Original Research Article

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

Comment [P1]: Survey or surveys???

ABSTRACT:

Aims: to investigate the ethnomedicinal uses of *Prosopis africana* and to screen the antimicrobial property as well as the phytochemical constituents of its leaves, stems and roots barks extracts.
Study design: Ethnobotanical surveys, antibacterial activity and phytochemical screening of extracts of *Prosopis africana* (Guill. & Perr.) Taub

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 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 traditional healers of Zounweogo province in national language Moore and were carried out on the ethnomedicinal uses of *P. Africana* in bacterial infections, the plant parts used and the mode of administration. The antimicrobial activity of different extracts of the leaves, the stem and root barks was evaluated by using agar diffusion method and the determination of the minimal inhibitory concentration (MIC) of extracts. The phytochemical constituents of all extracts was also screened.

Results: Thirty-six (36) traditional healers composed of 64% women and 36% men were surveyed. The results showed that the leaves and the stem bark are the most commonly used in bacterial infections while the roots are used for other therapeutic purposes. The leaves methanol extracts showed the best antibacterial activity on all bacterial strains : *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). The phytochemical screening revealed the presence of alkaloid salts, tannins, sterols and triterpenes, saponosides, flavonic glycosides and leucoanthocyans in the leaves, stem bark and root extracts.

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

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1. INTRODUCTION

Worldwide and particularly in Africa, bacteria, viruses, parasites and fungi cause infectious diseases. Their management is a major concern in daily medical practice. Indeed, according to the latest World Health Organization report on the causes of death of our planet, more of the 50 million people who died in 2012, 9 million were due to infectious diseases. Unfortunately, about half of them were from developing countries like Burkina Faso [1]**.** Among them, bacteria occupied an important place in the occurrence of these diseases [2]**.** Indeed, various bacteria including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* are notably involved in these infections [3, 4]. For that, the scientific world has discovered many remedies in order to remain the damage caused by these microorganisms

in patients. The objectives of these remedies were to reduce the incidence of infectious diseases, especially in developed countries[5]
Despite these scientific advances, people are turning to the products of traditional medicine to heal themselves. It is the case of in low-countries in where people use traditional medicines and pharmacopoeias in addition to modern medicine. A renewed interest in this traditional pharmacopoeia nowadays is perceptible because of the emergence of multidrug-resistant strains[4, 6]**.** Moreover, medicinal plants are main sources of novel biomolecules and constitute a new alternative in the prospecting of new treatments against infections[7, 8]**.** Among the plants of interest in traditional medicine, *Prosopis africana* (Guill. & Perr.) Taub. (Mimosaceae) was cited to have properties for the care of bacterial infections[9]. Thus, for a better knowledge of the use of this plant against bacterial infections, an ethnobotanical survey was conducted among traditional healers in the South Central region of Burkina Faso. After that, a laboratory study was undertake to determine the phytochemical composition and the *in vitro* potential activity of *Prosopis africana* stem barks, leaves and roots against infectious bacteria.

2. MATERIAL AND METHODS

2.1 Description of the study area

The ethnobotanical survey was conducted in the department of Zounweogo, in southern of Burkina Faso100 km from Ouagadougou, between 11°00′ and 12°00′ N and 1°00′ and 2°00′ W (Figure 1). At the last general census, population of Zounweogo province was estimated to 244.714 inhabitants[10]**.** Mossi group was dominates on the ethnic level in the region and the main language is "*mooré"*. Agriculture and livestock are the main economic activities of the population. The local health system includes traditional medicine, first resort of patients and modern medicine, which is characterized by remoteness of health centers, deficient in specialists and material resources, costs related to health care and drugs, malnutrition, etc.

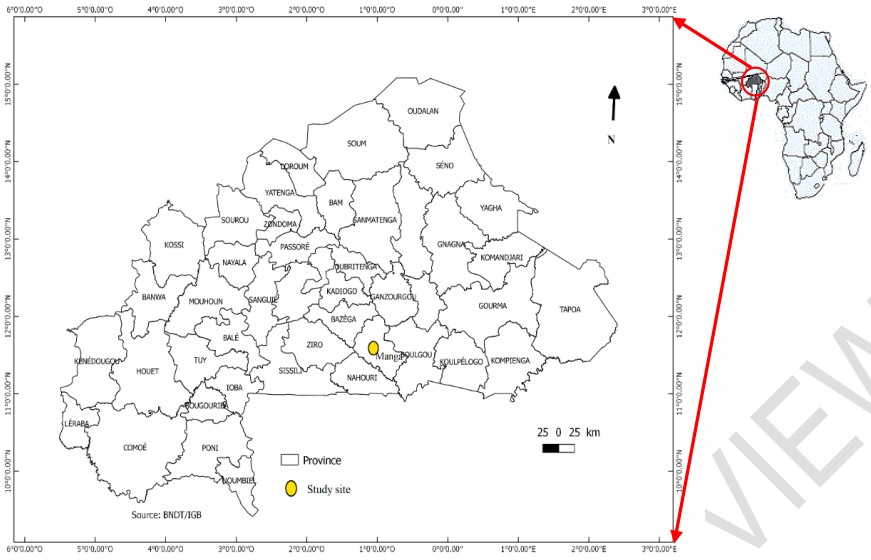

Figure 1. Location of the study site in Burkina Faso

2.2 Ethnobotanical Survey

The ethnobotanical survey was conducted during june 2014 with 36 traditional healers randomly assigned to the association of traditional healers 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 semi-structured questionnaires [11]. The interviews were conducted in the principal local language which is “mooré”. These interviews were mainly related to age and sex of the traditional healer; the bacterial infections treated by traditional healers using *Prosopis africana*; the plant organs used, the methods of preparation and the mode administration of recipes

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2.3 Plant material

Prosopis africana stem barks, leaves and roots barks were collected from the province of Zounweogo in June 2014 with the presence of a traditional healer. The plant material was 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 during 30 minutes, filtered, frozen and lyophilized.
- **Aqueous maceration:** 100 g of dried powder was extracted in 1000 mL of distilled water. 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%). The mixture was homogenized and left to macerate under stirring at room temperature for 24 hours

2.4 Bacterial strains

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

2.5 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 a complete dissolution. The media and all equipment used during handling were wrapped in aluminum foil and autoclaved at 121ºC 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 [12]**.**, 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 1-5.10^8 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[13, 14]**.** Sterile Wathman 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 a negative control while ampicillin, aztreonam, streptomycin and tetracycline were used as 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) ≥ 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) [12]. Ten microlitres (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 (10 µL) 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 [12]. 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 (n=3).The MIC of the extracts was determined by adding 50 µL (0.2 mg/mL) of an iodonitrotetrazolium salt solution (INT) after 30 minutes of incubation in the dark. Living microorganisms reduce the INT (colourless) by producing a pink colour.

2.9 Phytochemical screening

The preliminary phytochemical screening was made possible by conventional liquid reactions. These reactions were based on the coloring, precipitation or formation of foams. They are described by the [15]. They consisted in the search for alkaloids, anthocyanins, flavonoids, quinones, saponins, steroids, terpenoids and tannins.

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 traditional healers surveyed in Zounweogo province were at least 60 (61.11%) years old with a high proportion of women (Table I). Results showed that among the infectious diseases treated with *Prosopis Africana* by traditional healers in Zounweogo, the diarrhea was the most cited (40%)

followed by dermatosis (18%) and tooth decay (16%) (See Figure 1). Leaves of *Prosopis africana* were the most frequently cited in the recipes formulation (72.35%) following by stem barks (15.35%) and roots (12.30%). Moreover, the decoction was the most mode of preparation (81.4%) followed by maceration (18.60%) Results also showed five mode of administration used by the traditional healers of this region, which were body bath, purgation; oral route; mouthwash and inhalation. The body bath (40.24%) has represented the most mode of administration and inhalation (7.2%) was the less one (Table II).

Table I:

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

n=36

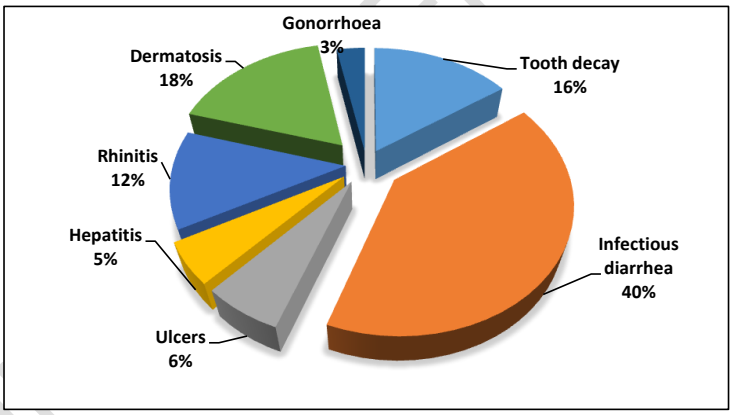

Figure 2: Diagram represented diseases given by traditional healers in province of Zounweogo

Parts (%)			Formulation (%)			Administration route (%)			
Leaves	Stem barks	Roots	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 II: 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 barks, the leaves and the root extracts were evaluated for their potential antibacterial activity on *Pseudomonas aeruginosa* PAO1*, Escherichia coli* ATCC 25922*, Escherichia coli* ATCC 35218 *and Staphylococcus aureus* ATCC 25923*.* Results were presented in Table III and Table IV, which gave the different values of the minimum inhibitory concentrations (MIC) and the inhibition diameters of the extracts respectively (See below). Ethanolic extracts from leaves and stem barks had the best antibacterial effects. Indeed, the best minimum inhibitory concentrations were observed with leaves ethanol extracts (0.39 mg/mL) and stem barks extract (0.78 mg/mL) on *Staphylococcus aureus* ATCC 25923. Root extract Root extracts have been inactive on all strains.The best inhibition diameters were observed with leaves ethanol extract (13±10 mm,) on *Escherichia coli* ATCC 25922 and *Escherichia coli* ATCC 35218. The smallest inhibition diameters were observed by root extracts on all strains

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

Table III: Minimum inhibitory concentrations of extract

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

	Raw Extracts (10 µL)								Standards (10 µg)				Solvent
Strain	**SbAM**	**SbAD**	**SbMM**	**LeAM**	**LeAD**	**LeMM**	**RtAM**	**RtMM**	**Amp**	**Az**	**Strep**	**Tetra**	**DMSO (10%)**
E. coli 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
E. coli 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
P. aeruginosa 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
S. aureus 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 IV: Inhibition diameters (mm) of stem barks, leaves and roots extracts of *Prosopis africana*

SbAM : Stem barks 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

3.2 Phytochemical screening

Preliminary chemical screening results reported in Table V revealed the presence of tannins in stem barks and leaves. Sterols and triterpenes, reducing compounds, leucoanthocyanosides in roots, stem barks and leaves. Carotenoids and flavonoid glycosides were revealed in the leaves and alkaloid salts were presented in the roots.

Table V: Preliminary chemical screening

	Samples of samples		
Solvents/Chemical groups	**Roots**	**Stem barks**	**Leaves**
Dichloromethane (DCM)			
Alkaloids base	nd	nd	+
Flavonics aglycones	nd	nd	+
Emodols (Aglycones anthracénosides)	nd	nd	nd
Carotenoids	nd	nd	+
Coumarins	nd	nd	nd
Stérols et triterpènes	+	+	+
Methanol/ Non-hydrolyzed			
AlKaloïds 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			
Alkaloïds salts	+	nd	nd
Reducing compounds	+	+	+
Polyphénols (tanins)	nd	+	+
Saponosides	+	+	nd

nd : Not detected; + : Presence

4. Discussion

The ethnobotanical Survey conducted in the South-Central region of Burkina Faso showed that the majority of traditional healers were adults. This study revealed that stem barks and leaves are the most commonly used parts of *Prosopis africana* against bacterial infections and the main method of preparing drugs was decoction. This could be explained by the fact that the active ingredients in recipes used by traditional healers are not thermolabile, decoction allows them to extract as many active ingredients as possible [16]. Surveys in African countries had established decoction as the most popular form of preparation in african traditional medicine [9, 17–19]**.** 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 diarrhea, dental caries, dermatoses and dysentery [9].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 [20, 21]**.** 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 [22, 23]. The antibacterial activity of stem barks extracts in solid and liquid media could be explained by the presence of tannins and steroids and triterpenes [24].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 leaves 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 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 decoction. The antibacterial study revelated better activity of methanol leaves extracts on all strains compared to the others parts. The antibacterial effects observed could be related to the therapeutic properties of tannins, sterols and triterpenes, saponosides, flavonic glycosides, leucoanthocyans, identified in this study. Thus, this study provides scientific basis and justification for the use of different parts of this plant in bacterial infectious treatment

CONSENT

Traditional healers participated to the survey through integrated consent.

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