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Antioxidant Capacity and Phenolic Composition of *Crateva adansonii* DC. (Capparaceae)

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Abstract: ***Crateva adansonii DC. (C. adansonii) is well known in Burkina Faso as edible and medicinal plant. This study aims at determining the antioxidant capacity of its leaves and to investigate their phenolic composition. Three methods, ferric reducing antioxidant power (FRAP), ABTS radical cation scavenging capacity, and free radical scavenging capacity (DPPH) were used to determine the antioxidant capacity of the leaves fractions. The phenolic compounds were using HPLC-MS method. The best ferric reducing antioxidant power was found in n-BuOHF with a concentration of 818.247 µmol AAE/g. The same trend was observed with the ABTS radical cation scavenging capacity, where the n-BuOHF (15.036 µmol TE/g) presented the highest activity. On the other hand, the MeOHF showed the most potent DPPH radical scavenging activity (202.811 µmol QE/g). The leaves of C. adansonii were disclosed to be a rich source of phenolic compounds. Among the ten phenolic compounds, p-coumaric, ferulic and sinapic acids, isoquercitrin, quercitrin, quercetol and kaempferol were identified as major phenolic compounds. C. adansonii leaf fractions exhibit remarkable antioxidant capacity, and this activity may correlate to the content and the type of phenolic compounds.***

Keywords: *Crateva adansonii*; Antioxidant activity; Phenolic composition; HPLC-MS.

1. Introduction

Crateva adansonii, a tropical tree is widely distributed all over India, Myanmar, and Sri Lanka [1]. Traditionally, the leaves, flowers, and root and stem barks are used for their medicinal properties. In India, the bark is useful in some cases of urinary complaints and fever, and in some mild forms of skin diseases [1]. In Bénin, the decoction of this species was used by traditional breeders to treat the digestive disorders of the bred animals, such as ruminants and *Thryonomys swinderianus*. A decoction of the fresh leaves and branches is taken orally to treat hypertension [2]. In Burkina Faso, the leaves of *C. adansonii* are highly exploited by the local population to prepare a sauce. In traditional medicine, the leaves are given internally to treat memory loss, seizures, rheumatism and a weak immune system. Roots and bark in the form of decoction are used to treat calculus affections, stomach troubles, fever, seizures and malaria [3].
C. adansonii is well-known for its various pharmacological properties, like diuretic, anti-inflammatory, laxative, antioxidant, anti-oxaluric, hepatoprotectant, lithonotriptic, antirehumatic, antiperiodic, antimycotic, contraceptive, antipyretic, antilithitic and antihelminthic properties [4], [5]. Moreover, phytochemical investigations of *C. adansonii* have shown the presence of lupeol acetate, varunaol, spinasterol acetate, taraxasterol and 3-epilupeol in the root bark [4]. A triterpene, diosgenin and two alkaloids, cadabicine and cadabcine diacetate were isolated from the bark of *C. adansonii*. The leaves contain isovitexin, proanthocyanidins, myricetin and phenolic acids, p-hydroxyl benzoic acid, vanillic acid, ferulic acid and sinapic acid [4].

Due to the widespread use of the *C. adansonii* by the local population of Burkina Faso, the present study aims at determining the antioxidant capacity of its leaves and to investigate their phenolic composition. The ferric reducing antioxidant power (FRAP), the ABTS radical cation scavenging activity (ABTS), and the free radical scavenging activity (DPPH) were used to evaluate the antioxidant capacity. Total phenolics and total flavonoids were quantified using the Folin-Ciocalteu and aluminum chloride reagents, respectively, and the phenolic profile of the most active antioxidant fraction was determined by high-performance liquid chromatography-mass spectrometry (HPLC-MS).

2. Material and Methods

2.1. Chemicals and reagents

HPLC grade methanol, and analytical grade acetic acid and hydrochloric acid were purchased from Merck (Germany). Standards: caftaric acid from Dalton (USA), gentisic acid, ferulic acid, sinapic acid, patuletin, luteolin from Roth (Germany), caffeic acid, chlorogenic acid, p-coumaric acid, hyperoside, isoquercitrin, rutoside, myricetol, fisetin, quercitrin, quercetol, kaempferol, apigenin were from Sigma (Germany). Folin-Ciocalteu reagent, NaH2PO4, Na2HPO4,

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sodium carbonate, aluminum trichloride and gallic acid were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). 2,2-Diphenyl-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate) ABTS, trichloroacetic acid, potassium persulfate, acetonitrile, methanol, acetone, n-hexane, n-butanol, dichloromethane and ethyl acetate were supplied by Fluka Chemie (Buchs, Switzerland). Potassium hexacyanoferrate [K3Fe(CN)6] was from Prolabo (Paris, France) and ascorbic acid was from Labosi (Paris, France).

2.2 Plant materials

The leaves of *Crateva adansonii* were collected in May 2011 at Gampella, 25 Km east from Ouagadougou (Burkina Faso). The plant was botanically identified by Prof. Millogo-Rasolodimby from the Biology Department of the University of Ouagadougou. A voucher specimen (MR-06) was deposited in the OUA herbarium of the CIB (Centre d'Information sur la Biodiversité), UFR/SVT of the University of Ouagadougou.

2.3 Extraction and fractionation

Dried and powered of the leaves (50 g) were used to prepare the aqueous acetone extract [10]. This extract was subject to sequential liquid-liquid fractionation to the *n*-hexane fraction (*n*-HF), dichloromethane fraction (DCMF), the acetonitrile fraction (ACNF), the ethyl acetate fraction (EAF), the methanol fraction (MeOHF) and the *n*-butanol fraction (*n*-BuOHF). For HPLC-MS analysis, the *n*-BuOHF was used for the phenolic compounds analysis by HPLC-MS as describe by Meda *et al*. [11].

2.4 Antioxidant activity determination

The antioxidant activity determination using the iron (III) to iron (II) reduction (FRAP) assay, the ABTS•+ (2,2'-azinobis-3- ethylbenzothiazoline-6-sulphonate) radical cation and the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging methods were done as described by Meda *et al*. [10]. Data were expressed in µmol ascorbic acid equivalent (AAEAC) per g of fraction, µmol trolox equivalent (TEAC) per g of fraction and µmol of quercetin equivalent (QEAC) per g of fraction, respectively.

2.5 Phytochemical analysis

2.5.1 Determination of total phenolics and total flavonoids

Total phenolics and total flavonoids of the fractions were determined using Folin-Ciocalteu and aluminium trichloride methods. The experimental procedure was fully described in Meda *et al*. [10].

2.5.2 HPLC-MS analysis of polyphenols

The *n*-butanol fraction was screened for 18 phenolic compounds analyzation using Agilent 1100 HPLC Series system. The Apparatus and chromatographic conditions have been described [11], [12]. Calibration curves in the 0.5–50 µg/mL range with good linearity (R2 > 0.999) for a five point plot were used to determine the concentration of polyphenols in the plant samples. The Agilent ChemStation (vA09.03) and DataAnalysis (v5.3) software were used for the acquisition and analysis of chromatographic data [12].

2.6 Statistical Analysis

The data were expressed as Mean ± Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p<0.0001$ and linear regression) was carried out with XLSTAT 7.1.

3. Results

3.1 Antioxidant activity

The antioxidant activity of the fractions from leaves aqueous acetone extracts are summarized in Figure 1.

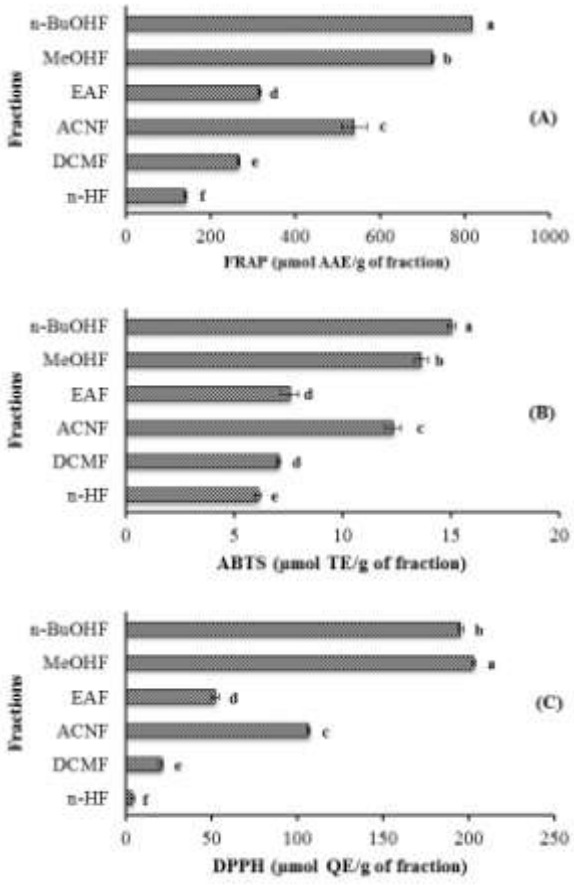

Figure 1: Antioxidant activities obtained using the FRAP **(A)**, ABTS **(B)** and DPPH **(C)** methods on *C. adansonii* leaf fractions. *n*-HF: n-hexane fraction; DCMF: dichloromethane fraction; ACNF: acetonitrile fraction; EAF: ethyl acetate fraction; MeOHF: methanol fraction; *n*-BuOHF: n-butanol fraction. Values are mean ± SD (n = 3). Different letters indicate significant difference ($p < 0.0001$)

The ability of the aqueous acetone fractions from *C. adansonii* leaves to reduce Fe(III) to Fe(II) ranged from 140.716 to 818.247 µmol AAE/g of fraction (fig 1. A). The best activities were found in the *n*-BuOHF and MeOHF 818.247 and 724.513 µmol AAE/g of fraction, respectively. The fraction obtained from n-hexane presented the lowest activity (140.716 µmol AAE/g of fraction).

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From the ABTS assay, the results are shown in Fig 1. (B) and indicate that the *n*-BuOH fraction was the most powerful (15.036 µmol TE/g of fraction), while the lowest activity was obtained with n-HF (6.102 µmol TE/g of fraction). Interesting activities were also obtained from MeOHF and ACNF with values of 13.637 and 12.348 µmol TE/g of fraction, respectively. No significant difference was found with the fractions obtained from ethyl acetate and dichloromethane ($P > 0.0001$). It was found that *n*-BuOHF scavenges twice the ABTS radical cation compared with EAF and DCMF.

In contrast, the results presented in Fig 1. (C) indicate that the MeOHF showed the most potent DPPH free radical scavenging activity (202.811 µmol QE/g of fraction) followed by *n*-BuOHF (198.470 µmol QE/g of fraction). The lowest activities were found with DCMF (20.707 µmol QE/g of fraction) and *n*-HF (3.777 µmol QE/g of fraction).
In general, the finding was that the antioxidant activity of the aqueous acetone fractions of *C. adansoni*i using the three methods was significantly higher in the fractions obtained from *n*-BuOH and MeOH solvents than in the other fractions ($p<0.0001$).

3.2 Phytochemical screening

The total phenolics per g of fraction ranged from 18.960 to 207.040 mg GAE (Table 1).

Table 1: Total phenolics and flavonoids in leaf fractions of *C. adansonii*

Fractions	Total phenolics (mg GAE/g of fraction)	Total flavonoids (mg QE/g of fraction)
n-HF	18.960 ± 0.115^{f}	0.644 ± 0.251^{e}
DCMF	54.133 ± 3.453^{e}	5.706 ± 0.564^{d}
ACNF	154.863 ± 2.802^{b}	35.370 ± 1.044^{b}
EAF	66.610 ± 1.784^{d}	17.151 ± 0.490^{c}
MeOH	207.040 ± 3.388^{a}	$33.477.861^{b}$
n-BuOHF	135.347 ± 1.288^{c}	38.357 ± 1.234^{a}

Values are mean ± SD (n = 3). Different letters in the same column indicate significant difference ($p < 0.0001$).

The highest total phenolics were obtained in the MeOHF with 207.040 mg GAE/g of fraction, followed by ANCF (154.863 mg GAE/ g of fraction) and *n*-BuOHF (135.347 mg GAE/ g of fraction). The *n*-HF contains the lowest total phenolics with a concentration of 18.960 mg GAE/g of fraction. These results showed that the phenolic compounds are more extractible by methanol than the other solvents.

Concerning the total flavonoids per g of fraction, the levels varied from 0.644 to 38.357 mg QE/g of fraction. Compared to the phenolic contents, the highest total flavonoids of 38.357 mg QE/ g of fraction were found in the *n*-BuOHF, followed by ACNF (35.370 mg QE/ g of fraction). The DCMF and *n*-HF presented the lowest total flavonoids, with contents of 5.706 and 0.644 mg QE/ g of fraction, respectively.

Based on the highest total flavonoids and antioxidant activities, the *n*-butanol fraction was selected for further identification and quantification of the individual phenols and flavonoids by HPLC-MS. In this study, eighteen phenolic compounds were investigated and the chromatographic profiles of the phenolic acids and flavonoids of *n*-BuOHF are presented in Figure 2.

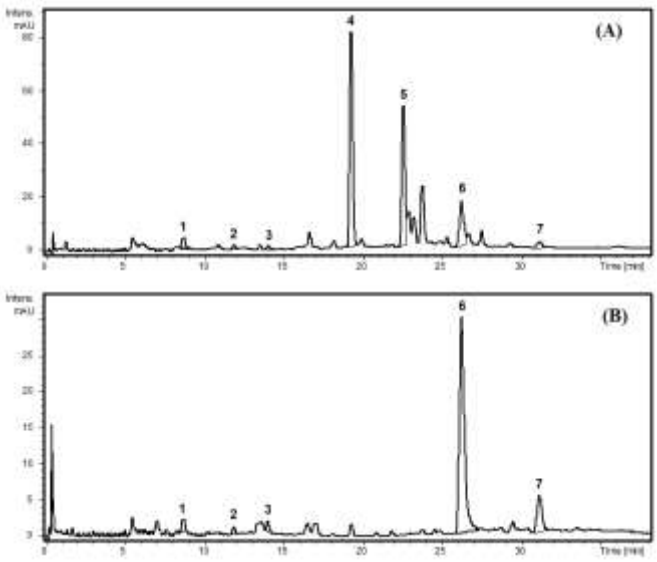

Figure 2: HPLC-MS chromatograms of *n*-butanol leaves fraction from *C. adansonii*. **(A)**: Non-hydrolyzed sample; **(B)**: Hydrolyzed sample. **1:** *p*-coumaric acid; **2:** Ferulic acid; **3**: Sinapic acid; **4:** Isoquercitrin; **5:** Quercitrin; **6:** Quercetol; **7:** Kaempferol.

Following analysis, six phenolic acids and four flavonoids were identified (Table 2).

Table 2: Identification and quantification of polyphenols in *n*-BuOHF from *C. adansonii* by HPLC-MS

Polyphenols	µg/g extracts	
	Non-hydrolyzed sample	Hydrolyzed sample
Gentisic acid	×	×
Caffeic acid	×	×
Chlorogenic acid	×	×
p-coumaric acid	750.2	268.8
Ferulic acid	192.2	131.6
Sinapic acid	250.6	185.8
Isoquercitrin	34405.2	Not found
Quercitrin	28116.8	Not found
Quercetol	3679.4	7610.4
Kaempferol	627.2	1396.6

NH: Non-hydrolyzed sample; H: Hydrolyzed sample; **×:** Qualitative

Three free cinnamic acid derivatives (*p*-coumaric acid, ferulic acid and sinapic acid) were quantified in the non-hydrolysed sample (NHS). *p*-Coumaric acid (750.20 µg/g) was the most abundant phenolic acid, followed by ferrulic acid (192.20 µg/g). Due to overlap, gentisic acid, caffeic acid and chlorogenic acid were only identified selectively through MS detection in the non-hydrolyzed and the hydrolyzed samples (HS).

The pattern of flavonoids indicated the presence of two quercetols in their glycosidic form: isoquercitrin (34405.20 µg/g) and quercitrin (28116.80 µg/g). Quercetol and kaempferol were also quantified with concentrations of

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3679.40 and 627.20 µg/g, respectively. Moreover, the fraction obtained from *n*-BuOH could contain the glycosides of quercetol and kaempferol due to the increase of their levels after hydrolysis.

4. Discussion

The healing properties of many medicinal plants have been typically attributed to their phenolic contents, mostly flavonoids and phenolic acids, due to their probable role in the prevention of diseases associated with oxidative stress (cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases). Flavonoids may help provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body [8], [9].

In this study, it was found that the *n*-butanol and methanol fractions exhibited interesting antioxidant capacities. This report suggests that the active antioxidant components of *C. adansonii* are well extracted by methanol and n-butanol. The leaves of *C. adansonii* were disclosed to be a rich source of phenolic compounds. Among the ten phenolic compounds, p-coumaric, ferulic and sinapic acids, isoquercitrin, quercitrin, quercetol and kaempferol were identified as major phenolic compounds.

The antioxidant activity of many compounds of botanical origin is proportional to the phenolic content, suggesting a causative relationship between the total phenolic content and antioxidant activity [6], [10], [11]. Positive correlations were found in this study between the total flavonoids and FRAP (R2 = 0.88), ABTS (R2 = 0.92) and DPPH (R2 = 0.82). Thus, this good antioxidant capacity could be attributed to the total flavonoids contained in *C. adansonii* leaves. Moreover, the antioxidant activity of phenolic compounds was correlated to their chemical structures [12], [13]. In general, the free radical scavenging and antioxidant activity of phenolics (e.g. flavonoids, phenolic acids) mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the nucleus, and is also affected by other factors, such as glycosylation of aglycones, other H-donating groups (-NH, -SH), etc. For example, flavonol aglycones, such as quercetin, myricetin and kaemperol, which contain multiple hydroxyl groups, have higher antioxidant activity than their glycosides, such as rutin, myricitrin, and astragalin [14]. These flavonol aglycones have been identified in the n-BuOHF of C. adansonii leaves. That may explain the sharp increase of antioxidant activity in this fraction. Ferulic acid prevents the lipid oxidation in food and free-radical-induced diseases such as cancer and atherosclerosis or aging caused by oxidative tissue degeneration [15], [16], and *p*-coumaric acid is able to protect rabbit corneal-derived cells from UVB-induced oxidation damage [17]. Gentisic acid is suggested to possess an antioxidant effect on LDL oxidation [18]. Kaempferol prevents osteoblast degeneration in osteoporosis or other degenerative disorders, and can be safely used as a chemo-preventive agent in colorectal cancer [19], [20]. Furthermore, isoquercitrin was shown to possess a wide range of biological effects (allelopathic, antioxidant, atheroprotective, and anti-inflammatory activities) and quercitrin prevents the lipid peroxidation in vitro [21], [22]. The leaves of *C. adansonii* could therefore contain bioactive substances useful in the treatment of the diseases associated with oxidative stress, justifying the widespread use of this species in the treatment of memory loss, seizures, rheumatism, weak immune system, calculus affections, stomach troubles, fever, seizures and malaria in Burkina Faso traditional medicine.

5. Conclusions

Fractions obtained from the aqueous acetone extract of *C. adansonii* leaves were analyzed for their antioxidant capacity and their phenolic composition. The results obtained in the present study indicate that *C. adansonii* leaf fractions exhibit remarkable ferric reducing power, ABTS radical cation scavenging capacity, and free radical scavenging capacity. The content and the type of phenolic compounds are responsible for this overall antioxidant activity. The leaves of *C. adansonii* were disclosed to be a rich source of phenolic compounds. Among the ten phenolic compounds, p-coumaric, ferulic and sinapic acids, isoquercitrin, quercitrin, quercetol and kaempferol were identified as major phenolic compounds. The finding was that *C. adansonii* leaves could be a potential source of natural antioxidants that could have great importance as a therapeutic agent in the prevention of cancer, aging, atherosclerosis, urolithiasis, inflammation and neurodegenerative diseases.

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