

Ethnobotanical survey, antibacterial activity and phytochemical screening of extracts of *Prosopis africana* (Guill. & Perr.) Taub.

ABSTRACT:

SAMPLE

Aims: To investigate the ethnomedicinal uses of *Prosopis africana* (Guill. & Perr.) Taub and to screen the antimicrobial property as well as determine the phytochemical constituents of leaves, stems and root bark.

Study design: Ethnobotanical surveys, antibacterial activity and phytochemical screening of extracts of *P. africana*

Place and Duration of Study: The ethnobotanical survey was conducted during June 2015 in Zounweogo Province. The experiments were conducted at the Department of Medicine and Traditional Pharmacopeia-Pharmacy (MEPHATRA-PH) of the Institute of Research in Health and Laboratory of Applied Biochemistry and Chemistry (LA.BIO.C.A), University Joseph KI-ZERBO

Methodology: The semi-structured questionnaires were administrated to 36 traditional healers and elucidated out on the ethnomedicinal uses of *P. africana* in treating bacterial infections, the plant parts used and the mode of administration. The antimicrobial activity of different polar extracts of the leaves, the stem and root were evaluated by using the agar diffusion method and the determination of the minimal inhibitory concentration (MIC) of extracts via microdilution method. The phytochemical constituents of all extracts were also screened via Ciulei method.

Results: The traditional healers consisted of 64% women and 36% men were surveyed. *P. africana* is used to treat tooth decay, childhood diarrhoea and chronic wounds. Leaves and the stem bark are the most commonly used plant part in treating bacterial infections. while the roots are primarily used for other therapeutic purposes. The main method of administration was decoction. Methanol extracts of the leaves showed better antibacterial activity on all bacterial strains than aqueous extracts : *Escherichia coli* ATCC 25922 (MIC = 390 µg/ml; diameter of inhibition = 13.00 ± 1.00 mm), *Staphylococcus aureus* ATCC 25923 (MIC = 390 µg/ml; diameter of inhibition = 12.33 ± 1.53 mm), *Escherichia coli* ATCC 35218 (MIC = 3120 µg/ml; diameter of inhibition = 13 ± 1.00 mm), *Pseudomonas aeruginosa* PAO1 (MIC = 12500 µg/ml ; diameter of inhibition = 12.33 ± 0.58 mm). Alkaloid salts, tannins, sterols and triterpenes, saponosides, flavonic glycosides and leucoanthocyanins were found in extracts of the leaves, as well as in the barks of the stem and root.

Conclusion: These results demonstrated that *P. africana* is a potent source of antimicrobial compounds and could justify its traditional use of in the folklore medicine of Zounweogo Province.

1. INTRODUCTION

Infectious diseases currently remain a treat to public health worldwide despite the efforts made in their prevention and treatment. This is due in large part to the appearance of a high percentage of antimicrobial-resistant strains among common infections (urinary tract infections, pneumonia, bloodstream infections, etc.) in all regions of the world [1]. For example, health centres and hospitals nowadays record high rates of nosocomial infections caused by resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) or multidrug-resistant Gram-negative bacteria. Another example is multidrug-resistant tuberculosis; 480 000 new cases were identified in 2013, and cases of extensively drug-resistant tuberculosis were acknowledged in 100 countries[2]. To fight against such threat, the world is in urgent need of new antimicrobial agents that could have novel mechanisms of action. Moreover, the situation is difficult in many countries where conventional medicine and current

advances in infectious disease control are not accessible to most of the population. This is particularly the case in Africa (and other developing countries) where traditional medicine (including use of medicinal plants) remains the primary sources of health care, with one traditional healer for 500 people, against one doctor for 40 000 people [2].

In Burkina Faso, *Prosopis africana* (Guill. & Perr.) Taub is used against infectious diseases [3, 4]. An ethnobotanical survey that we recently conducted in Zounweogo province permitted to know the use of *P. africana*.

From this survey, stem bark, and leaves are cited by traditional healers.

As part of the valorization of Burkina Faso medicinal plants, and to verify whether the stem bark and leaves (the most cited for their use against bacterial infections) are active in vitro, we investigated the phytochemical composition of the plant extracts, as well as their antibacterial activity.

2. MATERIAL AND METHODS

2.1 Description of the study area

The ethnobotanical survey was conducted in the province of Zounweogo, in southern of Burkina Faso, between 11°00' and 12°00' N and 1°00' and 2°00' W (Figure 1). At the last general census of 2016, the population of Zounweogo province was estimated to 244.714 inhabitants [5]. The daily lives of the Mossi tribal group, who dominates the study area, centres on agriculture and livestock. The local primary health system is centred around plant-based medicine, dispensed traditional healers due to the remoteness of state-sponsored health care centres, as well as a dearth of specialists and material resources at these centres.

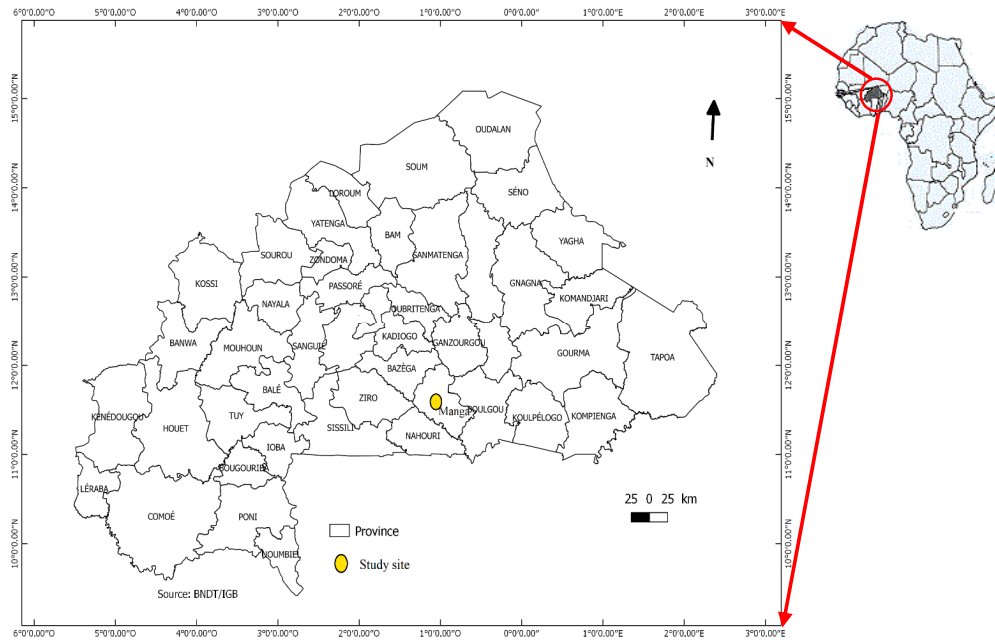


Figure 1. Location of the study site in Burkina Faso

2.2 Ethnobotanical survey

The ethnobotanical survey was conducted during **June 2015** with 36 traditional healers randomly assigned by the traditional healers **association** of Zounweogo province. The choice of this locality was mainly related to the trust already established between this traditional healers association and the Institute for Research in Health Sciences (IRSS). Data were collected using **a** semi-structured questionnaire. The interviews were conducted in the principal local language **of** "mooré". These interviews were mainly related to age and sex of the traditional healer; the bacterial infections treated using *P. africana*, the plant parts used, the methods of preparation and the mode administration of **decoction**.

2.3 Plant material

P. africana stem bark, leaves and roots bark were collected from the province of Zounweogo with the **aid** of a traditional healer. The plant material was **taxonomically** identified under the voucher N° 6852 by a botanist and a specimen was deposited at the herbarium of University Joseph KI-ZERBO. The fresh material collected was dried in a greenhouse with air circulation and then powdered until use

2.4 Extractions procedure

The different extracts were prepared as follow:

- **Aqueous decoction:** 100 g of dried powder was extracted in 1000 ml of distilled water. The melange was boiled for 30 minutes at **100 °C**, filtered with **Whatman filter paper No. 1**, frozen at **-80 °C** and lyophilized.
- **Aqueous maceration:** 100 g of dried powder was extracted in 1000 ml of distilled water. The raw material was left stirring during 24 hours at room temperature.
- **Methanol maceration:** 100 g of dried powder was mixed with 1000 ml of methanol (100%). filtered with **Whatman filter paper No. 1**, frozen at **-80 °C** and lyophilized,

2.5 Bacterial strains

Four **pathogens** were used for the experiment **namely** *Pseudomonas aeruginosa* PAO1 (from Pseudomonas Genetic Stock Center), *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923 (American Types Collection Culture).

2.6 Culture medium preparation

Muller Hinton Agar (MH) was prepared by dissolving 14 g of agar powder in 500 ml of distilled water and then bringing the mixture to a boil under magnetic agitation until the powder was completely dissolved.

The Luria-Bertani (LB) liquid medium was prepared by dissolving 25 g of the LB powder in 1000 ml of distilled water and bringing the mixture to a boil under magnetic agitation to obtain complete dissolution. The media and all equipment used during handling were wrapped in aluminium foil and autoclaved at 121°C for 15 minutes. After sterilization, the materials were unpacked in a fume hood. All handling was done under sterile conditions under the hood.

2.6 Standard inoculum preparation

Suspensions of bacterial inoculum were prepared according to the technique described by [6], by dispersing pure strains of bacteria in nutritious broth and cultured at 37°C for 24 h. The turbidity of the microbial suspension was adjusted with a densitometer to a standard of 0.5 McFarland equivalent to about $1-5 \cdot 10^8$ bacterial cells counted / ml. This suspension was diluted to one hundredth, thus constituting the standard inoculum.

2.7 Determination of bacterial growth inhibition by disc method

Inhibition zone diameter of discs soaked with extracts from trunk barks, leaves and roots of *Prosopis africana* was determined by the disc method [7, 8]. Sterile Whatman N°1 paper discs (6 mm), soaked with 10 µL of leaf, bark or root extracts (25 mg/ml) solubilized in 10% DMSO, were deposited on an inoculated 100 µL agar of a bacterial suspension (10^6 to 10^7 CFU/ml). DMSO 1% was used as negative control while ampicillin, aztreonam, streptomycin and tetracycline were used as a positive control. All Petri dishes were incubated for 24 hours, at the end of which time an inhibition diameter was measured around the discs. Extracts that produced an inhibition diameter (including that of the disc) \geq 9 mm, were considered to have antibacterial activities. All tests were repeated in triplicate.

2.8 Minimum inhibitory concentration (MIC) determination

The broth microdilution method has been adapted for the determination of the minimum inhibitory concentration (MIC) using a microplate (96 wells) [6]. Ten microliters (10 µl) of leaf, bark and root extracts (500 mg/ml) were diluted to one-half with 190 µl broth to obtain a concentration range of 25 to 0.3906 mg/ml. Ten microliters of DMSO 1% were added in each well. Ten microliters (10 µl) of inoculum (10^6 to 10^7 CFU/ml) were added to the test medium. The negative control consisted of 180 µl of Luria-Bertani broth (MLB), 10 µl of DMSO 1% and 10 µl of inoculum [6]. The microplates were covered with sterile covers, shaken to mix the contents of the wells and incubated at 37°C for 24 hours. All tests were repeated three times. The MIC of the extracts was determined by adding 50 µl (0.2 mg/ml) of an iodinitrotetrazolium salt solution (INT) after 30 minutes of incubation in the dark. Living microorganisms reduce the INT (colourless) by producing a pink colour.

2.9 Preliminary phytochemical screening

The preliminary phytochemical screening for alkaloids, anthocyanins, flavonoids, saponins, steroids, terpenoids and tannins was made possible by conventional liquid reactions. These reactions were based on the colouring, precipitation or formation of foams as described by Ciulei [9].

2.10 Statistical analysis

For statistical analysis, Microsoft Excel was used to obtain the means and standard deviations of the results. Prism Graph Pad version 5.00 software was used to measure the degree of significance of the results using the ANOVA one-way comparison test. A significant difference was considered for $p < 0.5$.

3. RESULTS

3.1 Ethnobotanical survey

Most of the 36 traditional healers surveyed in Zounweogo province were at least 60 (61.11%) years old with a high proportion of women (33.3%) (Table 1). Among the infectious diseases treated with *Prosopis africana* by traditional healers, diarrhoea was the most reported (40%) infection, followed by

dermatosis (18%) and tooth decay (16%) (Figure 2). Leaves were the most frequently used plant part (72.35%) following by stem bark (15.35%) and root bark (12.30%). Moreover, decoctions were the most employed mode of preparation (81.4%) followed by maceration (18.60%). Five modes of administration are used by traditional healers of this region, which were, body bath, purgation, oral route, mouthwash and inhalation. Body bath (40.24%) was represented as the most mode of administration, while inhalation (7.2%) was the least employed method (Table 2).

Table 1:

Age	Male	Female	Total
[30-60[8.33	30.56	38.89
≥60	27.78	33.33	61.11

Distribution (%) of traditional healers by age

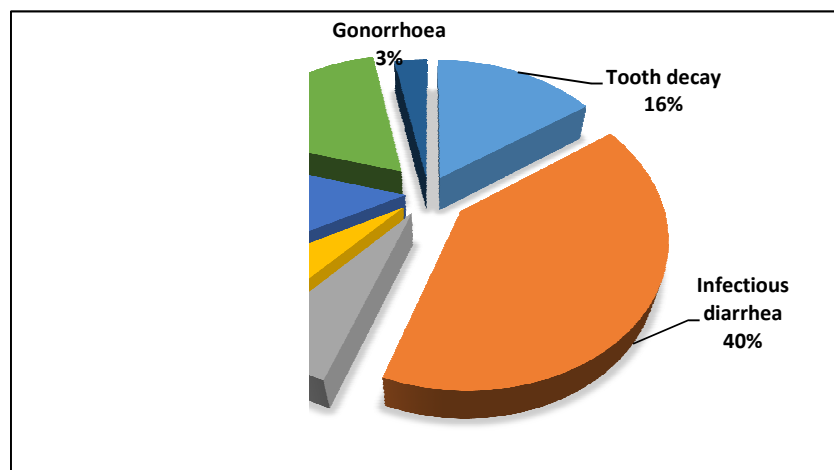


Figure 2: Diagram represented diseases given by traditional healers in the province of Zounweogo

Parts (%)			Formulation (%)			Administration route (%)			
Leaves	Stem bark	Root bark	D	M	Bb	P	OR	Mw	Ih
72.35	15.35	12.30	81.40	18.60	40.24	28.39	14.53	9.35	7.49

Table 2: Usage mode of *Prosopis africana* in the treatment of bacterial infections

D: Decoction; M: Macerate; Bb: Body bath; P: Purgation; OR: Oral Route; Mw: Mouthwash; Ih : Inhalation

3.2 Antibacterial activity

The stem bark, the leaves and the root bark extracts were evaluated for their potential antibacterial activity on *P. aeruginosa* PAO1, *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *S. aureus* ATCC 25923. Results are presented in Table 3 and Table 4, which gives the different values of the minimum inhibitory concentrations (MIC) and the inhibition diameters of the extracts respectively. Methanolic extracts from leaves and stem bark had the best antibacterial effects. Indeed, the best minimum inhibitory concentrations were observed with the leaf methanol extracts (0.39 mg/mL) and stem bark extract (0.78 mg/mL) on *S. aureus* ATCC 25923. Root extracts have been inactive on all strains. The best inhibition diameters were observed with leaves methanol extract (13±10 mm) on *E. coli* ATCC 25922 and *E. coli* ATCC 35218. The smallest inhibition diameters were observed by root extracts on all strains.

Strain	Extracts MIC (mg/ml)							
	AD		AM		MM			
	Sb	Le	Sb	Le	Rt	Sb	Le	Rt
<i>E. coli</i> ATCC 25922	6.25	03.12	06.25	06.25	>25	06.25	00.39	>25
<i>E. coli</i> ATCC 35218	06.25	12.50	06.25	12.50	>25	06.25	03.12	>25
<i>P. PAO1</i>	12.50	25.00	25.00	12.50	>25	25.00	12.50	>25
<i>S. aureus</i> ATCC 25923	3.12	6.25	12.50	6.25	>25	00.78	00.39	>25

Table 3: Minimum inhibitory concentrations of extract

AD Aqueous decoction; **AM**: Aqueous macerate; **MM**: Methanolic macerate ; **Sb** : Stem bark , **Le**: leaves ; **Rt** : Roots ; **MIC** : Minimal Inhibition Concentration

Strain	Raw Extracts (10 µL)							Standards (10 µg)				Solvent	
	SbAM	SbAD	SbMM	LeAM	LeAD	LeMM	RtAM	RtMM	Amp	Az	Strep	Tetra	DMSO (10%)
<i>E. coli</i> ATCC 25922	9.3±0.6	10.7±0.6	11 ±1	09±00	9.7±0.6	13±10	NS	NS	NS	17±0.6	NS	NS	06±00
<i>E. coli</i> ATCC 35218	10.0±10	09±00	11 ±1	11.7±0.6	10.3±0.6	13±10	NS	NS	NS	21±1	NS	NS	06±00
<i>P. aeruginosa</i> PAO1	9.3±0.6	09±00	09±0	9.7±0.6	09±00	12.3±0.6	NS	NS	NS	18.7±0.6	NS	NS	06±00
<i>S. aureus</i> ATCC 25923	10.3±0.6	09±00	11±1	9.7±0.6	10.3±0.6	12.3±1.5	NS	NS	17±10	NS	14±00	14±00	06±00

Table 4: Inhibition diameters (mm) of stem bark, leaves and root bark extracts of *Prosopis africana*

SbAM : Stem bark aqueous Macerate, **SbAD** : Stem barks Aqueous Decoction, **SbMM**: Stem barks Methanolic Macerate, **LeAM** : Leaves Aqueous Macerate, **LeAD** : Leaves Aqueous Decoction, **LeAM** : Leaves Aqueous Macerate; **LeMM** : Leaves Methanolic Macerate ; **RtAM** : Root Aqueous Macerate, **RtMM** : Root Methanolic Macerate, **NS**: Non-Sensitive; **Amp**: Ampicillin, **Az**: Aztreonam, **Strep**: Streptomycin, **Tetra**: Tetracyclin

3.3 Phytochemical screening

Preliminary chemical screening results reported in Table 5 revealed the presence of tannins in stem barks and leaves. Sterols and triterpenes, reducing compounds, and leucoanthocyanosides were documented in root bark, stem bark and leaves. Carotenoids and flavonoid glycosides were revealed in the leaves while alkaloid salts were found in the root bark.

Table 5: Preliminary chemical screening

Solvents/chemical groups	Samples of samples		
	Root bark	Stem bark	Leaves
Dichloromethane (DCM)			
Alkaloids base	nd	nd	+
Flavonics aglycones	nd	nd	+
Emodols (Aglycones anthracénosides)	nd	nd	nd
Carotenoids	nd	nd	+
Coumarins	nd	nd	nd
Sterols and triterpenes	+	+	+
Methanol/ Non-hydrolyzed			
Alkaloids salts	+	nd	nd
Reducing compounds	+	+	+
Leucoanthocyanosides	+	+	+
Polyphénols (tannins)	nd	+	+
Saponosides	nd	nd	nd
Methanol/Hydrolysis			
Flavonic glycosides	nd	nd	+
Glycosides of anthracenosides	+	nd	nd
Coumarin derivatives	nd	nd	nd
Glycosides of sterols and triterpenes	+	+	+
Aqueous extracts			
Alkaloids salts	+	nd	nd
Reducing compounds	+	+	+
Polyphénols (tannins)	nd	+	+
Saponosides	+	+	nd

nd : Not detected; + : Present

Discussion

The survey was based on knowledge related to bacterial diseases by the traditional healers. The majority of traditional healers interviewed were women. It could explain by the fact that in African societies, women are more interested in infectious diseases [10]. Leaves, stem bark and root bark are the main parts used in traditional medicine. This could be justified by the fact that these parts of plants are most accessible throughout the year. The strong use of the decoction could be explained by the fact that this form makes it possible to extract faster the active ingredients. Also, the decoction attenuates or cancels the toxic effect of certain recipes [11]. The surveys in African countries had established decoction as the most popular form of preparation in African traditional medicine [3, 12–14].

All extracts of crude leaves, stem bark and root bark extracts of *P. africana* exhibited some level of antibacterial properties against the tested microorganisms to include *Escherichia coli* 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* PAOI, and *Staphylococcus aureus* ATCC 25923. Results are presented in Table 3 and Table 4, which gives the different values of the minimum inhibitory concentrations (MIC) and the inhibition diameters of the extracts respectively.

Leaves extracts were more active on most of the bacterial strains used compared to stem barks extracts using the disc and microdilution method. The antibacterial potential of *Prosopis africana* stem barks and leaves extracts may justify their use in traditional medicine for the treatment of diseases such as green infant diarrhoea, dental caries, dermatoses and dysentery [3]. The results obtained also showed that the methanolic extract from the leaves was the most active of the extracts on all strains tested. The best antibacterial activity of this extract could be explained by its richness in flavonoids. Indeed, this type of polyphenolic compounds was known to have antimicrobial properties [15, 16]. Several authors have already demonstrated the antibacterial properties of *Prosopis africana* by linking them to the presence of certain bioactive groups such as steroids, tannins, flavonoids, alkaloids, terpenoids [17, 18]. The antibacterial activity of stem barks extracts in solid and liquid media could be explained by the presence of tannins and steroids and triterpenes [19]. These authors have also shown that such compounds had antibacterial activities on *Escherichia coli*, *staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*. Indeed, all strains showed significantly different sensitivities to all stem barks and leave extracts. This difference in sensitivity could be explained by the fact that the wall of Gram- bacteria contains a lipid layer making them less permeable and therefore more resistant than Gram+ bacteria that do not have this protection. The ineffectiveness of root extracts on all bacterial strains might be due to the absence of tannins in the stem bark and leaves. The tannins could act in synergy with the other compounds. The ineffectiveness of the extracts could also be due to the poor diffusion of the extracts through the agar, and also the physiological state of the bacteria

5. CONCLUSION

The ethnobotanical survey revealed that leaves and stem barks are the most commonly used and the main method of preparation is a decoction. The antibacterial study revealed better activity of methanol leaves extracts on all strains compared to the other parts. The antibacterial effects observed could be related to the therapeutic properties of tannins, sterols and triterpenes, saponosides, flavonic glycosides, leucoanthocyanins, identified in this study. Thus, this study provides a scientific basis and justification for the use of different parts of this plant in bacterial infectious treatment.

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Competing interests

Authors have declared that no competing interests exist

AUTHORS' CONTRIBUTIONS

B.A : managed the literature searches, write the protocole, and wrote the first draft of the manuscript

M R D and C.S: collected data and managed analyses of study
C.E, O.N, S.D.S, M.K.H: read and approved the final manuscript.
M.K. : Validation

CONSENT

Traditional healers participated to the survey through integrated consent.

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