined using the agar-well diffusion method. All the extract and fractions were diluted in Dimethyl Sulfoxide (DMSO) 25% to obtain series of concentrations of 20, 10, 5, 2.5, 1.25 and 0.625  $mg mL^{-1}$ . The MIC was taken as the lowest concentration of extract or fractions that caused a clear to semi-clear inhibition zone around the hole after 24 h incubation at 37°C.

Sterile petri dishes (d = 10 cm, Bibby Sterilin, UK) were prepared with a base layer of Müller-Hinton agar (Difco). Bacteria at density of  $10^6$ - $10^8$  cfu were inoculated on solid agar. Holes (6 mm) were made in the agar with a sterile cork borer and filled with 50  $\mu$ L of different dilutions of the extract and fractions. Petri dishes were incubated at 37°C for 24 h. The diameters of the circular inhibition zones obtained were measured. Commercial antibiotic discs of Gentamicin and Amoxicillin were used as positive controls. DMSO 25% was used as a negative control.

**Statistical analysis:** Data are expressed as  $\bar{X} \pm SE$  (n = 5). Significant differences are determined by the Newman-Keuls Test Pharmacological Calculation System.

## RESULTS AND DISCUSSION

The phytochemical screening revealed the presence of flavonoids, sterols/triterpens and tannins in the acetone extract of the leaves. The presence of these metabolites in some *Combretum* species has already been reported by Nacoulma (1996), Baba-Moussa *et al.* (1999), Simon *et al.* (2003) and Batawila *et al.* (2005).

*Combretum niroense* leaves extracted with acetone yielded 6.5%. Three solvents of different polarities: n-hexane, ethyl acetate and n-butanol were used to extract phenolic compounds from the acetone extract. Yields of fractions are shown in Table 1.

Ethanol, methanol and acetone are the commonly used solvents to extract chemical compounds of the plant. Sun and Ho (2005) reported that acetone extracts more phenolic compounds than methanol and ethanol.

The total phenolics and total flavonoids contents in the acetone extract and the different fractions are consigned in Table 1. Table 1 shown that n-butanol extracted more phenolic compounds (375  $mg GAE g^{-1}$ ) than any other solvent. Table 1 also showed that the highest amount of flavonoids is found in the ethyl acetate fraction (239  $mg QE g^{-1}$ ). n-hexane, a much less polar solvent, contains the lowest quantity of phenolic and flavonoid compounds. The results showed that the acetone extract of leaves of *Combretum niroense* contained  $204.2 \pm 16.1$   $mg GAE g^{-1}$  of extract (i.e., 20.42% of the total extract) of phenolic compounds as against  $37.80 \pm 1.04\%$  in the leaves of *Combretum micranthum* (Karou *et al.*, 2005). These results showed that *Combretum micranthum* contains more phenolic compounds than *Combretum niroense*. However many research works reported that the contents in metabolites, phenolic and flavonoid compounds as well, are related to the nature of the soil and the microclimate (Millogo-Kone, 2008). The leaves of *Combretum niroense* and those of *Combretum micranthum* were harvested almost at the same period of the year (June-August) but on different areas. Kana *et al.* (2005) demonstrated that flavonoid contents increased in October to December, with the tendency to decrease in May to June. Chaves *et al.* (1997) demonstrated that UV irradiation was the major inducer of

Table 1: Total phenolics and total flavonoids in extract/fractions of *Combretum niroense*

Extract fractions	Yield (%)	Total phenolic content (mg GAE $g^{-1}$ of extract or fractions)	Total flavonoids content (mg QE $g^{-1}$ of extract or fractions)
AE	6.50	$204.2 \pm 16.1^c$	$106.3 \pm 4.8^c$
EF	29.63	$251.5 \pm 9.2^b$	$239.4 \pm 16.6^b$
BF	37.04	$375.8 \pm 13.8^a$	$182.5 \pm 8.9^a$
HF	31.48	$70.0 \pm 6.5^d$	$50.1 \pm 4.6^d$

AE: Acetone extract, EF: Ethyl acetate fraction, BF: Butanol fraction, HF: Hexane fraction. Different letters in the same column indicate significant difference ( $p < 0.01$ )

the enhanced flavonoid secretion in the exudate. These results underline the ecophysiological role of the flavonoids in the protection of the plant against the damaging effects of UV irradiation.

Antioxidant activities of acetone extract and the various fractions from acetone extract were determined by using two test systems: the iron (III) to iron (II) reduction method (FRAP) and the ABTS method which consists in measuring the relative antioxidant ability of extract and fractions to scavenge the radical cation ABTS<sup>+</sup> produced by the oxidation of 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonate).

Statistical studies revealed that there is no significant difference between the results obtained with butanol fraction following the two methods (Table 2). Also, one can notice that with both methods (FRAP and ABTS), the highest antioxidant activity was obtained with the butanol fraction (1.86±0.13 mmol g<sup>-1</sup> through FRAP method and 1.80±0.41 mmol g<sup>-1</sup> for ABTS method) followed by the acetone extract (1.58±0.02 and 0.96±0.28 mmol g<sup>-1</sup>, respectively for FRAP and ABTS methods), the ethyl acetate fraction (1.04±0.02 and 0.71±0.11 mmol g<sup>-1</sup>, respectively for FRAP and ABTS methods) and the n-hexane fraction (0.43±0.02 and 0.24±0.08 mmol g<sup>-1</sup>, respectively for FRAP and ABTS methods). Recent investigations have shown differences between the test systems for the determination of antioxidant activity (Schlesier *et al.*, 2002; Nsimba *et al.*, 2008). It was therefore recommended to use at least two methods, what we did.

Table 2: Antioxidant activity of extract or fractions obtained from *Combretum niroense*

Extract/fractions and references compounds	FRAP (mmol ascorbic acid equivalents g <sup>-1</sup> )	ABTS (mmol ascorbic acid equivalents g <sup>-1</sup> )
AE	1.58±0.02 <sup>b</sup>	0.96±0.28 <sup>b</sup>
EF	1.04±0.02 <sup>c</sup>	0.71±0.11 <sup>b</sup>
BF	1.86±0.13 <sup>a</sup>	1.80±0.41 <sup>a</sup>
HF	0.43±0.02 <sup>d</sup>	0.24±0.08 <sup>c</sup>
Gallic acid	-	13.4±0.11
Quercetin	-	7.81±0.21

AE: Acetone extract, EF: Ethyl acetate fraction, BF: Butanol fraction, HF: Hexane fraction. ABTS: 2, 2'-azinobis-3-ethyl-benzothiazoline-6-sulphonate, FRAP: Iron (III) to iron (II) reduction activity. Different letters in the same column indicate significant difference (p<0.01)

Table 3: Antibacterial effects of *Combretum niroense* extract or fractions at 20 mg mL<sup>-1</sup>

Strains	AE	EF	BF	HF	Gentamicin	Amoxicillin
<i>E. coli</i>	8.0±0.0	R	10.5±0.5	R	21	16
<i>S. aureus</i>	12.0±0.0	12.5±0.5	12.0±0.0	9.0±0.0	R	R
<i>K. pneumoniae</i>	12.0±0.0	10.0±0.0	13.5±0.5	10.0±0.0	-	-
<i>Salmonella</i> sp.	10.0±0.0	12.0±0.0	12.5±0.5	9.5±0.5	-	-
<i>E. coli</i> ATCC 25922	10.5±0.5	10.0±0.0	13.0±0.0	10.5±0.5	21	16
<i>S. typhimurium</i> ATCC 13311	13.5±0.5	11.5±0.5	15.0±0.0	10.5±0.5	25	24
<i>S. aureus</i> ATCC 6538	18.5±0.5	15.5±0.5	18.5±0.5	14.5±0.5	18	36
<i>P. mirabilis</i> ATCC 35659	20.5±0.5	19.0±1.0	22.0±0.0	12.5±0.5	-	-

AE: Acetone extract, EF: Ethyl acetate fraction, BF: Butanol fraction and HF: Hexane fraction, R: Resistant. -: Non determined. The values of Table 3 represent the diameters of the zones of inhibition in mm. The diameter of the hole is 6 mm

Several reports revealed relationship between antioxidant activity and the total phenolics and total flavonoids contents (Anna *et al.*, 2003; Negro *et al.*, 2003; Ramandeep and Geoffrey, 2005). The results of the present study confirmed these reports; butanol fraction contained the highest amount of phenolics and it exerted the highest antioxidant activity. It was also reported that antioxidant activity could be related to the nature of phenolics (Singh *et al.*, 2007; Yu *et al.*, 2007).

The results consigned in Table 3 are related to the diameters of the zones of inhibition. Table 3 shown that gentamicin and amoxicillin, two broad spectrum antibiotics, exerted strong inhibitions against the tested strains *E. coli*, *S. aureus* ATCC 6538 and *S. typhimurium*. But clinical isolate *S. aureus* showed resistance to the two antibiotics while it displayed susceptibility to all the extract and fractions. Indeed, with clinical isolate *S. aureus* the acetone extract, the ethyl acetate fraction and the butanol fraction gave, respectively 12, 12.5 and 12 mm. Those values showed no significant difference. Though efficient against all the tested strains, the butanol fraction exhibited a low antibacterial activity against *E. coli*. The butanol fraction was more effective than all the tested extracts while the hexane fraction had revealed to be the less active fraction. There was no significant difference (p<0.05) between the acetone extract and the ethyl acetate fraction against the clinical isolates and even for *E. coli* ATCC 25922.

The butanol fraction gave the lowest Minimal Inhibitory Concentrations (MIC). A very low MIC corresponds to a very strong antibacterial activity. The highest MICs (ranging from 10-20 mg mL<sup>-1</sup> and even more) were obtained with *E. coli* which means that this strain is the less sensitive to the tested extract and fractions. *S. aureus* 6538 and *P. mirabilis* ATCC 35659 showed very sensitive to all the tested extracts (Table 4). The resistance of clinical isolate *S. aureus* to many antibiotics had been demonstrated by Zéba (2006). Many research works had been conducted on methicillin resistant *S. aureus* and showed that medicinal plants exerted inhibition against methicillin resistant

Table 4: Minimum inhibitory concentrations (MIC) in mg mL<sup>-1</sup>

Strains	AE	EF	BF	HF
<i>E. coli</i>	20	>20	10	>20
<i>S. aureus</i>	5	5	5	20
<i>K. pneumoniae</i>	2.5	5	1.25	5
<i>Salmonella</i> sp.	5	5	5	10
<i>E. coli</i> ATCC 25922	5	10	2.5	10
<i>S. typhimurium</i> ATCC 13311	2.5	10	1.25	10
<i>S. aureus</i> ATCC 6538	0.625	0.625	0.625	2.5
<i>P. mirabilis</i> ATCC 35659	0.625	0.625	0.625	2.5

AE: Acetone extract, EF: Ethyl acetate fraction, BF: Butanol fraction and HF: Hexane fraction

*Staphylococcus* strains. Indeed, Voravuthikunchai and Kitpipit (2005) tested 10 medicinal plants against 35 clinical isolates of methicillin *S. aureus* and a serotyped *S. aureus* ATCC 25923. Nine out of the 10 medicinal plants showed effectiveness with MICs ranging from 0.05 to 3.2 mg mL<sup>-1</sup>. In the literature, no antimicrobial study had been reported on *Combretum niroense* but it was reported that many *Combretum* species have medical applications against various bacterial infections such as gonorrhoea, syphilis and diarrhoea (Fyhrquist *et al.*, 2002). Martini *et al.* (2004) showed that *Combretum erythrophyllum* exerted antimicrobial activity against Gram-positive and Gram-negative bacteria. They isolated, by bioassay-guided fractionation, seven flavonoids (apigenin, genkwanin, 5-hydroxy-7,4 dimethoxyflavone, rhaninocitrin, kaempferol, quercetin-5,3'-dimethylether, rhaninocitrin, rhaninazin) and demonstrated that all of them had good activity against *Vibrio cholerae* and *Enterococcus faecalis*.

## CONCLUSION

The results obtained through this study clearly demonstrated that all the tested extract/fractions of Acetone extract and various fractions from acetone extract of *Combretum niroense* Aubrév. ex keay exerted antioxidant and antibacterial activities at different levels. The butanol fraction obtained from the acetone extract possessed the highest phenolic contents and demonstrated the best antioxidant and antibacterial activities. Those extracts had shown effectiveness against microorganisms responsible of infectious diseases thus justifying the successful use of *Combretum niroense* for the treatment of diarrhea, dysenteries in traditional medicine.

Further investigations will be performed by:

- The isolation and identification of pure compounds in the extracts, especially in the butanol fraction of acetone extract
- Testing these compounds against pathogenic bacteria
- Determining their antioxidant activity

## ACKNOWLEDGMENTS

We are grateful to the International Foundation for Science (IFS) and also to the International Atomic Energy Agency (IAEA) for providing the facilities.

## REFERENCES

- Anna, M.N., P.P. Riitta, A. Marjukka and O.C. Kirsi-Marja, 2003. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem.*, 81: 485-493.
- Arias, M.E., J.D. Gómez, N. Cudmani, M.A. Vattuone and M.I. Isla, 2004. Antibacterial activity of ethanolic and aqueous extract of *Acacia aroma* Gill ex Hook et. *Life Sci.*, 75: 191-202.
- Baba-Moussa, F., K. Akpagana and P. Bouchet, 1999. Antifungal activities of seven West African *Combretaceae* used in traditional medicine. *J. Ethnopharmacol.*, 66: 335-338.
- Batawila, K., K. Kokou, K. Koumaglo, M. Gbéassor, B. de Foucault, P. Bouchet and K. Akpagana, 2005. Antifungal activities of five *Combretaceae* used in Togolese traditional medicine. *Fitoterapia*, 76: 264-268.
- Chaves N., J.C. Escudero and C. Gutierrez-Merino, 1997. Role of ecological variables in the seasonal variation of flavonoid content of *Cistus ladanifer* Exudate. *J. Chem. Ecol.*, 23: 579-603.
- 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, pp: 67.
- Cole, G.M., G.P. Lim, F. Yang, B. Teter, A. Begum, Q. Ma, M.E. Harris-White and A. Frautschy, 2005. Prevention of Alzheimer's disease: Omega-3 fatty acid and phenolic anti-oxidant interventions. *Neurobiol. Aging*, 26: S133-S136.
- Eloff, J.N., 1998. The presence of antibacterial compounds in *Anthocleista grandiflora* (Loganiaceae). *South Afr. J. Bot.*, 64: 209-212.
- Fyhrquist, P., L. Mwasumbi, C.A. Hægström, H. Vuorela, R. Hiltunen and P. Vuorela, 2002. Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combretum* (*Combretaceae*) growing in Tanzania. *J. Ethnopharmacol.*, 79: 169-177.
- Hinneburg, I., H.J.D. Dorman and R. Hiltunen, 2006. Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem.*, 97: 122-129.

- Kana, I., T. Yoko and A. Sayumi, 2005. Seasonal variation in flavonoid contents of green pepper and other vegetables. *J. Jap. Soc. F. Sci. Technol.*, 52: 190-195.
- Karou, D., H.M.J. Dicko and A.S. Traore, 2005. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. *Afr. J. Biotechnol.*, 4: 823-828.
- Kloucek, P., Z. Polesny, B. Svobodova, E. Vlkova and L. Kokoska, 2005. Antibacterial screening of some Peruvian medicinal plants used in Calleria District. *J. Ethnopharmacol.*, 99: 309-312.
- Lamien-Meda, A., C.E. Lamien, M.M.Y. Compaoré, R.N.T. Meda, M. Kiendrebeogo, B. Zeba, J.F. Millogo and O.G. Nacoulma, 2008. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. *Molecules*, 13: 581-594.
- Liu, R.H., 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.*, 78: 517S-520S.
- Martini, N. and J.N. Eloff, 1998. The preliminary isolation of several antibacterial compounds from *Combretum erythrophyllum* (Combretaceae). *J. Ethnopharmacol.*, 62: 255-263.
- Martini, N.D., D.R.P. Katerere and J.N. Eloff, 2004. Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae). *J. Ethnopharmacol.*, 93: 207-212.
- Millogo-Koné, H., 2008. Study of the phytochemical parameters and the biological activities of *Parkia biglobosa* (Jacq) Benth (Mimosaceae). Ph.D Thesis, University of Ouagadougou.
- Nacoulma, O.G., 1996. Medicinal plants and their traditional uses in Burkina Faso. Ph.D Thesis, University of Ouagadougou.
- Negro, C., L. Tommasi and A. Miceli, 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresour. Technol.*, 87: 41-44.
- Nsimba, R.Y., H. Kikuzaki and Y. Konishi, 2008. Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus* spp. seeds. *Food Chem.*, 106: 760-766.
- Ojala, T., S. Remes, P. Haansuu, H. Vuorela, R. Hiltunen, K. Hahtela and P. Vuorela, 2000. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. *J. Ethnopharmacol.*, 73: 299-305.
- Ordoñez, A.A., N.M. Cudmani, D. Gomez, M.A. Vattuone and M.I. Isla, 2003. Antimicrobial activity of nine extracts of *Sechium edule* (Jacq) Swartz. *Microbiol. Ecol. Health Dis.*, 15: 33-39.
- Ramandeep, K.T. and P.S. Geoffrey, 2005. Antioxidant activity in different fractions of tomatoes. *Food Res. Int.*, 38: 487-494.
- Riboli, E. and T. Norat, 2003. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am. J. Clin. Nutr.*, 78: 559S-569S.
- Salvat, A., L. Antonnacci, R.H. Fortunato, E.Y. Suarez and H.M. Godoy, 2001. Screening of some plants from northern Argentina for their antimicrobial activity. *Lett. Applied Microbiol.*, 33: 293-297.
- Schlesier, K., M. Harwat, V. Bohm and R. Bitsh, 2002. Assessment of antioxidant activity by using different *in vitro* methods. *Free Radical Res.*, 36: 177-187.
- Simon, G., J. Dewelle, O. Nacoulma, P. Guissou, R. Kiss, D. Dalozé and J.C. Braekman, 2003. Cytotoxic pentacyclic triterpenes from *Combretum nigricans*. *Fitoterapia*, 74: 339-344.
- Singh, R., S. Singh, S. Kumar and S. Arora, 2007. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Food Chem. Toxicol.*, 45: 1216-1223.
- Sun, T. and C.T. Ho, 2005. Antioxidant activities of buckwheat extracts. *Food Chem.*, 90: 743-749.
- The World Health Organization, 2002. WHO global burden of disease (GBD) estimates (revised). <http://www.who.int/healthinfo/bodestimates/en/>.
- Thiombiano, A., 2005. The Combretaceae in Burkina Faso: Taxonomy, ecology, dynamic and regeneration of species. Ph.D. Thesis, University of Ouagadougou.
- UNAIDS, World Health Organization, 2007. AIDS epidemic update. [http://data.unaids.org/pub/EPISlides/2007/2007\\_epiupdate\\_en.pdf](http://data.unaids.org/pub/EPISlides/2007/2007_epiupdate_en.pdf).
- Voravuthikunchai, S.P. and L. Kitpipit, 2005. Activity of medicinal plant extracts against hospital strains of methicillin-resistant *Staphylococcus aureus*. *Clin. Microbiol. Infect.*, 11: 510-512.
- Yu, J.O., Z.X. Liao, J.C. Lei and X.M. Hu, 2007. Antioxidant and cytotoxic activities of various fractions of ethanol extract of *Dianthus superbus*. *Food Chem.*, 104: 1215-1219.
- Zéba, B., 2006. Study of the enzymatic support of the bacterial resistance to antibiotics: Implimentation to  $\beta$ -lactamines/  $\beta$ -lactamases; epidemiology. Ph.D Thesis, University of Ouagadougou.