Original Research Article

Phytochemical composition and antioxidant activity of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* used against seed-borne fungi in Burkina Faso

ABSTRACT

Aims: Hydro-ethanolic extracts of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* from Burkina Faso were investigated for their phytochemical composition and their antioxidant activities.

Methods: High-performance thin-layer chromatography (HPTLC) method was used for phytochemical screening. The total phenolic, total flavonoid and anthocyanin contents of extracts were assessed. The antioxidant potentials using 2,2-diphenyl-l-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays of the extracts were also evaluated.

Results: Phenolic compounds, flavonoids and anthocyanins were present in all these plant extracts. Tannins were only found in *Acacia gourmaensis* extract. *Acacia gourmaensis* extract exhibited the highest total phenolics (807.58 ± 28.63 mg GAE/g), total flavonoids (271.39 ± 0.58 mg QE/g), total anthocyanins (83.16 ± 0.14 µg/g) contents and had the highest antioxidant activity by DPPH (330.84 ± 16.23) and FRAP methods (3211.11 ± 52.24). *Balanites aegyptiaca* and *Securidaca longepedunculata* showed the lows phenolic compounds (80.72 ± 2.11 mg GAE/g and 76.69±1.84 mg GAE/g respectively); total flavonoids (88.7 ± 1.65 mg QE/g and 104.54 ± 9.65 mg QE/g respectively), anthocyanins (24.49±1.43 µg/g and 24.57±0.52 µg/g respectively) contents and had the low antioxidant activity for DPPH method (46.83 ± 3.01 and 56.20 ± 3.79 mg AAE/100g respectively) and FRAP method (102.06 ± 5.09 and 57.78 ± 0.99 mg AAE/100g respectively).

Conclusion: *Balanites aegyptiaca*, *Securidaca longepedunculata*, and *Acacia gourmaensis* represent natural sources of phenolic compounds, antioxidant activity and antifungal properties.

Keywords: Balanites aegyptiaca, Securidaca longepedunculata Acacia gourmaensis, Phytochemical compounds, Antioxidant activity, Antifungal activity.

1. INTRODUCTION

Fungi are the cause of important crop diseases. Many of these pathogens are carried on or inside seeds and can reduce seed germination and seedling emergence [1]. Seed-borne pathogens may cause seed abortion, seed rot, seed necrosis, reduced or eliminated germination capacity as well as seedling damage [2].

Plant extracts are considerable natural sources of antimicrobial compounds for the control of human and plant diseases [3]. Natural plant products are an important source of new chemicals in agriculture [4]. Plant-derived pesticides are available and cost effective in countries where synthetic pesticides are expensive [5]. A considerable number of natural products and medicinal plants contain some active phytochemical ingredients such as phenolic compounds, flavonoids, alkaloids, tannins, coumarins, curcuminoids or terpenes that induce various biological activities in animals including antioxidant, anti- inflammatory and anti-cholinesterase effects [6,7]. Plants used for their antifungal properties in Burkina Faso include *Balanites aegyptiaca*, *Securidaca longepedunculata*, and *Acacia gourmaensis* [8,9,10].

Balanites aegyptiaca (L.) Delile, known as desert dates belonging to the Zygophyllaceae family, is one of the most common wild plant species in the drylands of Africa and South Asia [11]. The plant (leaves, roots and bark, fruits) is used in phytotherapy for its potential antimicrobial effect [12]. Almost all parts of this plant are used in traditional medicine for anti-inflammatory, anti-helminthic, insecticidal, anti-molluscicidal, anti-fungal, anti-bacterial activities [12]. It is traditionally employed in the treatment of jaundice, yellow fever, syphilis, diarrhea, epilepsy, cough and wound healing [13].

Securidaca longepedunculata Fres, commonly known as violet tree, is a savanna grown medicinal plant belonging to the Polygalaceae family. It is commonly used as a medicine in many parts of Africa for the treatment of rheumatic conditions, fever, headache and various other inflammatory conditions [14]. Dried roots powder is also used as a pest control agent in storage, and methanol extracts of the roots have the potential to protect against insect pests and microbial agents [15][16].

Acacia gourmaensis A. Chev. is the member of the Fabaceae family known in Burkina Faso. The aqueous extract of this plant is used for antifungal activity [17].

In recent studies, *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* hydro-ethanolic extracts are used against seed-borne fungi in Burkina Faso [9,10]. It is therefore necessary to study the phytochemistry and biological properties of these plant extracts to confirm their antifungal activity.

The aim of the present work was to evaluate the phytochemical composition as well as the antioxidant activity of hydro-ethanolic extracts of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis*.

2. MATERIAL AND METHODS

2.1. Plant material

Fresh stem bark of *Balanites aegyptiaca, Securidaca longepedunculata* and *Acacia gourmaensis* was harvested from different trees during May 2018 in Mogtedo localized in the Plateau-Central region of Burkina Faso. The plant material was washed with tap water to remove debris and dust particles and then rinsed with sterile distilled water. They were dried under shade at 25°C, pulverized with a pestle and mortar, then kept in a sterile transparent polyethylene bag and stored at 4°C until used.

2.2. Phytochemical composition

2.2.1. High-performance thin-layer chromatography (HPTLC) screening

Phytochemical screening of standard solution and samples (Se, Ba and Ag) extracts was performed on $20\,\mathrm{cm} \times 10\,\mathrm{cm}$ silica gel $60\,\mathrm{F}_{254}$ HPTLC (glass) plate (Merck, Darmstadt, Germany). 2 µL of each extract were applied as 5 mm bands with a semi-automatic plate spotter (CAMAG, Linomat V, Switzerland) set to dispense along a line 10 mm from the

bottom edge of the plate. The distance between tracks was 10 mm. Distances from left and right edge of the plate were 20 mm. The plates were placed in a 20×20 cm vee-bottomed TLC tank (saturation time 30 min) containing ethyl acetate: formic acid: acetic acid: water (100:11:11:26) and ethyl acetate: water: methanol: n-hexane (11.9:1.6:1.4:3.5) respectively for flavonoids and tannins. The developed plates were then dried with an air dryer (cold air) for 5 min. Concerning flavonoids, the plate was heated at 105°C for two (02) min and sprayed with the Neu reagent. Evaluation was performed under UV 366 nm. As for the tannins, the plate was sprayed with a 2 % $FeCl_3$ reagent. Evaluation was performed under white light.

2.2.2. *Determination of total phenolic content*

Total phenolic content was determined, according to Singleton *et al.* [18]. Different plants extracts (25 µL, 100 µg/mL in Methanol) were mixed with Folin Ciocalteu reagent (105 µL, 0.2 N) and 5 min later with sodium bicarbonate (100 µL, 75 g/L). After 1-hour incubation, the absorbance of each mixture was measured at 760 nm against a blank with a microplate reader. A standard calibration curve was plotted using Gallic acid (0-100 mg/L). Polyphenol content was expressed as mg of Gallic acid equivalent per g of extract (mg GAE/g).

2.2.3. Determination of total flavonoids content

The total flavonoids content was estimated according to the method of Dowd as adapted by Arvouet–Grant *et al.* [19]. Different extracts of plants (75 µL, 100 µg/ mL) were mixed with aluminum trichloride (75 µL, 2% in methanol). Absorbances were read at 415 nm after 10 min of incubation against a blank using a microplate reader. Total Flavonoids content was expressed as mg of quercetin equivalent per g of extract (mg QE/g) using a standard calibration curve of quercetin (0-150 mg/L).

2.2.4. Total anthocyanins assays

Anthocyanin contents of samples were carried out by spectrophotometer (SHIMAZU) following the pH-differential method [20]. Two buffers were used in this method: potassium chloride pH1.0 (0.025 M) and acetate buffer pH4.5 (0.4 M). Briefly, 0.2 mL of sample extract was added to 1.8 mL of each buffer. Each mixture was read against a blank at 510 and 700 nm. Absorbance (A) was evaluated following the formula:

$A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$

The concentration of total monomeric anthocyanins in the sample was determined according to the calculation of cyanidin-3-glucoside concentration below:

$$TAC\ (mg/L) = \frac{(A \times M \times FD \times 1000)}{(\varepsilon \times l)}$$

A: Absorbance; **M:** Molecular Weight; (449.2); **DF**: Dilution Factor; **ε**: Molar Absorptivity (26900).

2.3. Antioxidant activity

2.3.1. DPPH Radical Scavenging Activity

The ability of plant extracts to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was evaluated at 517 nm, as described by Sombié *et al*. [21]. The DPPH Radical Scavenging Activity was expressed as µg of ascorbic acid equivalent per g of extract (µg AAE/g of extract).

2.3.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The ability of the plant extracts to reduce iron (III) to iron (II) was measured at 700 nm following the procedure described by Sombié *et al*. [21]. Iron (III) reducing activity was determined as µg quercetin equivalent per g of dry seed extract (µg QE/g of extract).

3. RESULTS AND DISCUSSION

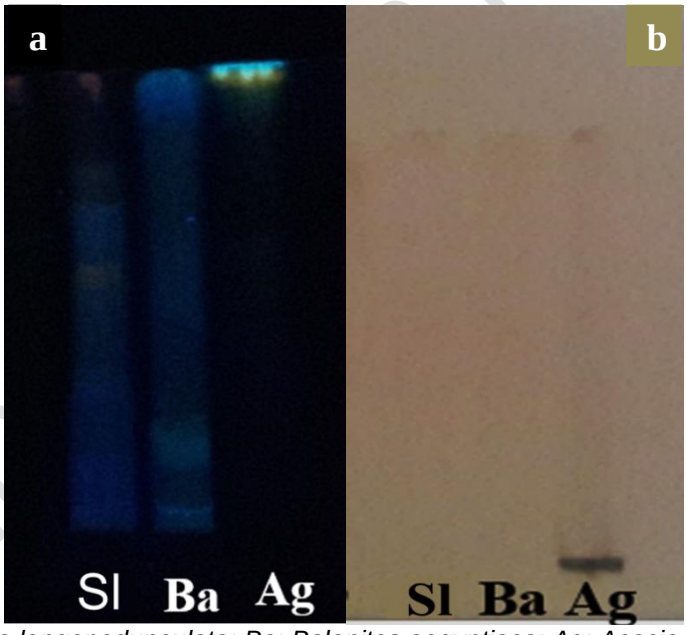
Three local plants, *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* from Burkina Faso used as antifungal extracts were studies to confirm their biological activities.

Hydro-ethanolic extract of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaencis* screened for their phytochemical composition by HPTC were presented in Fig 1. Two major phytochemical groups are identified in the tree plants extracts: flavonoids and tannins.

Flavonoids are secondary metabolites and are considered to be one of the most common groups of natural constituents found in plants [22]. Kahkonen *et al*. [23] stated that flavonoids are probably the most important natural phenolics due to their broad spectrum of chemical and biological activities, including antioxidant, antimicrobial activity and free radical scavenging properties.

In this study, flavonoids were found in all these three species with a relative abundance and less differentiation in *Balanites aegyptiaca* and *Securidaca longepedunculata* (Fig. 1).

Tannins are polyphenolic secondary metabolites of higher plants [24]. They are associated with plant defense mechanisms [25]. The group of vegetable tannins is composed of two classes, the‘‘hydrolysable’’ and the ‘‘condensed’’ tannins according to their chemical structure and properties. It has been demonstrated that condensed tannins have more antimicrobial properties [24]. The present study reported that tannins are more present in *Acacia gourmaensis* extract (Fig. 1). Previous studies revealed the presence of tannin only in *Securidaca longepedunculata* leaves extracts [16,26]. However, tannin is seldom found in *Balanites aegyptiaca* extract [13,27].



Sl: Securidaca longepedunculata; Ba: Balanites aegyptiaca; Ag: Acacia gourmaensis.

Fig 1: Phytochemical screening of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* (a): flavonoids, (b): tannins

Further confirmation by quantitative assays of phenolic compounds, flavonoids, and anthocyanins content of three plant species is shown in Fig 2.

Phenolic compounds are commonly found in plants and have been reported to have a wide range of biological activities, including antioxidant properties [28,29]. In this study, the total

polyphenol content of *Acacia gourmaensis* hydro-ethanolic extract was 807.58±28.63 mg GAE/g and showed a highly significant difference ($P < 0.05$). *Balanites aegyptiaca* and *Securidaca longepedunculata* had low total phenolic contents of 80.72±2.11 and 76.69±1.84 mg GAE/g extract, respectively. Another investigation in Burkina Faso reported law total polyphenol content (532 mg GAE/g) in stem bark methanolic extract of *B. aegyptiaca* [30]. Muanda *et al*. [31] showed that the aqueous methanol root extract of *Securidaca longepedunculata* content was 9.86 mg GAE/g.

Total flavonoids were ranged from 271.39±58.46, 104.54±9.65 to 88.70±1.65 mg QE/g of extract respectively for *Acacia gourmaensis, Securidaca longepedunculata, and Balanites aegyptiaca*. *Acacia gourmaensis* hydro-ethanolic extract contained significantly higher total flavonoid contents ($P < 0.05$) than the two other plants studied in this work. Previously in Burkina Faso, Karama *et al.* [32] were shown that *S. longepedunculata* methanolic extract content more flavonoids in leaves from warm period (40.96 ± 0.19 mg QE/g) than leaves collected in cold period (28.74 ±0.39 mg QE/g). In other hands, Ouedraogo *et al*., [30] founded 14±0.02 mg GAE/g flavonoids in the stem bark methanolic extract of *B. aegyptiaca*.

Anthocyanins are water-soluble pigments derived from flavonoids via the shikimic acid pathway [33]. Anthocyanins are attractive compounds due to their biological properties, mainly as antioxidants or antifungal properties [34,35]. The Anthocyanins content in our hydro-ethanolic plant extracts were 83.16±0.14, 24.57±0.52 and 24.49±1.43 µg/g respectively for *A. gourmaensis, S. longepedunculata and B. aegyptiaca*. *Acacia gourmaensis* extract contained a significantly higher content of anthocyanin among all the other plants ($P < 0.05$).

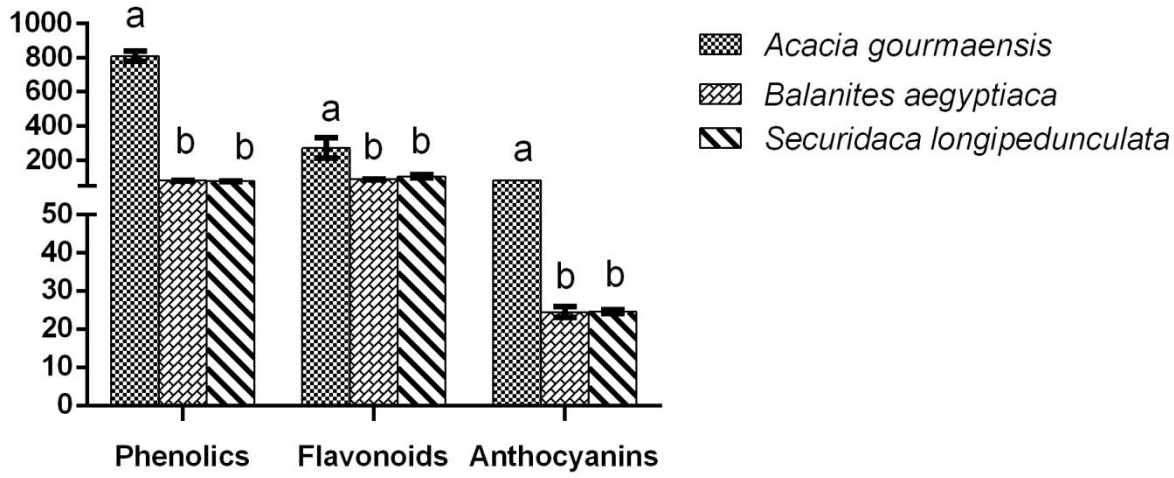


Fig 2. Phytochemical content of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis*

The antioxidants activities of three plant extracts were analyzed using the free radical scavenging capacity 2,2-diphenyl-1-picrylhydrazyl (DPPH), the ferric reducing antioxidant power (FRAP) (Fig. 3).

The DPPH radical scavenging activity test is an 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 [36]. Chemicals which are able to change the color of the DPPH free radical from violet to yellow can be considered as antioxidants and, therefore, radical scavengers [37]. *Acacia gourmaensis* hydro-ethanolic extract of the present study showed high DPPH radical scavenging activity (330.84±16.23 mg AAE/100 g of powder) with a significant difference than two other plants ($P < 0.05$). *Securidaca longepedunculata* and *Balanites aegyptiaca* extract showed low DPPH scavenging activity with 56.20± 3.79 and 46.83± 3.01 mg AAE/100 g respectively.

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 assay determined the reducing power of plant extracts resulting from the ability of their components to donate electrons and, therefore, participate in redox reactions [21]. In the present study, *Acacia gourmaensis* hydro-ethanolic extract had a significantly ($P < 0.05$) higher reducing power capacity (3211.11±52.24 mg AAE/100 g of powder) followed by *Balanites aegyptiaca*, and *Securidaca longepedunculata* (102.06±5.09, and 57.78±0.99 mgAAE/100 g of powder, respectively).

This study confirmed the antioxidant activity of *Balanites aegyptiaca*, and *Securidaca longepedunculata* as reported by previous workers [31,38].

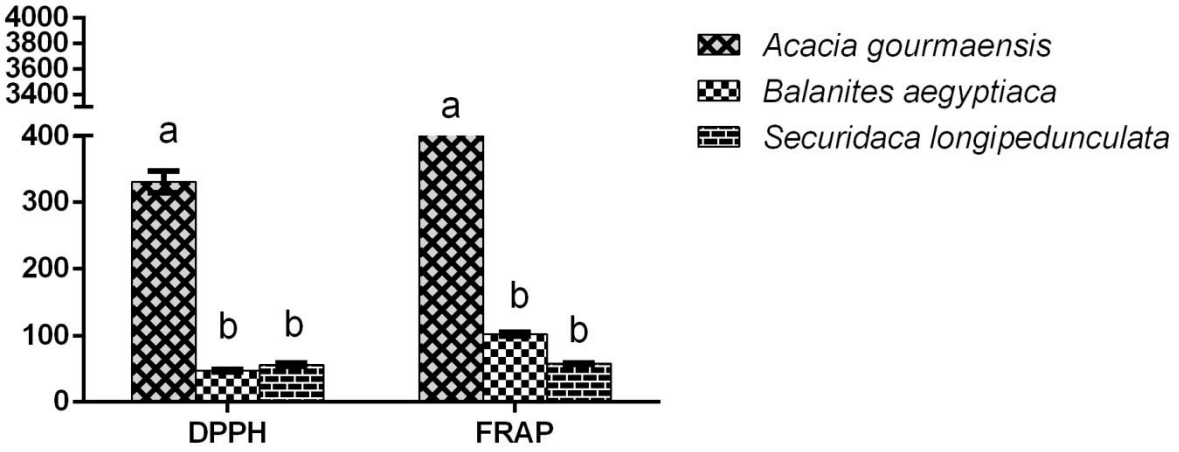


Fig 3: Antioxidant activity of *Balanites aegyptiaca*, *Securidaca longepedunculata*, and *Acacia gourmaensis*

In this study, for the first time, phytochemical composition and antioxidant activity of *Acacia gourmaensis* extract have been examined. However, previous studies demonstrated that *A. gourmaensis* have some antifungal activities [10,17].

The present study showed that the extracts possessing the highest phenolic contents were also found to have the highest flavonoids, and anthocyanins contents. The same trains were observed with antioxidant activity from the DPPH and FRAP methods. The *Acacia gourmaensis* hydro-ethanolic extract was the most efficient in phytochemical content and antifungal activity followed by *Securidaca longepedunculata* and *Balanites aegyptiaca*

Indeed, phenolic compounds (flavonoids, tannins, anthocyanins) have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation, and also as a potential antimicrobial agent [39].

In the view of antimicrobial activity, Junaidu *et al*., [26] attributed antifungal effects of *S. longepedunculata* by the presence of the active phytochemicals like flavonoids tannins, saponins, alkaloids and glycosides in the extracts. Recent studies revealed that *S. longepedunculata* hydro-ethanolic extract had an important antifungal activity [10,26]. Likewise, Tula *et al*. [27] reported that leaves of *Balanite aegyptiaca* aqueous extracts possessed polyphenols, saponins, steroids, and flavonoids. However, flavonoids and tannins were not found with *B. aegyptiaca* alcoholic extracts [12,38]. Very recent studies showed antifungal activity of *B. aegyptiaca* [8,10].

Regarding phytochemical content and antioxidant activity, *Acacia gourmaensis* is supposed to have more antifungal activity, as described other others in previous studies [6]. However, the opposite was found in our previous study. Indeed, we have done a comparison of antifungal activity of *Acacia gourmaensis, Securidaca longepedunculata* and *Balanites aegyptiaca.* This study showed that *Acacia gourmaensis* have the lowest antifungal activity followed by *Securidaca longepedunculata* and *Balanites aegyptiaca* [9,10]. Indeed, Demirci *et al*. [40] reported that plant extracts antimicrobial properties could be inhibited by certain compounds used as a source of energy by microorganisms. Moreover, antimicrobial activity is more linked to specific molecules and synergy effects of bioactive constituents in plant extracts [41].

4. CONCLUSION

This study has shown that *Acacia gourmaensis*, *Securidaca longepedunculata*, and *Balanites aegyptiaca* from Burkina Faso, have some phytochemicals, antioxidant with known antifungal activities. The hydro-ethanolic extracts of *Acacia gourmaensis*, which had the highest total phenolics, flavonoids and anthocyanins contents, were found to possess the strongest radical scavengers in both DPPH and FRAP assays. There is not a positive correlation between *Acacia gourmaensis* phytochemical content, antioxidant activity and antifungal activity. Nevertheless, the present study provides some information on the phytochemical and antioxidant activity of *Acacia gourmaensis*, *Securidaca longepedunculata* and *Balanites aegyptiaca* which paves the way for further research to identify the active compounds responsible for the biological activity of the plants.

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