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A comparative study of phytochemical profile and antioxidant activity of Sahelian plants used in the treatment of infectious diseases in northern part of Burkina Faso: *Acacia seyal* Delile and *Acacia tortilis* (Forssk.) Hayne subsp. *raddiana* (Savi)

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***Acacia seyal* and *Acacia tortilis* are used in the treatment of infectious diseases in traditional medicine by population in Northern Burkina Faso. Phytochemical screening by tube test and on HPTLC plates showed the presence of important chemical compounds in these plants. 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 this biological activity is related to phenolic content. The trunk bark of *A. tortilis* had an important antioxidant activity with IC_{50} value of 0.01±0.01 µg/mL due to its highest content ($p<0.05$) of total phenolic compounds (383.19±0.07 mg GAE/g), of condensed tannins (18.21±0.04%) and flavonoids (66.09±0.06 mg QE/g). This antiradical activity was comparable to that of Trolox used as reference. Radical scavenging activity of leaves of *A. seyal* was also significant with IC_{50} value of 0.02±0.01 µg/mL. Its total phenolic compounds, condensed tannins and flavonoids were estimated at 371.43±0.09 mg GAE/g, 14.24±0.00% and 52.72±0.10 mg QE/g, respectively. This study shows that local plants adapted to drought could make an interesting source of molecules with antioxidant property in the prevention and the treatment of infectious diseases.**

Key words: Sahel plants, infectious diseases, phenolic compounds, radical scavenging.

INTRODUCTION

Climatic warming is the most serious environmental problems which has led to the development of highly transmissible and virulent infectious diseases such as Ebola, Dengue, Zika, or Severe Acute Respiratory Syndrome (Debil, 2013).

These emerging or re-emerging diseases have occurred in recent decades and constitute real threats to public health (Chidiac and Ferry, 2016). The tropical regions of

Africa affected by dryness are the most vulnerable. Increasing temperatures in these regions favor the proliferation of mosquitoes. Malaria and dengue are the most common vector infectious diseases in these areas (Githeko et al., 2000). Indeed, climate change is responsible for 6% of malaria cases in some low-income countries and the capacity of mosquitoes carrying the dengue virus to transmit this infection has increased by 9.4% since 1950 (Kraemer et al., 2015). According to World Health Organization, the incidence of these diseases will increase each year with climatic warming (WHO, 2014). Burkina Faso has experienced drought since 1970, which has allowed desertification to gain ground every year. Previous studies showed that infectious diseases are the most common in this country (Besancenot et al., 2004). The high cost of imported medicines and the cultural attachment to effectiveness of recipes based on plant makes that at least 80% of rural people living in developing countries depend on traditional medicine for their needs in healthcare (OMS, 2013). The renewed interest in herbal medicines in developed countries could be explained by the emergence and expansion of various pathologies of bacterial and/or viral origin, which often resist to conventional medicine treatments. It is also due to undesirable effects of some synthetic pharmaceutical drugs (Moore et al., 1985). In addition, thanks to World Health Organization's politics of promoting traditional medicine, herbal medicines occupy today a considerable place in international pharmaceutical trade (OMS, 2013). The *Acacia* genus of Fabaceae-Mimosoideae family is widely spread in arid zones, tropical forests and driest regions of the world including Sahelian countries of Africa. It contains more than 1350 species, present in bushes form and sometimes as large trees (Ibrahim and Aref, 2000). Acacia species are used in planting and sylvo-pastoral programs to mitigate the effects of desertification. They are the most available and play an important socio-economic and therapeutic role in the Sahel regions. *Acacias* are used daily by local populations as food, medicine, energy, building materials, fodder for cattle and as source of large quantities of gum arabic (*Acacia senegal* and *Acacia seyal*) (Guinko, 1997). Previous work showed that *Acacia* spp. have very efficient pharmacological properties. Indeed, the presence of some chemical groups in these species, such as tannins known for their astringent, antiparasitic and antibacterial properties as well as anthocyanins and flavonoids recognized for their anti-inflammatory, antioxidant and antiradical effects would justify the preferential choice of the genus Acacia in the treatment of some infections (MacRae et al., 1989; Okuda et al., 1991).

Other studies showed that one of the consequences of exposure of populations to high intensities of solar radiation is the increase of oxidative stress which causes an overproduction of free radicals in body and the alteration or suppression of cellular immunity. This reinforces the risks of infectious diseases (Favier, 2006). It is therefore important to promote alternative and local therapy based on plants potential antioxidant in order to reinforce the resilience of the population facing the effects of climate.

The objective of this study was to compare the chemical profile and the antioxidant activity of *A. seyal* and *Acacia tortilis* used in the treatment of infectious diseases in Northern Burkina Faso.

MATERIALS AND METHODS

Plant

A. seyal called Bulbi in Fulfulde language is a thorny bush, sometimes a tree up to 12 m tall. Leaves are twice composed and the bark often smooth breaks loose by irregular plates. *A. tortilis* or *Acacia* false gum called Tchiluki is also a thorny which can reach more than 15 m high. The peak is displayed in parasol, leaves are twice pinnate and the bark is often red-brown. *A. seyal* and *A. tortilis* are used in some regions of Africa to treat different diseases in traditional medicine such as jaundice, bilious fevers, skin allergies, diabetes, hypertension or as diuretic (Jaouadi et al., 2015). These two species are used by populations in Northern Burkina Faso in traditional recipes for the treatment of infectious diseases such as yellow fever, dysentery, gonorrhea, schistosomiasis, ulcers, and pulmonary infections but especially against malaria and dengue.

The different parts (leaves and trunk bark) of *A. seyal* and *A. tortilis* were harvested in Mamassirou village (Soum province) in March 2019. After identification by the Botanical team of Ouaga 1 Pr Joseph Ki-ZERBO University, specimens were deposited in a herbarium under registration 4S/2019 and 5S/2019, respectively. Each plant material was washed and dried in an airy room, protected from sunlight for a week and finely ground by a mechanical grinder.

Chemical material

The chemicals products used were: 1,1-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, Aluminium trichloride and Acetic acid (Labosi, France), n-Hexane (SDS, France), Gallic acid, Quercetin (Sigma-Aldrich, Germany) and $AlCl_3$ (Fluka Chemika, Switzerland)

Extraction and phytochemical screening

One hundred grams of dry matter was extracted by maceration at

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low temperature (4°C) for 24 h with methanol. The extraction was repeated twice with the marc obtained after each filtration. In total, 1 L of solvent was used. The different 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 (1982) for tube tests and on HPTLC plates according to the analytical technique used by Kavit et al. (2013). Plates with aluminium 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 et al. (1999) and used by Ramde-Tiendrebeogo et al. (2012). 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 of dry material (mg GAE/g).

Determination of tannins content

Condensed tannins

The condensed tannins were estimated according to method described by Price et al. (1978) and used by Ba et al. (2010). 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 min of incubation in a water bath at 20°C. The condensed tannins content T (%) was determined using the following formula:

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

where $5.2\ 10^{-2}$ = 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 (1987) and used by Mboko et al. (2017). 1 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 s. The hydrolysable tannins content T (%) was determined using the following formula:

$T\% = A.\ PM.\ V.\ FD/\mathcal{E}_{mole}.\ P$

where A = Absorbance, $\mathcal{E}_{mole}$ = 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 et al. (2009). 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 min later, 0.3 mL of $AlCl_3$ 10% (m/v) was added. The whole was incubated at room temperature for 6 min. 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 increasing concentration of quercetin solution (Y=0.0001X+0.0014; R^2=0.9891) following the same procedure. 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 1, 1-diphenyl-2-picrylhydrazyl (DPPH) test

The antiradical activity by DPPH test was evaluated according to the method of Kim et al. (2003) used by Ramde-Tiendrebeogo et al. (2012). Ten numbered tubes (1-10) were primed. The DPPH radical was dissolved in methanol (2 mg/50 mL). 0.5 mL of the extract was 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:

$I\% = (A_0\text{-}A_S)/A_0 \times 100$

where I = percentage of inhibition, A_0 = absorbance of control, and A_S = 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 One Way Analysis of Variance (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

Chemical screening of extracts

Preliminary phytochemical screening using tube tests revealed the presence of chemical groups including alkaloids, carbohydrates, flavonoids, saponins, sterols, tannins, phenolic acids, and triterpenes (Table 1). The determination of constituents by HPTLC and the revelation of the spots, showed that certain compounds

Table 1. Chemical composition of leaves and trunk bark extracts of *Acacia seyal* and *Acacia tortilis*.

Chemical group	*Acacia seyal*		*Acacia tortilis*	
	Leaves	Trunk bark	Leaves	Trunk bark
Alkaloid bases	-	-	-	-
Alkaloid salts	+	+	+	+
Carbohydrates	+	+	+	+
Polyphenols	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Sterols	+	+	+	+
Triterpenes	+	+	+	+
Tannins	+	+	+	+
Carotenoids	+	+	+	+
Anthracenosids aglycones	-	-	-	-
Phenolic acids	+	+	+	+

+ Present, - absent.

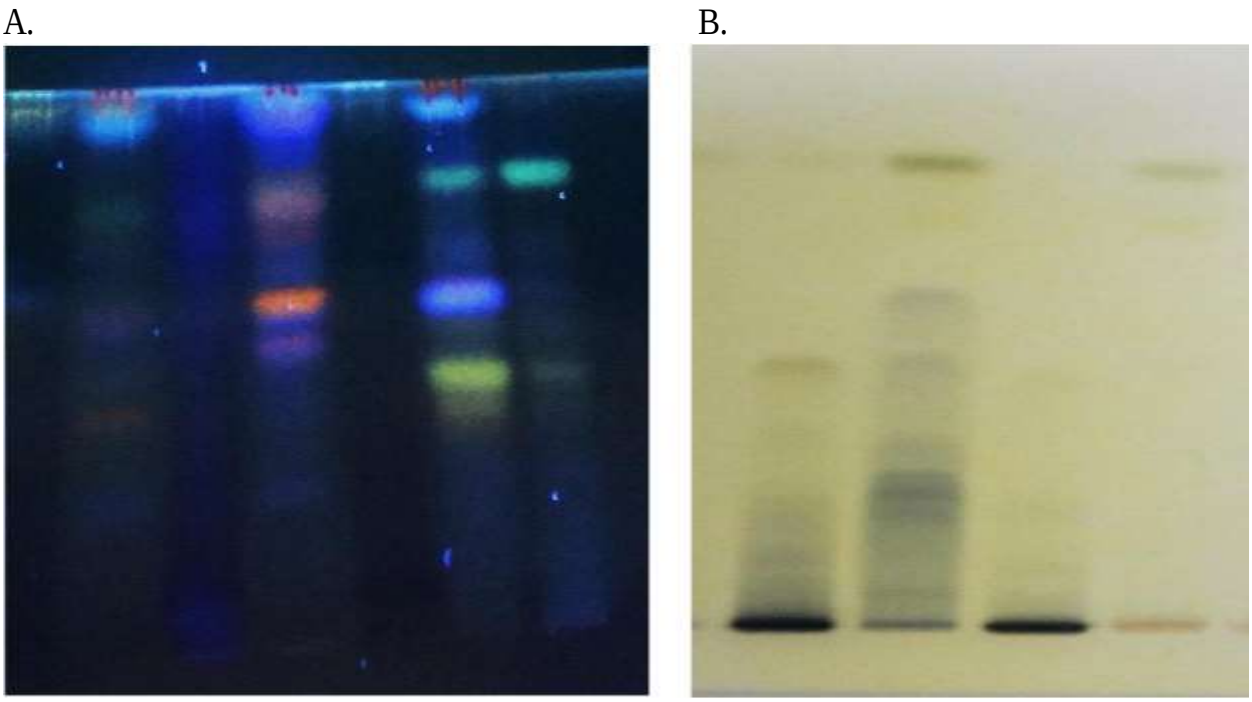

Figure 1. Determination of phenolic compounds of leaves and trunk bark extracts of *Acacia seyal* and *Acacia tortilis* on HPTLC plates.

react positively to Neu reagent, to 3% H_2SO_4 in EtOH (96%) and to $FeCl_3$ 2% (Figure 1A and B). Flavonoids were yellow-orange and phenolic acids blue. Terpenes were purple while sterols were brown. The presence of tannins was confirmed.

Antioxidant activity and phenolic contents

The contents of phenolic compounds and the concentration inhibiting 50% of DPPH are regrouped in Table 2. The trunk bark of *A. tortilis* showed the highest

Table 2. Phenolic contents of leaves and trunk bark and antiradical activity by the DPPH test.

Plant	*Acacia seyal*		*Acacia tortilis*		*Reference substance*
	Leaves	Trunk bark	Leaves	Trunk bark	Trolox
Total phenolic mg GAE/g	371.43 ± 0.09[a]	310.33 ± 0.06[ab]	307.89 ± 0.15[ab]	383.19 ± 0.07	-
Condensed tannins (%)	14.24 ± 0.00	8.85 ± 0.07	7.37 ± 0.00	18.21 ± 0.04	-
Hydrolyzable tannins (%)	7.11 ± 0.20	12.77 ± 0.07	11.59 ± 0.09	10.21 ± 0.08	-
Flavonoids mg QE/g	52.72 ± 0.10	41.15 ± 0.04	43.52 ± 0.14	66.09 ± 0.06	-
Antiradical activity IC_{50} (µg/mL)	0.02 ± 0.01[c]	0.03 ± 0.02[c]	0.03 ± 0.01[c]	0.01 ± 0.01	0.01 ± 0.00

Values are mean±SEM (n = 3); [a]p < 0.05 against *Acacia tortilis* trunk bark; [b]p<0.05 against leaves of *Acacia seyal*; [c]p < 0.05 against Trolox. GAE, Gallic acid equivalent; QE, quercetin equivalent.

content of total phenol compounds (383.19±0.07 mg GAE/g) (P < 0.05) than its leaves and the different parts of *A. seyal*. In addition, it had the highest percentage of condensed tannins (18.21±0.04%). *A. seyal* trunk bark had the highest percentage of hydrolysable tannins (12.77±0.07%). Flavonoid content ranged from 66.09±0.06 mg QE/g for the highest at the lowest 41.15±0.04 mg QE/g obtained, respectively with the trunk bark of *A. tortilis* and *A. seyal*. The best activity of DPPH scavenging (P < 0.05) was obtained with the extract of *A. tortilis* trunk bark (0.01±0.01 µg/mL, IC_{50}) which had higher level of total phenolic, condensed tannins and flavonoids. This antiradical activity was comparable to that of Trolox used as reference. The leaves of *Acacia seyal* which had phenolic content, condensed tannins and flavonoids estimated to 371.43±0.09 mg GAE/g, 14.24±0.00% and 52.72±0.10 mg QE/g, respectively showed significant antiradical activity with IC_{50} value equal to 0.02±0.01 µg/mL compared to its trunk bark and *A. tortilis* leaves.

DISCUSSION

Oxidative stress is an imbalance of the oxidant-antioxidant balance in favor of oxidants. It develops when free radicals or oxidative molecules are produced faster than they can be neutralized by the body (Houssaini et al., 1997). Oxidative stress is secondary to the establishment of pathology but contributes to its immune or vascular complications (Atamer et al., 2008). Previous studies showed that reducing oxidative stress can greatly improve the health status of populations (Rorive et al., 2005). It is therefore important to evaluate the antioxidant potential of natural resources that can act in the prevention or treatment of infectious diseases. Results showed that A. seyal and *A. tortilis* have an antioxidant property due to their ability to trap free radical DPPH. But, methanol extract of the trunk bark of *A. tortilis* which had the highest content of total phenolic compounds (383.19±0.07 mg GAE/g), of condensed tannins (18.21±0.04%) and flavonoids (66.09±0.06 mg QE/g) also had the best antioxidant activity (0.01±0.01 µg/mL, IC_{50}) comparable to that of Trolox (0.01±0.00 µg/mL, IC_{50}) used as reference. The present results are in agreement with previous studies which reported the antioxidant activity of some *Acacia* spp. and showed that this antioxidant activity is related to phenolic content (Abdel-Farid et al., 2014). The result obtained with a raw extract of A. tortilis trunk bark shows significant antiradical activity of the active principle contained in this plant. Tannins and flavonoids are recognized for their important antioxidant activity. Indeed the protective effects of flavonoids in biological systems are linked to their ability to transfer electrons to free radicals, to activate antioxidant enzymes or to inhibit oxidases (Bruneton, 2009; Santi et al., 2018). Previous work showed that Vicenin and Rutin which are two flavonoids isolated in A. tortilis have the ability to remove hydroxides and peroxides (Seigler, 2003; Jaouadi et al., 2015). The tannins have antiradical and antioxidant properties expressed by their inhibiting effect on lipid peroxidation and radical-scavenging ability on DPPH radical (Bouchet et al., 1998). The antiradical activity obtained with *A. seyal* leaves is quite remarkable (0.02±0.01 µg/mL, IC_{50}). The use of its leaves is to encouraged due to the fact that *A. seyal* being an important source of gum arabic marketing, using its leaves allows to avoid destruction of the plant and to preserve its durability. Other phytoconstituents exist in both plants as shown in Table 1, such as alkaloids, triterpenes, sterols, saponins, and phenolic acids. This could justify their multiple uses in traditional medicine.

A. seyal and A. tortilis are woody plants, perennial, widely distributed and adapted to drought of Sahelian countries. They contribute to soil protection against erosion by wind and runoff. Also, the richness of these plants in total phenolic compounds, tannins and flavonoids with antiradical properties as demonstrated by this study as well as by other authors makes them interesting source of bioactive molecules. It is therefore urgent to promote the therapeutic experience of Sahelian local populations in order to develop improved traditional medicines or to isolate new bioactive molecules for the

prevention and treatment of infectious diseases that continue making more victims each year.

Conclusion

The objective of this study was to compare the phytochemical profile and antioxidant activity of two Sahelian plants (*A. seyal* and *A. tortilis*) used in the treatment of infectious diseases by populations in Northern Burkina Faso. Results showed significant antiradical activity of *A. tortilis* trunk bark and the leaves of *A. seyal* related to the importance of their phenolic contents. 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 infectious diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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