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ORIGINAL ARTICLE

Comparative study of phytochemical profile and antioxidant activity of sahelian plants used in the treatment of childhood diseases in northern Burkina Faso: case of *Petroselinum crispum* (Mill.) Fuss, *Ocimum gratissimum* L and *Acacia senegal* (L.) Willd

Alphonsine Ramde -Tiendrebeogo [1, 3 *], Moumouni Koala [1], Lazare Belemnaba[1], Marius Lompo[1] and Innocent Pierre Guissou [2]

1 Department of Traditional Medicine and Pharmacopoeia-Pharmacy / Research Institute in Health Sciences (MEPHATRA-PH / IRSS), National Center for Scientific and Technological Research (CNRST), 03 BP 7034 Ouagadougou 03, Burkina Faso

2 St Thomas d'Aquin University (USTA), Faculty of Health Sciences (FSDS) / 06 BP 10212 Ouagadougou 06 Burkina Faso

3 International Mixed Unit -Environment, Health, Societies (UMI 3189, ESS) CNRS / UCAD / UGB / USTTB / CNRST

* Corresponding author; E-mail: ramdalphonsine@gmail.com

ABSTRACT

Petroselinum crispum (PC), Ocimum gratissimum (OG) and Acacia senegal (AS) are three plants used by mothers in the treatment of childhood diseases in traditional medicine in northern region of Burkina Faso. Phytochemical screening by tube test and on HPTLC plates showed that these plants are rich in chemical compounds which explains their multiple use as food supplements and for their therapeutic virtues. Determination of total phenolic content using method of Folin-Ciocalteu Reagent (FCR), and antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) test showed that the extracts of the different plants have an antiradical activity due to their ability to trap free radical DPPH. But, the trunk bark of AS which showed the highest content ($p < 0.05$) of total phenolic compounds (376.47 ± 0.23 mg GAE/g), of tannins (17.91 ± 0.02% condensed, 11.97 ± 0.29% hydrolysable) and of flavonoids (37.17 ± 0.01 mg QE/g), also had the best antioxidant activity ($p < 0.05$) with IC_{50} value of 0.02 ± 0.01 µg/mL. This study shows that local plants adapted to drought could makes an interesting source of molecules with antioxidant property in the prevention and the treatment of childhood diseases and contribute to the health security of populations living in the Sahel region.
***Keywords**: Sahel plants, childhood diseases, phenolic compounds, radical scavenging.*

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INTRODUCTION

Environmental health risks remain a preoccupation for developing countries more particularly those of the Sahel where the prevalence of many diseases sensitive to the change of temperatures is increasing considerably [1]. Children particularly those living in poor countries are more vulnerable to health risks. Diarrhea remains a major cause of morbidity and mortality of children under 5 years of age. In sub-Saharan Africa specifically, about 800 000 children still die each year from diarrhea [2]. This diarrheal mortality is frequently associated with malnutrition or measles [3].

Located in the heart of West Africa, Burkina Faso is a sahelian country. Climate change exposes this country to permanent risks of dryness, intense heat but also to inundations. Diarrhea represents 11.5% of the main causes of child mortality and measles epidemics exist despite a remarkable vaccination coverage. The intestinal parasitic infections have a high prevalence and a negative impact on the socioeconomic plan of the country [4, 5].

Less than 40% of young children with malnutrition-related diseases have access to modern treatments in sub-Saharan Africa [6]. The World Health Organization estimates that between 2030 and 2050 climate change will cause about 250 000 additional deaths every year, among which 95 000 cases due to undernourishment of children [7]. It is therefore imperative to find palliative solutions including the valorization of sahelian plants with nutritional and / or therapeutic virtues in order to contribute to food and health security of the poorest countries.

Petroselinum crispum (Mill.) (Apiaceae) Fuss or parsley and *Ocimum gratissimum* L. (Lamiaceae) also called African basilisk are two aromatic herbs used by women of northern Burkina Faso in culinary preparations but also for therapeutic aims to treat different diseases in children, especially against malnutrition, diarrheal, measles, fever of teething, mycosis or to give a good body to children. For that, women add the leaves of these two plants in the preparation of boiled or soup for their children. Previous studies showed that *Petroselinum crispum* and *Ocimum gratissimum c*ontain vitamins (A, B, C) and minerals (Ca, P, Na, K, Fe). These two aromatic herbs contain phenolic and terpene compounds with antimicrobial and antifungal properties against a wide range of bacteria and fungi. They constitute therefore a natural alternative to chemical additives [8-12]. In addition, *Petroselinum crispum* and *Ocimum gratissimum* are highly effective against fever and diarrheal diseases, epistaxis, measles an child malaria [13-16].

The third specie *Acacia senegal* is from Fabaceae-Mimosoideae family, recognized for its main source of gum arabic production. It is used for many food industry applications. The different parts of *Acacia senegal* are also used by women from northern Burkina Faso in the treatment of childhood diseases including intestinal parasitosis, diarrhea-vomiting, malaria, or as a laxative [17, 18].

Other work showed that species of the Acacia genus have very efficient pharmacological effects due to their high tannin content, which gives them astringent, antiparasitic, antibacterial and antidiarrheal properties. Acacia species are also rich in flavonoids recognized for their antioxidant, antiradical, anti-bacillary and vermicides effects [19-21].

In addition to undernourishment, one of the consequences of children's exposure to heat in hot regions is the production of a large amount of free radicals which accumulate in their bodies without the ability to neutralize or eliminate them. In fact, previous authors have reported an alteration of the antioxidant system with high oxidative stress and decreased antioxidant defenses in malnourished children compared to those in good healthy [22, 23]. It is therefore important to promote local plants which are potential antioxidant nutrients for the management of childhood diseases.

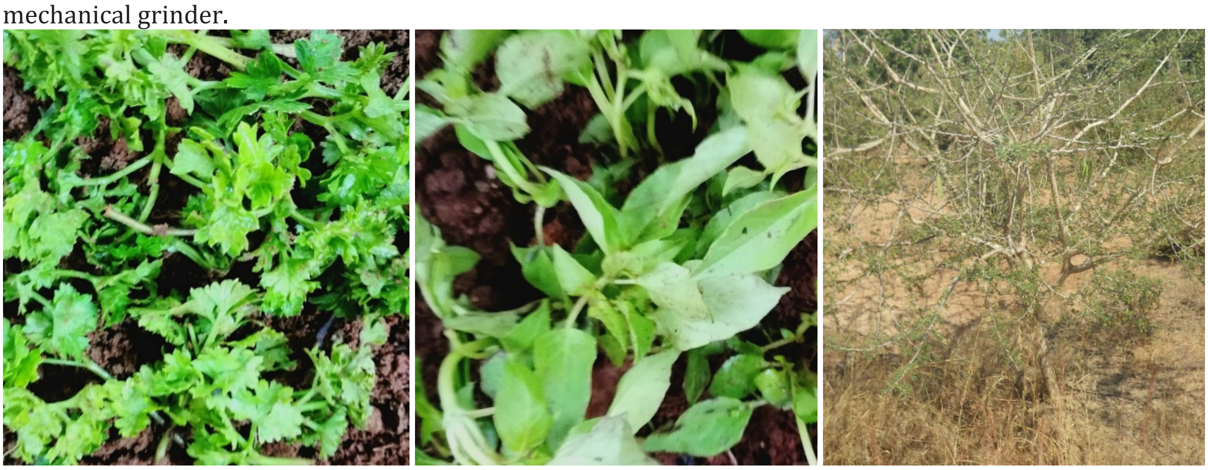
The objective of this study was to compare the phytochemical profile and the antioxidant activity of *Petroselinum crispum*, *Ocimum gratissimum* and *Acacia senegal*, three plants used in the treatment of childhood diseases.

MATERIALS AND METHODS

Materials

Plant material

The leaves of the two aromatic herbs, *Petroselinum crispum* (PC) and *Ocimum gratissimum* (OG) (Figure 1A and 1B) were harvested in a vegetable garden located on the edge of the pond of Dori (Seno province) in February 2019. The different parts (leaves, trunk bark and fruits) of *Acacia senegal* (AS) (Figure 1C) were harvested in the village of Komandjangou (Soum province) in the same period. After identification by the Botanical team of Ouaga 1 Pr Joseph Ki-ZERBO University, specimens were deposited in a herbarium under registration H1S/2019, H2S/2019 and 3S/2019 respectively. Each plant material was washed and dried in an airy room protected from sunlight for a week and finely ground into powder by a mechanical grinder.



1A. *Petroselinum crispum* **1B.** *Ocimum gratissimum* **1C.** *Acacia senegal*

Figure 1. Plants used in the treatment of childhood diseases in northern region of Burkina Faso

Chemicals materials

The chemicals products used were: 1-diphenyl-2-picrylhydrazyl (DPPH)) (Sigma,St Louis, MO, USA), 6-hydroxy 2,5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) and Folin-Ciocalteu Reagent (Sigma Chemical Company, Steinheim, Germany), NEU Reagent (Natural Products - Poly Ethylene Glycol), NaOH, Na_2CO_3 , Methanol (E. Merck, Darmstadt, Germany), Ethyl acetate (SSI, France), Sulfuric acid, Aluminum trichloride and Avcetic acid (Labosi, France), n-Hexane (SDS, France), Gallic acid, Quercetin (Sigma-Aldrich, Germany) and $AlCl_3$ (Fluka Chimika, Switzerland).

Methods

Extraction and phytochemical screening

One hundred (100) grams of dry matter was extracted by maceration at low temperature (4 °C) for 24 hours with methanol. The extraction was repeated twice with the marc obtained after each filtration. In total one (01) liter of solvent was used. The (03) filtrates were collected and then concentrated to dryness (100 mL) on a rotary evaporator at a temperature below 40 °C.

Phytochemical screening of extracts was carried out by following the method described by Ciulei [24] for tube tests and on HPTLC plates according to the analytical technique used by Kavit, Patel [25]. Plates with aluminum support Silica Gel 60 F_{254} were used. The spots of extracts were deposited by using the system of Linomat 5 (Camag, Muttez; Switzerland) spray on automated instrument for HPTLC. Eluent system (Ethyl acetate: Formic acid: Acetic acid: Water, 100 : 11 : 11 : 26, v/v/v/v) was used for the migration of flavonoids, phenolic acids, sterols and triterpenes. Another system (Ethyl acetate: Water: Methanol: n-Hexane, 11,9 : 1,6 : 1,4 : 3,5, v/v/v/v) was used for the migration of tannins. Flavonoids and phenolic acids were revealed with Neu's reagent in the presence of UV light (366 nm), sterols and triterpenes were revealed by 3% H_2SO_4 in EtOH (96%) and tannins by $FeCl_3$ 2%.

Determination of total phenolic content

Total phenolic content was estimated using the Folin–Ciocalteu Reagent (FCR) as described by Singleton, Orthofer [26] and used by Ramdé-Tiendrébéogo, Tibiri [27]. The reaction mixture consists of 1 mL of extract, 1 mL of FCR 2N and 3 mL of 20% sodium carbonate solution. This mixture is left to stand at room temperature for 40 min and then the absorbance was measured (spectrophotometer UV, Shimadzu) at 760 nm. In the control tube, the extract volume was replaced by distilled water. Calculation was based on a calibration curve obtained with increasing concentrations of gallic acid (Y= 0.0664X-0.0009; R^2 = 0.9991).The total phenolic content was expressed as milligrams of Gallic Acid Equivalent per gram (mg GAE /g) of dry material.

Determination of tannins content

Condensed tannins

The condensed tannins were estimated according to method described by Price, Van Scoyoc [28] and used by Ba, Tine [29]. The reagent was vanillin 1% (1 g of vanillin dissolved in 100 mL of 70% sulfuric acid). 2 mL of this reagent was added to 1 mL of extract. The absorbance of the mixture was read at 500 nm (spectrophotometer UV, Shimadzu) after 15 minutes of incubation in a water bath at 20 °C. The condensed tannins content T (%) was determined using the following formula:

$$T\% = 5.2\ 10^{-2} \times \frac{A.V}{P}$$

$5.2\ 10^{-2}$ = Equivalent constant of cyanidine, A= Absorbance, V = Extract volume and P = Sample weight.

Hydrolyzable tannins

The hydrolyzable tannins were estimated according to method described by Mole and Waterman [30] and used by Mboko, Matumuini [31]. One (01) mL of the extract and 3.5 mL of the reagent ($FeCl_3$ 10^{-2} M in HCl 10^{-3} M) were mixed. The absorbance of the mixture was read at 660 nm (spectrophotometer UV, Shimadzu) after 15 seconds. The hydrolysable tannins content T (%) was determined using the following formula:

$$T\% = \frac{A\ .\ PM\ .\ V\ .\ FD}{\varepsilon_{mole}\ .\ P}$$

A = Absorbance, $\varepsilon_{mole} = 2169$ (for gallic acid), PM = Weight of gallic acid (170.12 g/mol), V = volume of extract, P = Sample weight and FD = Dilution factor.

Determination of total flavonoid content

Total flavonoid content was determined according to method of Alothman, Bhat [32]. The extract was prepared at a concentration of 1 mg/mL in methanol. 1 mL of this extract was mixed with 3 mL of double-distilled water followed by 0.3 mL of $NaNO_2$ at 5% (m/v). 5 minutes later, 0.3 mL of $AlCl_3$ 10% (m/v) was

added. The whole was incubated at room temperature for 6 minutes. 1 mL of NaOH 1 N was added. The absorbance of the mixture was measured at 510 nm using a microplate reader (MP 96 spectrophotometer, SAFAS).
Calculation was based on a calibration curve obtained with quercetin as reference following the same procedure as the sample. The flavonoid content of the sample, expressed as milligrams of Quercetin Equivalent per gram of plant material (mg QE/g) was obtained by relating the absorbance read on the calibration curve.

Antiradical activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test
The antiradical activity by DPPH test was evaluated according to method of Kim, Lee [33] used by Ramdé-Tiendrébéogo, Tibiri [27]. Ten numbered tubes (1-10) were primed. The DPPH radical was dissolved in methanol (2 mg/50 mL). 0.5 mL of extract were put in tube 1 to which 2 mL of methanolic solution of DPPH radical (0.04 mg/mL) was added. Concentrations range of extracts or standard (quercetin) were prepared by cascade dilution. After 10 min incubation at 37 °C protected from light**,** the absorbance of residual DPPH was measured at 517 nm (spectrophotometer SAFAS). Antiradical activity of a sample (calculated by the following formula) is given as percentage of reduced DPPH:

$$I\% = \frac{A_0 - A_s}{A_0} \times 100$$

I = Percentage of inhibition, A_0 = Absorbance of control, As absorbance of sample. For each sample the concentration (µg/mL) required to reduce by 50% the activity of DPPH (IC_{50}) was determined.
Statistical analysis
The results are expressed as mean ± SEM (n = 3). The Data were analyzed using ANOVA (One Way ANOVA) followed by Dunnett's posttest for multiple comparisons (Graph Pad Prism version 5.0 for Windows, Graph Pad Software, San Diego California USA). Differences were considered statistically significant for a p value less than 0.05.

RESULTS AND DISCUSSION
Phytochemical profile of extracts
Preliminary phytochemical screening using tube tests reveal the presence of chemical groups including alkaloids, carbohydrates, flavonosids, saponins, steroids, tannins, acids phenols, triterpenoids (Table 1).
Table 1. Phytochemical profile of extracts.

	Herbaceous plants		**Woody plant**		
	Petroselinum crispum (Mill.) Fuss	*Ocimum gratissimum* L	*Acacia senegal* (L.) Willd		
Chemical groups	Leaves	Leaves	Leaves	Trunk bark	Fruits
Alkaloid salts	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Polyphenols	+	+	+	+	+
Flavonosids	+	+	+	+	+
Saponins	**+**	+	+	+	+
Steroids and terpenoids	**+**	+	+	+	+
Tannins	+	+	+	+	+
Carotenoïds	+	+	+	+	+
Anthracenosids aglycones	-	-	-	-	-
Acid phenolic	+	+	+	+	+

+ present, - absent
The determination of constitutes by HPTLC and the revelation of the spots showed that certain compounds react positively to Neu reagent, to 3%H_2SO_4 in EtOH (96%) and to $FeCl_3$ 2%. Flavonoids were yellow-orange and phenol acids blue. Terpenes were purple while sterols were brown. The presence of tannins was confirmed (Figure 2A and 2B).

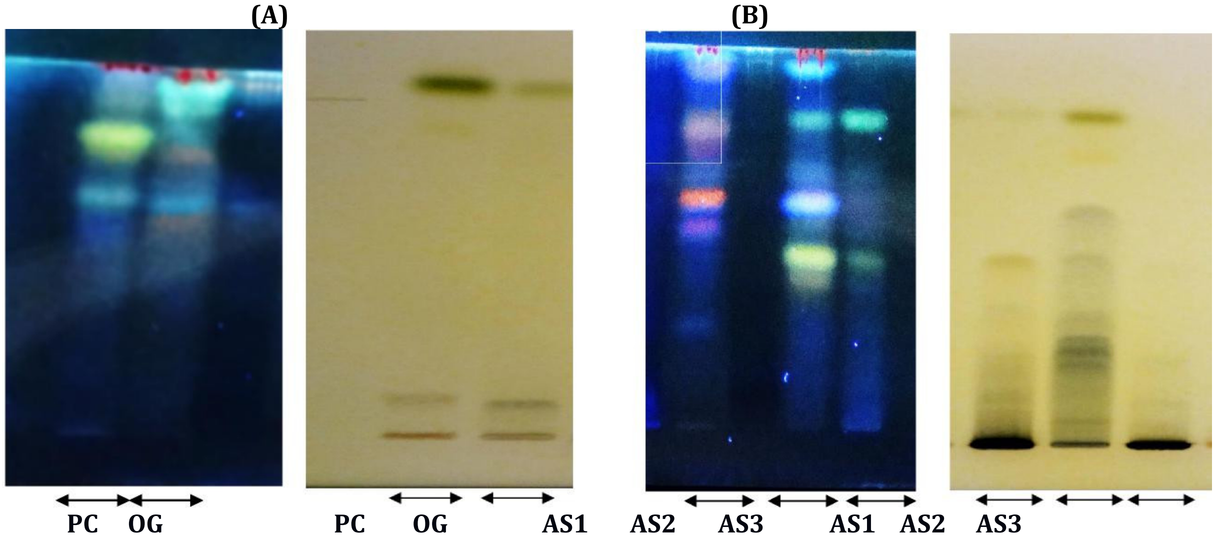


Figure 2. HPTLC of: (**A**) leaves of *Petroselinum crispum* (PC) and *Ocimum gratissimum* (OG); (**B**) parts of *Acacia Senegal* (AS); AS1 = Leaves; AS2 = Trunk bark; AS3 **=** Fruits

Antioxidant activity and phenolic content

The contents of phenolic compounds and the concentration inhibiting 50% of DPPH are regrouped in Table 2. The trunk bark of AS showed the highest content ($P < 0.05$) of total phenol compounds (376.47 ± 0.23 mg GAE /g) compared to leaves or fruits. At leaves level, those of OG had higher total phenolic contents (167.08 ± 0.06 mg GAE /g) than those of AS, then of PC. AS fruits had the lowest contents of total phenolic (97.25 ± 0.03 mg GAE / g). Tannin (condensed and hydrolyzable) and flavonoid contents were also higher in the trunk bark of AS, respectively 17.91 ± 0.02%, 11.97 ± 0.29% and 37.17 ± 0.01 mg QE/g. At leaves level they were classified as follows OG > PC > AS. The lowest levels of tannins and flavonoids were noted in AS fruits. DPPH scavenging activities of all the extracts were concentration dependent (Table 2). The best activity ($P < 0.05$) was obtained with the extract of AS trunk bark (0.02± 0.01 µg/mL, IC_{50}) which had higher level of total phenolic content. The other extracts showed a DPPH scavenging activity largely lower as compared to those of Trolox (0. 01± 0.00 µg/mL, IC_{50}) a reference antioxidant.

Table 2:Phenolic contents of the used parts of plants and radical scavenging activity by DPPH test.

	Herbaceous plants		**Woody plant**			**References substances**
	Petroselinum crispum (Mill.) Fuss Leaves	*Ocimum gratissimum* L Leaves	*Acacia senegal* (L.) Willd Leaves	Trunk bark	Fruits	Trolox
Total phenolic mg GAE/g	102.77 ± 0.02 [ab]	167.08 ± 0.06 [a]	163.39 ± 0.01[ab]	376.47 ± 0.23	97.25 ± 0.03[ab]	
Condensed tannins (%)	4.75 ± 0.00	5.81 ± 0.01	3.50 ± 0.00	17.91 ± 0.02	2.20 ± 0.01	
Hydrolyzable tannins (%)	3.78 ± 0.22	5.04 ± 0.06	2.44 ± 0.10	11.97 ± 0.29	3.34 ± 0.07	
Flavonoids mg QE/g	28 ± 0.03	35.81 ± 3.03	17.32 ± 1.98	37.17 ± 0.01	14.21 ± 0.06	
Antiradical activity against DPPH IC_{50} (µg/mL)	0.81 ± 0.06[c]	0.09 ± 0.02[c]	0.66 ± 0.05[c]	0.02 ± 0.01[c]	0.93 ± 0.07[c]	0.01 ± 0.00

With GAE = Gallic Acid Equivalent; QE = Quercetin Equivalent; All values are expressed as mean ±SEM (n = 3 of triplicate); a, $p < 0.05$ against *Acacia senegal* trunk bark; b, $p<0.05$ against leaves of *Ocimum gratissimum*; c, $p < 0.05$ against Trolox

DISCUSSION

Oxidative stress is involved in many diseases as a triggering factor or associated with complications. It results in cellular lesions causing physiological disorders and promoting the appearance of pathological processes such as oxidation of lipoprotein membrane, glycoxidation and oxidation of DNA, causing cell death [34]. Antioxidants protect the organism from the harmful action of free radicals. Some antioxidants are produced by our own body and others like vitamins C, E and beta-carotene are ingested [35].

In this study, the antiradical test with DPPH showed that the extracts of the different plants have an antioxidant property due to their ability to trap free radical DPPH. But, the trunk bark of the woody plant AS which showed the highest content of total phenolic compounds (376.47 ± 0.23 mg GAE/g), of tannins (17.91±0.02% condensed, 11.97±0.29% hydrolysable) and of flavonoids (37.17 ± 0.01 mg QE/g) also had the best antioxidant activity (0.02 ± 0.01 µg/mL, IC_{50}). Our results are in agreement with the work of the previous authors who showed that plants with best antioxidant activity contain high levels of phenolic compounds [36, 37]. Compared to the antiradical activity of the reference control Trolox (0.01± 0.00 µg/mL,IC_{50}), this result obtained with a raw extract of the trunk bark of AS shows significant antiradical activity of the active principle contained in this plant. Previous studies had also reported the therapeutic importance of woody versus herbaceous forms [38, 39].

The antioxidant property of medicinal plants is associated with the presence of several phytoconstituents including phenolic compounds such as tannins and flavonoids which would be responsible for the antiradical activity [40, 41]. Other work showed that increasing the dose of antioxidant in the diet of children suffering from malnutrition leads to a reduction of oxidative stress and an improvement in the state of health [42, 43]. The presence of flavonoids in the plants studied is very important because these compounds are recognized for their effectiveness against fever, edema and inflammation of the mucous membranes in children. In fact, the protective effects of flavonoids in biological systems are linked to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes, reducing radicals of alpha-tocopherol or to inhibit oxidases [44] .The presence of tannins in the extracts is very beneficial for mothers who use these three plants. Tannins have the property to precipitate proteins, they are astringent, antibacterial, antidiarrheal. Hydrolysable or condensed tannins have antiradical and antioxidant properties expressed by their inhibiting effect on lipid peroxidation and radical scavenging ability on DPPH radical [45]. The presence of alkaloids and saponins in extracts justifies the use of these plants for the care of children. The first are antimalarial while the second are very good antitussives, expectorants [46-48]. As for steroids, they are useful against the delays in growth [49].

In addition to their therapeutic virtues, the two herbs PC and OG are used as food supplements in children.The absence of anthracenosids aglycones could guarantee the non-toxic nature of these plants and therefore preserve the health of children. Previous work on the essential oil of OG has shown that it contains chemical compounds such as thymol, tert-Butanol, and O-cymene for the most part. Thanks to these chemical constituents, essential oil of OG has an inhibitory power of lipoxygenase L-1 and cyclooxygenase-1, two enzymes involved in the production of mediators of inflammation [11, 50].

Other authors have shown that the leaves of PC and OG are rich in vitamins (A, B, C, K), and minerals (Ca, P, Na, K, Fe) [12, 51].

Acacia senegal is a woody plant, perennial, widely distributed and adapted to the drought of Sahelian countries. It contributes to soil protection against erosion by wind and runoff. Also the richness of this plant in total phenolic compounds, tannins and flavonoids with antiradical properties as showed in this study and by previous authors [52, 53] makes it an interesting source of molecules with antioxidant property in the prevention and the treatment of childhood diseases.

CONCLUSION

The objective of this study was to compare the phytochemical profile and the antioxidant activity of two herbaceous (*Petroselinum crispum, Ocimum gratissimum*) versus one woody (*Acacia senegal*) used in the treatment of childhood diseases in the Sahel region of Burkina Faso. Results showed that the three plants are rich in chemical compounds which explains their multiple use in traditional medicine. However *Acacia senegal,* a perennial drought-adapted plant, had the best antioxidant activity related to its high levels of total phenolic compounds, tannins, and flavonoids. This study shows that local plants adapted to drought could contribute to health security of populations living in the Sahel region. Further bioguided studies should make it possible to isolate and identify new bioactive molecules for the treatment of the most common diseases in the Sahel countries.

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