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Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso

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Abstract: A total of fourteen (14) species of wild edible fruits from Burkina Faso were analyzed for their phenolic and flavonoid contents, and their antioxidant activities using the DPPH, FRAP and ABTS methods. The data obtained show that the total phenolic and total flavonoid levels were significantly higher in the acetone than in the methanol extracts. *Detarium microcarpum* fruit had the highest phenolic and the highest flavonoid content, followed by that of *Adansonia digitata, Ziziphus mauritiana, Ximenia americana and Lannea microcarpa*. Significant amounts of total phenolics were also detected in the other fruit species in the following order of decreasing levels: *Tamarindus indica > Sclerocarya birrea > Dialium guineense > Gardenia erubescens > Diospyros mespiliformis > Parkia biglobosa > Ficus sycomorus > Vitellaria paradoxa*. *Detarium microcarpum* fruit also showed the highest antioxidant activity using the three antioxidant assays. Fruits with high antioxidant activities were also found to possess high phenolic and flavonoid contents. There was a strong correlation between total phenolic and flavonoid levels and antioxidant activities.

Keywords: Wild fruits; Tropical fruits; phenolics; flavonoids; antioxidant capacity.

Introduction

Wild edible plants contribute significantly to the nutrition of rural West African inhabitants [1]. Although these foods are consumed by people throughout the year in fresh and dried forms, reliance on these foods increases during periods of cereal shortages. Nowadays, however, a nutritional transition is occurring in the poorest countries of the world resulting in the replacement of traditional plant-based diets that are rich in fruits and vegetables with diets that are rich in calories provided by animal fats and sugars, and low in complex carbohydrates [2].

Wild edible foods include fruits, leaves, flowers and seeds from spontaneous trees and shrubs. Of these, fruits are receiving increase interest from researchers working on wild edible plant species because of their nutritional value, vitamin and mineral contents. These fruits are being investigated as potential food supplements in the Sahelian region so as to increase quality of daily food for the rural population [3]. In West Africa, around 75 wild edible fruit species known by the Malinké ethnic group of Ivory Coast, have been inventoried by Ambé [4]. The same author found that many wild edible fruits are still unknown or insufficiently exploited in the region, despite their nutritional values.

Research on wild fruits and other wild edible plants is also intended to promote the preservation of these species, presently under threat by human activities. In addition to their nutritional value, the preservation of these fruits also has economical advantages, as there is a significant trade in some if these wild edible fruits [4]. Some of these wild fruits are also known to have medicinal properties. Any scientific evidence for the health benefits of such wild fruits in addition to their nutritional value would be an added value to the plants producing such fruits. Concerning their medicinal properties, the most commonly studied benefit is their antioxidant effects. Antioxidants play a crucial role in the prevention of chronic ailments such as heart disease, cancer, diabetes, hypertension, stroke and Alzheimer's disease by combating oxidative stress [5].

The dietary intake of fruits has a strong inverse correlation with the risk of developing coronary heart disease and cancer. In fruits, vitamins C, A and E, and polyphenols are known to be responsible for such antioxidant activity, with polyphenols being the most active. A number of studies have reported the content of some essential nutrients in wild edible fruits from western Africa [3,6], including energy levels, ascorbic acid, vitamins, metals and trace minerals.

In Burkina Faso, *Adansonia digitata* L. (Bombacaceae), *Detarium microcarpum* Guill. et Perr. (Caesalpiniaceae), *Dialium guineense* Willd (Caesalpiniaceae), *Diospyros mespiliformis* Hochst. (Ebenaceae), *Ficus sycomorus* L. (Moraceae), *Gardenia erubescens* Stapf. et Hutch. (Rubiaceae), *Lannea microcarpa* Engl. et K. Krause. (Anacardiaceae), *Parkia biglobosa* (Jacq.) R. Br. (Caesalpiniaceae), *Saba senegalensis* (A.Dc) Pichon. (Apocynaceae), *Sclerocarya birrea* (A. Rich) Hochst. (Anacardiaceae), *Tamarindus indica* L. (Caesalpiniaceae), *Vitellaria paradoxa* C.F. Gaertn. (Sapotaceae), *Ximenia americana* L. (Olacaceae) and *Ziziphus mauritiana* Lam. (Rhamnaceae) are well known wild edible fruit species, and their fruits are traditionally consumed as a food sources as well as for medicinal purposes (Table 1).

Table 1. Therapeutic uses of some of the studied edible fruits.

Fruits	Part studied	Therapeutic uses
Adansonia digitata L. (Bombacaceae)	Flesh with peel	Refreshing, tonic, diuretic, bitter-sweet, cystitis, dysentery, hepatic disorders, hypogalactia [20,21]
Detarium microcarpum Guill. et Perr. (Caesalpiniaceae)	Flesh with peel	Vitamin C and B deficiencies, dizziness, infectious diseases. Analgesic, digestive, tonic, stimulant, antibacterial, antiscorbutic [21]
Dialium guineense Willd (Caesalpiniaceae)	Flesh	-
Diospyros mespiliformis Hochst. (Ebenaceae)	Flesh with peel	Green fruits: dysentery, gastroenteritis, diarrhoea, menorrhagia, vomiting. Astringent, spasmolytic, antibacterial, haemostatic [21]
Ficus sycomorus L. (Moraceae)	Flesh with peel and seed	-
Gardenia erubescens Stapf. et Hutch. (Rubiaceae)	Flesh with peel	-
Lannea microcarpa Engl. et K. Krause. (Anacardiaceae)	Flesh with peel	Rachitism and scurvy [21]
Parkia biglobosa (Jacq.) R. Br. Caesalpiniaceae	Flesh	Constipation (Laxative), yellow fever, rickets, anorexia [21]
Saba senegalensis (A.Dc) Pichon var. senegalensis (Apocynaceae)	Flesh	Green fruits: Diuretic, galactogogue. Ripe fruits: Anorexia, antiscorbutic, stimulant, tonic [21]
Sclerocarya birrea (A. Rich.) Hochst. (Anacardiaceae)	Flesh with peel	Internal use: Pulp fruits (juice): constipation, nausea, anorexia, Astringent, diuretic, hypotensor, laxative, antiscorbutic, Anorexia, External use (juice) cutaneous eruptions. Antiviral, antibacterial, anti-inflammatory drug, astringent [21]
Tamarindus indica L. (Caesalpiniaceae)	Flesh	Constipation, abdominal pains, bowel obstruction, pregnancy vomiting, intestinal disorders, biliary, poisonings, puffiness, cardiac weakness, diabetes, parasites, paludism, fever, headache, diuretic, antispasmodic, febrifuge, hypotensor [21]
Vitellaria paradoxa C.F. Gaertn. (Sapotaceae)	Flesh with peel	Food source, laxative [21]
Ximenia americana L. (Olacaceae)	Flesh with peel	Pulp fruits: Constipation, refreshing, tonic, purgative, astringent [21]
Ziziphus mauritiana Lam. (Rhamnaceae)	Flesh with peel	Allergies, vitamin A and C deficiencies, constipation, insomnia, depression, nutritive, sedative, anti-allergenic [21]

Among these fourteen well known wild edible fruits from Burkina Faso, only *A. digitata* [7], *Z. mauritiana* [8], *S. birrea* [9,10], *T. indica* [11-13] and *D. microcarpum* [14], have been studied for their phytochemical contents and/or biological activities.

The present study aimed to promote the contribution of West African edible wild fruits in public health campaigns to encourage the eating of five to nine or more servings of fruits and vegetables daily, through evaluation of the total phenolic and flavonoid contents, and the antioxidant capacities of fourteen wild edible fruits from Burkina Faso. These data will be used to estimate the phytochemical and antioxidant intake of the local population and to understand the therapeutic uses of some of these fruits.

Several methods have been developed to monitor the total antioxidant capacity in biological samples [15-17]. These assays differ in how the different radicals and/or target molecules are generated and in the way the end points are measured. Because of this, more than one method is required to investigate the *in vitro* antioxidant potential of complex mixtures such as fruit extracts. We have therefore used the DPPH (2, 2-dipheny-l-picrylhydrazyl), FRAP (ferric reducing antioxidant capacity) and ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate) assays to evaluate antioxidant activity.

Results and Discussion

Total phenolics (TP) and total flavonoids (TF).

MeOH 70% and acetone 70% pH 2 are two solvents which are mainly used for phenolic extraction from plants, food or vegetables [16,18,19]. These two methods of extraction were performed with all the fruit samples to determine which leads to more efficient phenolic extraction. The fruit samples were kept at -20°C before analysis. Asami *et al*. [19] have shown that freezing fruit samples may lead to higher extraction efficiencies for total phenolics, because of enhanced plant cell rupture during ice crystal formation and melting, with improved solvent access and extraction.

The total phenolics of the MeOH extracts varied from 190.58 to 4946.67 mg GAE/100 g of fruit weight (fw) and that of acetone extracts were 231.33 – 5978.33 mg GAE/100 g of fw (Table 2), using a standard curve of gallic acid (R^2 = 0.9996). While no significant difference was observed between phenolics values of the two extracts from *X. americana* fruit, we found that the total phenolics of the acetone extract were significantly higher (P < 0.05) than those of methanolic extracts in all the other fruits except in shea tree fruit (*V. paradoxa*) where the MeOH extract (381.67 mg GAE/100 g) shows more phenolics than the acetone one (231.35 mg GAE/100 g).

This result shows that the phenolics from these fruits are more extractible by acidified aqueous acetone than aqueous MeOH. The highest phenolics content was measured in *D. microcarpum* fruit (5978.33 mg/100 g of fw, acetone extract) followed by that of *A. digitata, Z. mauritiana*, *X. americana*, *L. microcarpa* and *S. senegalensis* (4072.5, 3240.83, 2086.67, 1005.75 and 945.83 mg/100 g of fw with acetone extracts, respectively). Important amount of phenolics (308 – 888 mg GAE/100 g of fw, acetone extract) were also measured in the remaining fruits in the following decreasing order: *T. indica* > *S. birrea* > *D. guineense* > *G. erubescens* > *D. mespiliformis* > *P. biglobosa* > *F. sycomorus* > *V. paradoxa*.

Table 2. Total phenolic and Total flavonoid levels for fourteen fruit extracts (MeOH 70%, Acetone 70%, pH 2). Results are mean ± SD (n = 6).

| Fruits	Total phenolics (mg GAE/100 g of fruit)		Total flavonoids (mg QE/100 g of fruit)	
	MeOH	Acetone	MeOH	Acetone
A. digitata	3518.33 ± 17.80	4057.50 ± 57.34	31.70 ± 3.35	42.73 ± 0.46
D. microcarpum	4946.67 ± 79.41	5978.33 ± 87.50	116.05 ± 3.04	155.90 ± 1.89
D. guineense	579.00 ± 6.013	852.50 ± 23.41	19.45 ± 3.18	10.23 ± 0.83
D. mespiliformis	336.33 ± 9.14	591.67 ± 36.80	22.40 ± 0.28 [b]	27.10 ± 0.93 [b]
F. sycomorus	190.58 ± 11.89	308.83 ± 6.80	24.15 ± 1.81	33.15 ± 1.79
G. erubescens	298.50 ± 9.25	656.83 ± 10.91	11.70 ± 0.81	34.55 ± 2.04
L. microcarpa	240.58 ± 7.78	1006.83 ± 6.59	23.35 ± 1.39	35.35 ± 1.63
P. biglobosa	380.92 ± 8.43	526.17 ± 34.70	nd	nd
S. senegalensis	515.50 ± 32.21	945.83 ± 14.13	1.70 ± 0.35	5.30 ± 0.62
S. birrea	505.83 ± 6.08	872.33 ± 19.21	33.90 ± 0.35	28.00 ± 0.88
T. indica	957.33 ± 13.20	888.67 ± 21.96	2.18 ± 0.21	5.68 ± 0.10
V. paradoxa	381.67 ± 41.57	231.33 ± 20.74	20.70 ± 0.48	30.95 ± 0.41
X. americana	2230.00 ± 76.09 [a]	2086.67 ± 55.11 [a]	30.95 ± 3.76	23.60 ± 1.75
Z. mauritiana	2352.50 ± 52.70	3240.83 ± 44.21	56.88 ± 0.30	92.55 ± 1.76

nd: not detectable. Values with the same letter are not significantly different (P > 0.05).

Using the $AlCl_3$ reagent and quercetin as standard (R^2 = 0,9993), the total flavonoids varied from 1.7 mg QE/100 g (*S. senegalensis*) to 116.05 QE/100 g (*D. microcarpum*) for MeOH extracts (Table 2). The highest value for the acetone extract was 155.9 mg QE/100 g (*D. microcarpa*) and the lowest was 5.3 mg QE/100 g (*S. senegalensis*) with the following decreasing order: *D. microcarpum* > *Z. mauritiana* > *A. digitata* > *L. microcarpa* > *G. erubenscens* > *F. sycomorus* > *V. paradoxa* > *S, birrea* > *D. mespiliformis* > *X. americana* > *D. guineense* > *T. indica* > *S. senegalensis*. Flavonoids were not detectable in *P. biglobosa* fruits extracts using $AlCl_3$ reagent. When comparing the results of the two extracts, there was no significant difference between flavonoids value of the two extracts from the fruit of *D. mespiliformis*. For the remaining fruits, the total flavonoid content of the acetone extracts were significantly higher (P < 0.05) than those of the MeOH extracts except for those of *D. guineense, S. birrea* and *X. americana* where the MeOH extract had significantly (P < 0.05) higher flavonoids content. This shows that the flavonoids present in the studied fruits are more extractible by the acidified aqueous acetone than aqueous MeOH.

Antioxidant capacity.

The antioxidant capacities of the fruits were analyzed using the free radical scavenging capacity (DPPH), the ferric reducing antioxidant power (FRAP) and the ABTS radical cation scavenging capacity (ABTS) (Figure 1).

Figure 1. Antioxidant activities obtained using the DPPH (a), FRAP (b) and ABTS (c) methods on fourteen fruit extracts (MeOH and Acetone) viz. *A. digitata* (AD), *D. microcarpum* (DM), *D. guineense* (DG), *D. mespiliformis* (DMe), *F. sycomorus* (FS), *G. erubescens* (GE), *L. microcarpa* (LM), *P. biglobosa* (PB), *S. senegalensis* (SS), *S. birrea* (SB), *T. indica* (TI), *V. paradoxa* (VP), *X. americana* (XA) and *Z. mauritiana* (ZM). Results are mean ± SD (n = 6).
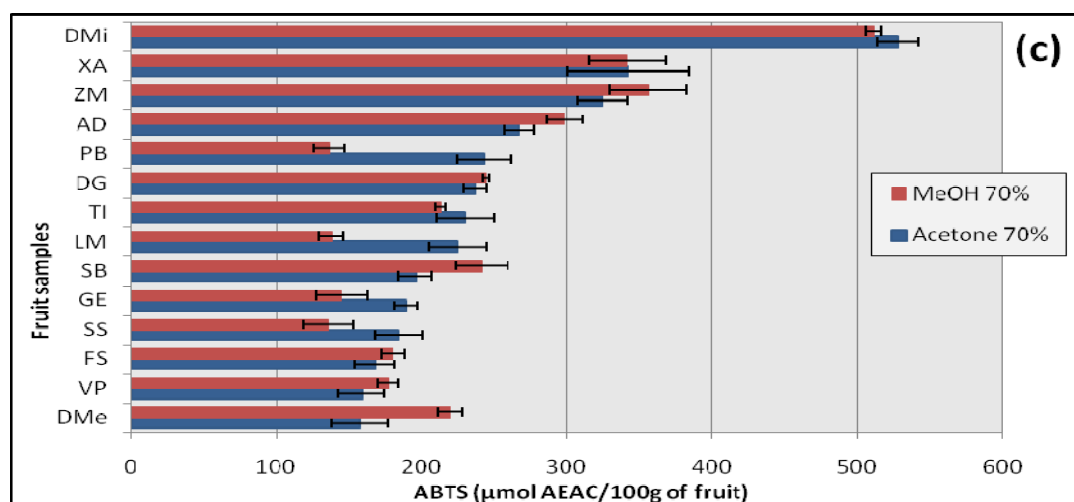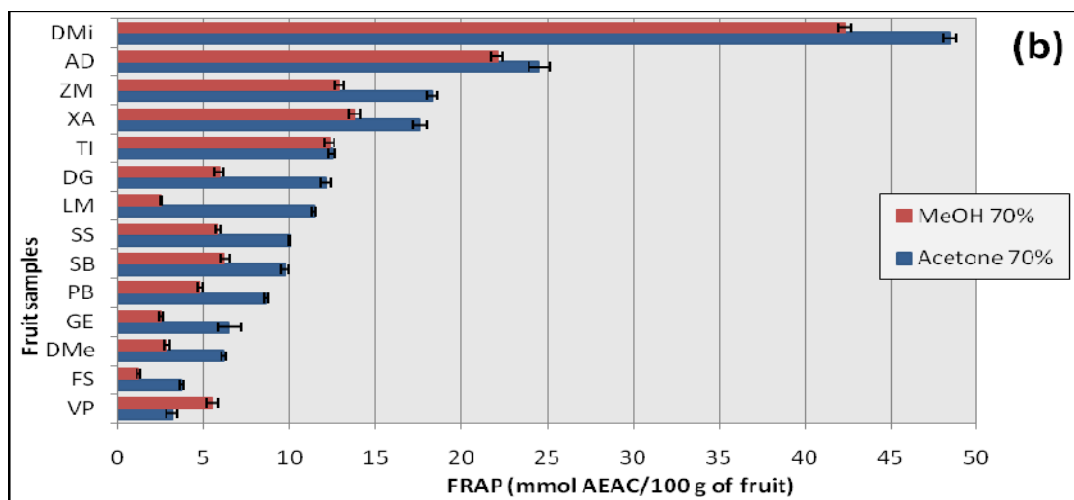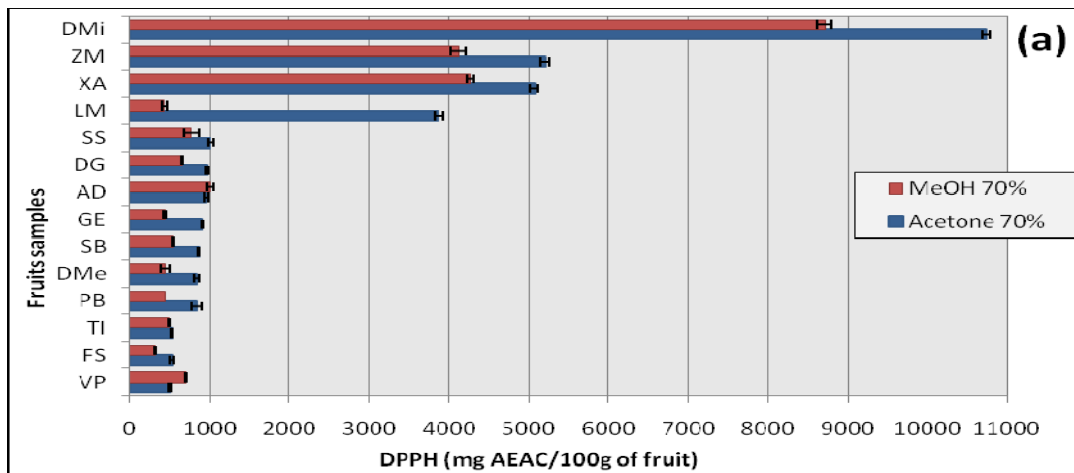
The DPPH test is the oldest indirect method for determining the antioxidant activity which is based on the ability of the stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols [17]. The bleaching of DPPH solution increases regularly with increasing amount of fruit in a given volume. The bleaching action is mainly attributed to the presence of antioxidant compounds like polyphenols in the solution. The antioxidant capacity evaluated by DPPH radical-scavenging ranged from 319.63 to 8709.5 mg AEAC/100 g of fw for MeOH extracts and from 499.48 to 10729.41 mg AEAC/100 g of fw for the acetone extracts (Figure 1.a), using ascorbic acid as standard ($R^2 = 0.9997$). The DPPH radical scavenging of the acetone extracts were significantly higher ($P < 0.05$) than those of MeOH extracts except for *A. digitata* and *V. paradoxa*. When considering the acetone extracts, the following decreasing order was found: *D. microcarpum > Z. mauritiana > X. americana > L. microcarpa > S senegalensis > D. guineense > A. digitata > G. erubenscens > S. birrea > D. mespiliformis > P. biglobosa > F. sycomorus > T. indica > V. paradoxa*. The antioxidant activity of the fruit from *D. microcarpum* was 2 to 24 fold higher than that of other fruits.

The FRAP assay measured the ability of phenolics to reduce Fe(3+) to Fe(2+). The results of the FRAP method were similar to those of DPPH method. The FRAP values ranged from 1.21 to 42.35 mmol AEAC/100 g of fw for MeOH extracts and from 3.12 to 48.45 mmol AEAC/100 g of fw for acetone extracts (Figure 1.b), using ascorbic acid as standard ($R^2 = 0.9999$). In this assay, no significant difference was observed between FRAP value of the two type of extracts from the fruits of *T. indica*. For the others fruits, the acetone extracts values were significantly higher ($P < 0.05$) than those of MeOH extracts, except for *V. paradoxa* where the MeOH extracts had the highest value. The highest FRAP values were obtained with the fruit of *D. microcarpum* followed by *A. digitata, Z. mauritiana, X. americana* and *T. indica* (48.45, 24.50, 18.28, 17.57 and 12.42 mmol AEAC/100 g, respectively). In other fruits, the acetone extracts have shown also important FRAP values.

The ABTS test measures the relative antioxidant ability of fruits to scavenge the radical-cation ABTS[.+] produced by the oxidation of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate [17]. In the ABTS assay, the antioxidant capacities ranged from 136 to 511.5 µmol AEAC/100 g of fw for MeOH extracts and from 157.50 to 528 µmol AEAC/100 g of fw for acetone extracts (Figure 1.c), using ascorbic acid as standard ($R^2 = 0.9990$). In this assay, the acetone extracts values were apparently higher than those of the MeOH extracts for *D. microcarpum, X. americana, P. biglobosa, T. indica, L. microcarpa, D. guineense* and *S. senegalensis* (528, 342.5, 243.5, 230, 225, 189.5, 184.5 µmol AEAC/100 g, acetone extracts) and inversely for the seven other fruits. Nevertheless, the t-test showed there was a significant difference ($P < 0.05$) only for the extracts from *A. digitata, D. mespiliformis, G. erubescens, L. micropcarpa, P. biglobosa* and *S. birrea* ($P = 0.05$). The following decrease order was obtained with acetone extracts: *D. microcarpum > X. americana > Z. mauritiana > A. digitata > P. biglobosa > D. guineense > T. indica > L. microcarpa > S. birrea > G. erubenscens > S seneglensis > F. sycomorus > V. paradoxa > D. mespiliformis*.

It is now well accepted that increasing individual fruit and vegetable consumption up to 600 g per day (the baseline of choice) can reduce the total worldwide burden of disease [2]. In developed countries, nutraceuticals, functional foods and other food products, with various claims for health benefits, form a rapidly growing segment of the market, as a response to increasing disease risk factors being identified [22].

In fruits, polyphenols constitute the main bioactive phytochemicals that have been proven effective in the prevention of certain chronic diseases such as coronary heart diseases, cancers and diabetes [19], because of their free radical-scavenging activities.

The results of this study showed important differences in the total polyphenol (TP) and total flavonoid (TF) content of these fruits, and also in their antioxidant capacity using the DPPH, FRAP and ABTS assays. Overall, the phenolic content was high in all the studied fruit samples as compared to the majority of temperate as well as other tropical fruits [5,18,23]. In the work reported here, the lowest TP values (231.33 and 308.83 mg GAE/100 g for *V. paradoxa* and *F. sycomorus*, respectively) were similar to the regularly consumed fruits in the United Kingdom [11], and to those of blueberries (670.9 mg GAE/100 g), dogwood berries (432 mg GAE/100 g) and sour cherries (429.5 mg GAE/100 g) from Bulgaria [24]. Furthermore, the TP value of tamarind (3.9 mg GAE/g) flesh from Singapore was two (2) fold lower than that of our study [12]. The TP of *S. birrea* fruit from Zimbabwe (2262 μg GAE/g of pulp, equivalent to 226.2 mg/100 g) was estimated by Ndhlala *et al*. [10]. This value is three fold lower than that of our acetone extract value (872.33 mg GAE/100 g) and two fold lower than the methanol extract value (505.83 mg GAE/100 g) from the same fruit. These variations could be explained by the climate and also the extraction solvent used.

Consumption of fruit is known to provide a wide variety of flavonoids, which play a protective role by reducing the risk for cancer and cardiovascular diseases [25]. In the present study, the contribution of flavonoids to the TP value of the fruits extracts varied from 0.23 % to 13.38 %. Our TF values were not comparable to those of others, due to differences in test methods used. For example, Ndhlala *et al*. [10] estimated the TF of *S. birrea* fruit from Zimbabwe (202 μg catechin/g of pulp) using a vanillin assay.

The fruit of *D. mirocarpum* with the highest TF, also possessed the highest TP. Considering the acetone extracts, the TF of this fruit was 1.7 to 29 fold higher than that of others fruits. The fruit of *Z. mauritiana* with 92.55 mg QE/100 g of TF also contained a remarkably high TP value.

A significant correlation (R = 0.83, p<<0.0001) was obtained between TP and TF levels. This correlation is high compared to others studies [26,27] on plant extracts (R = 0.43) and honey samples (R = 0.11).

For most fruits tested, the results for the antioxidant activities were similar to that of others reported in the literature, depending on the methods used. For example, Leong and Shui [28] reported < 5000 mg AEAC/100 g of DPPH values for Singapore market fruits. FRAP values of 13.5 – 218 mg AEAC/100 g were measured for Malaysian tropical fruits and for a large variety of fruits and vegetables [11,23,29]. The ABTS assay has also been used to estimate the antioxidant activity of numerous fruits and vegetables [11,12,28].

The antioxidant capacity of baobab (*A. digitata*) fruit pulp has been studied by Besco *et al*. [7] using a photochemiluminescence. The value of the total antioxidant capacity of baobab pulp was 9 times higher than that of orange pulp, with values of 240.5 and 24.3 μmol/g, respectively. However, our study shows that the antioxidant activity of *D. microcarpum* is two-fold higher than that of *A. digitata*. We can thus assume that the fruit of *D. microcarpum* is a promising source of antioxidants, in comparison with baobab fruit and the orange.

In this study, the fruits possessing highest phenolic contents were also found to have the highest antioxidant capacity from the DPPH, FRAP and ABTS methods. For example, in all the three different

antioxidant assays, the fruit of *D. microcarpum* was the most efficient followed by that of *Z. mauritiana, X. americana, L. microcarpa and S. senegalensis*. The same decreasing order was obtained for TP content.

We can also affirm that the strong antioxidant capacities of these fruit extracts were due to their high phenolic contents. This last assertion is confirmed by the correlation studies between the total phenolic and antioxidant activities.

Well pronounced correlations were observed between TP and the antioxidant activities: 0.82 (TP and DPPH), 0.96 (TP and FRAP) and 0.89 (TP and ABTS). These correlations also confirm the values of the Folin-Ciocalteu test as a tool for showing the availability of antioxidant compounds in fruits. Previous studies on the antioxidant activity of nectarine, peach, and plum from California [30] showed a close level of correlation between phenolic content and DPPH (R = 0.93 – 0.96). Several authors have found good correlations between total phenolics determined by the Folin-Ciocalteu method and the ABTS test: 0.99, 0.96, and 0.94 calculated from wines by Simonetti *et al*. [31], Landrault *et al*. [32], and De Beer *et al*. [33], respectively.

Good correlations were also observed between TF and antioxidant activities: 0.88 (TF and DPPH), 0.91 (TF and FRAP) and 0.82 (TF and ABTS). This means that most of the flavonoids estimated by the $AlCl_3$ method have antioxidant activity and are sensitive to the DPPH, FRAP and ABTS tests. Indeed Chang *et al*. [34] showed that aluminium chloride method is specific only for flavones and flavonols which are known to be present in fruits and to possess antioxidant activities [25,35]. Such a significant correlation between TF and antioxidant activity was also observed by Karadeniz *et al*. [36] with Turkish fruits.

High and significant correlations (R ≥ 0.90) were found between data from the DPPH, FRAP and ABTS antioxidant assays. These correlations confirmed the antioxidant activity of phenolic compounds from the tested fruits. Hinneburg *et al*. [15], however, found no correlation between the FRAP and DPPH assays on extracts from culinary herbs and spices.

The results from this study highlight the importance of the TP, TF and antioxidant capacities of some wild edible fruits from West Africa, as well as indicating the important need for further investigations into the identification of phenolics and flavonoids and *in vivo* antioxidant activities in these fruits. According to Leung [6], all the studied fruits are energy rich (71 - 303 calories/100 g) and almost all of them have therapeutic uses during their ripening period (Table 1). This study provides data to health professionals and food policy makers in West Africa for encouraging the population to consume more wild edible fruits as well as, promoting the preservation of such fruit species.

Conclusions

Fourteen (14) edible wild fruits were analysed for their total phenolic, flavonoid and antioxidant contents and activities respectively. The phenolics and flavonoids were more readily extracted using acidified aqueous acetone as solvent and the overall antioxidant values for the acetone extracts were significantly higher than those of the MeOH extracts. Total phenolics were higher in all the fruit samples studied, in comparison to the majority of other tropical fruits. The fruit of *D. microcarpum* had the highest total phenolic, flavonoid and antioxidant values. Fruits from *Z. mauritiana, X. americana, L. microcarpa and S. senegalensis* also exhibited high total polyphenolic and antioxidant

capacities. The correlations indicated that total phenolics and flavonoids are the major contributors to the antioxidant activity of these fruits. Further investigations will be important to identify individual phenolic compounds, as well as the antioxidant and biological activities of these wild edible fruits, particularly those of *D. microcarpum*.

Experimental

General

The experiments were performed using a Cecil CE 2041 spectrophotometer (Cecil Instruments, England). All reagents were of analytical grade: Folin Ciocalteu-reagent, NaH_2PO_4, Na_2HPO_4, sodium carbonate, aluminium trichloride ($AlCl_3$), gallic acid and quercetin were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). 2,2-Dipheny-l-picrylhydrazyl (DPPH); 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate) ABTS; trichloroacetic acid, potassium persulfate, acetone and methanol used were supplied by Fluka Chemie (Buchs, Switzerland). Potassium hexacyanoferrate [$K_3Fe(CN)_6$] was from Prolabo (Paris, France); ascorbic acid and iron trichloride were supplied by Labosi (Paris, France).

Samples

Fresh ripe fruit samples from *Adansonia digitata* L. (Bombacaceae), *Detarium microcarpum* Guill. et Perr. (Caesalpiniaceae), *Dialium guineense* Willd (Caesalpiniaceae), *Diospyros mespiliformis* Hochst. (Ebenaceae), *Ficus sycomorus* L. (Moraceae), *Gardenia erubescens* Stapf. et Hutch. (Rubiaceae), *Lannea microcarpa* Engl. et K. Krause. (Anacardiaceae), *Parkia biglobosa* (Jacq.) R. Br. (Caesalpiniaceae), *Saba senegalensis* (A.Dc) Pichon. (Apocynaceae), *Sclerocarya birrea* (A. Rich) Hochst. (Anacardiaceae), *Tamarindus indica* L. (Caesalpiniaceae), *Vitellaria paradoxa* C.F. Gaertn. (Sapotaceae), *Ximenia americana* L. (Olacaceae) and *Ziziphus mauritiana* Lam. (Rhamnaceae) were obtained in the central part of Burkina Faso. All fruits were harvested from March to August 2006 in the Gampela region, except *D. guineese*, which was a commercial sample. Fruits were also botanically identified by Prof. Millogo from the Plant Biology Department of the University of Ouagadougou. The parts of the fruits studied were chosen according to what is known to be consumed by locals.

For dry fruits, the flesh of *D. guineese* and *P. biglobosa*, and the flesh with peel of *A. digitata, D. microcarpum* and *Z. mauritiana* were manually ground and sieved to remove seeds, and the fine powder was kept in the freezer until extracted. The fresh fruits were cleaned under running tap water and kept at -20°C. The flesh of *S. senegalensis*, the flesh with peel of *D. mespiliformis*, *L. microcarpa*, *S. birrea, V. paradoxa, X. americana*, and flesh with peel and seeds of *F. sycomorus* were crushed just before extraction.

Phenolic extraction

For each fruit sample, two types of extraction were performed using 70% aqueous methanol [36] and 70% aqueous acetone pH 2 [18]. Briefly, powdered/ or crushed fruit (edible part with or without

skin and seeds, as would normally be eaten) were homogenized and 1g of the homogenate was extracted three times with 10 mL of each solvent by shaking vigorously for 10 min. The samples with solvent were centrifuged (4000 rpm for 15 min in a bench centrifuge). The three supernatants collected after centrifugation were combined and filtered through a Whatman no.1 filter, after which the filtrate was evaporate to dryness at 40°C under vacuum. The extracted phenolics were dissolved in methanol-water (4:1, 10 mL). For each solvent (methanol and acetone) two different extractions were performed on two independent samples from the initial homogenate.

Determination of total phenolics and total flavonoids

The total phenolics of each fruit extract were determined by the Folin-Ciocalteu method [37]. 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 g/L in water, 2 mL) was added. After 2 h of incubation, the absorbances were measured at 760 nm against a water blank. A standard calibration curve was plotted using gallic acid (0-200 mg/L). The results were expressed as mg of gallic acid equivalents (GAE)/100 g of fruit weight.

The total flavonoids were estimated according to the Dowd method as adapted by Arvouet-Grand *et al*. [38]. A diluted methanolic solution (2 mL) of each fruit extract was mixed with a solution (2 mL) of aluminium trichloride ($AlCl_3$) in methanol (2 %). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a methanol (2 mL) and plant extract (2 mL) without $AlCl_3$. Quercetin was used as reference compound to produce the standard curve, and the results were expressed as mg of quercetin equivalents (QE)/100 g of fruit weight.

Iron (III) to iron (II) reduction activity (FRAP)

The total antioxidant capacity of each fruit extract was determined using the iron (III) reduction method [15]. The diluted aqueous solution of each fruit extract (1 mL), at a concentration of 100 µg/mL, was mixed with phosphate buffer (0.2 M, pH 6.6, 2.5 mL) and 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution (2.5 mL). After 30 min incubation at 50°C, trichloroacetic acid (10 %, 2.5 mL) 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 an aqueous $FeCl_3$ (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/100 g of fruit weight.

DPPH radical method

The ability of the extract to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was evaluated as described in the literature [26]. The antioxidant content was determined using a standard curve of ascorbic acid (0 – 10 µg/mL). The results were expressed as mg of ascorbic acid equivalent antioxidant content (AEAC) per 100 g of fruit weight.

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 Re *et al.* [16]. $ABTS^{\cdot+}$ 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 ± 0.02 units at 734 nm using spectrophotometer.

For each fruit, the diluted methanol solution of the extract (10 µL) was allowed to react with fresh $ABTS^{\cdot+}$ solution (990 µL), 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 µmol ascorbic acid equivalents /100 g of fruit weight.

Statistical analysis

All data presented are means of six determinations (three replicates per each of the two independent extractions) along with standard deviations. Statistical analysis used the MS Excel software (CORREL Statistical function) to calculate quercetin, ascorbic acid and gallic acid equivalents, to determine inhibition percentage, and to establish linear regression equations. The Pearson Product Moment function of SigmaStat2.0 was used to determine correlation coefficients (R).

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