

Original Research Article

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

ABSTRACT:

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

Study design: Ethnobotanical surveys, antibacterial activity and phytochemical screening of extracts of *P. rosopis africana* (Guill. & Perr.) Taub

Place and Duration of Study:- The ethnobotanical survey was conducted during June 2015 (Methodology indicate 2014) in Zounweogo Pprovince (the Methodology section indicate Department not Province – make all the same), Burkina Faso. 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 36 traditional healers of Zounweogo province in national language Moore and elucidated information on were carried 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 barks of stem and root barks was evaluated by using the agar diffusion method and the determination of the minimal inhibitory concentration (MIC) of extracts (indicate method?). The phytochemical constituents of all extracts was also screened via ? (indicate method).

Results: The thirty-six (36) traditional healers consisted of 64% women and 36% men were surveyed. Indicate the bacterial infections treated with this species. The results showed that the 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. Indicate mode of administration as per methodology above. The leaves Methanol extracts of the leaves showed the best 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). The phytochemical screening revealed the presence of alkaloid salts, tannins, sterols and triterpenes, saponosides, flavonic glycosides and leucoanthocyan were found in extracts of the leaves, as well as in the barks of the stem bark and root extracts.

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

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, (Low quality information in opening statement of paragraph- remove –Get straight to the point as

quickly as possible. All redundant information should be removed from Introduction – only very pertinent information is to be provided.) Of the more of the than 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]. ~~This sentence is meaningless as the other half would be from developed countries.~~ Among them, bacteria occupied an important place in the occurrence of these diseases [2]. ~~(this sentence is meaningless – remove).~~ Indicate factors that lead to infectious diseases. ~~V~~Indeed, various bacteria including such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are notably involved in these infections [3, 4- renumber all subsequent citations]. ~~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].~~ Seeing that these 3 bacteria forms part of this investigation, more should be stated about them so that the reader understands the context of the bacteria and their adverse impact on society (economically and mortality).

~~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. Medicinal plants as sources of novel biomolecules are receiving A-renewed interest in this traditional pharmacopoeia nowadays is perceptible because of the emergence of multidrug-resistant microbial strains[4 (only one citation is sufficient, more citations does not make it more valid!,-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 treatment of bacterial infections[9]. More should be stated about the traditional use of this species in treating microbial infections, particularly bacterial infections & also related to leaves as well as the bark of roots and stems (in order to validate their use in this study). Indicate the problem statement that led you to investigate this particular species, especially in the study area. 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, ~~whereafter. After that, a laboratory study was undertake to determine~~ the phytochemical composition was determined. Lastly, and the in vitro (nothing is stated in the methodology about in vitro) potential activity of *Prosopis africana* stem barks, leaves and roots against infectious bacteria was elucidated. The study is of value because ... (indicate value of investigation for study area and other similar areas or traditional healers).~~

2. MATERIAL AND METHODS

2.1 Description of the study area

The ethnobotanical survey was conducted in the (department – the abstract indicate Province not department – which is it? Or is there a department of Zounweogo within the province of Zounweogo? - clarify) of Zounweogo, in southern of Burkina Faso ~~400 km from Ouagadougou~~, 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 above it was indicated as department) was estimated to 244.714 inhabitants[10] – why do we absolutely need to know the population number of this study area in order to understand the results? – if not absolutely necessary, then remove). The daily lives of the Mossi tribal group, who was dominates the study area, centers 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 primary health system is centered around includes traditional plant-based medicine, dispensed traditional healers, first resort of patients and modern medicine, which is characterized by due to the remoteness of state sponsored health care centers, as well as a dearth of deficient in specialists and material resources at these centers, costs related to health care and drugs, malnutrition, etc.

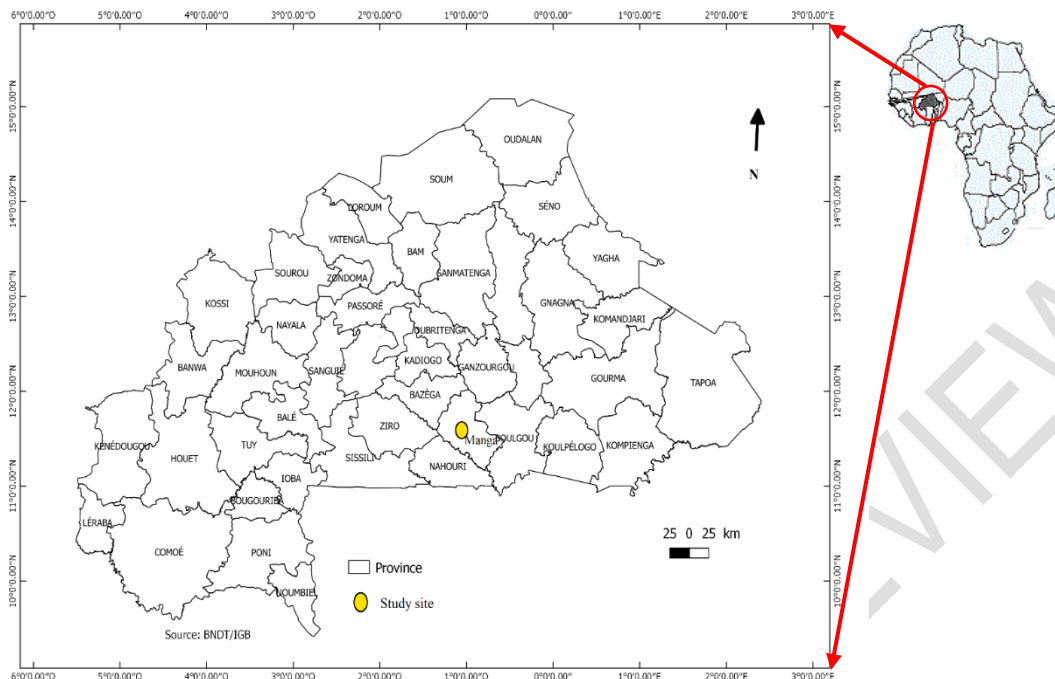


Figure 1. Location of the study site in Burkina Faso – do not put the legend in a text box

2.2 Ethnobotanical Survey

The ethnobotanical survey was conducted during June 2014 (Abstract indicate 2015) with 36 traditional healers randomly assigned by to the association of 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 questionnaires [14]. The interviews were conducted in the principal local language which is of –“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 parts used, the methods of preparation and the mode administration of decoction recipes

2.3 Plant material

Prosopis africana stem barks, leaves and roots barks were collected from the province of Zounweogo in June 2014 with the aid presence 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 extract methods descriptions are incomplete and does not lend itself to repeatability – which is the cornerstone of science – update section 2.4)

The different extracts were prepared as follow:

- **Aqueous decoction:** 100 g of dried powder was extracted in 1000 mL of distilled water. The melangemélange was boiled during for 30 minutes at what temperature?, filtered (how and with what? Whatman filter paper No. 1?), frozen (how?) and lyophilized (how?).

- **Aqueous maceration:** 100 g of dried powder was extracted ~~within~~ 1000 mL of distilled water. Raw material was left stirring ~~during for~~ 24 hours at room temperature. What happened thereafter?
- **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 (Should this number not be 2.4.1 ?) Bacterial strains

Four ~~types of pathogens/germs~~ were used for the experiment, ~~namely: 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). Was the laboratory certified as a level 2 biosafety facility? Clarify!

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 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 [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 $1-5 \cdot 10^8$ 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 [13, 14]. Sterile Whatman (check spelling! – one of many unforced errors that permeate this manuscript) 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) 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 [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 iodinitrotetrazolium salt solution (INT) after 30 minutes of incubation in the dark. Living microorganisms reduce the INT (colourless- You are mixing American and British English - adhere to only 1) by producing a pink colour.

2.9 Phytochemical screening

The preliminary phytochemical screening for alkaloids, anthocyanins, flavonoids, quinones, saponins, steroids, terpenoids and tannins was made possible by conventional liquid reactions. These reactions were based on the coloring, precipitation or formation of foams, as described by Ciulei. – 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 $p < 0.5$ – where is the ANOVA results?.

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 (33.3%) of women (Table 1 – use Arabic (I, 2, 3 ...) not Roman (I, II, III, IV...) lettering – correct throughout the manuscript!). Results showed that among the infectious diseases treated with *Prosopis africana* by traditional healers in Zounweogo, the diarrhea was the most reported infection (40%), followed by dermatosis (18%) and tooth decay (16%) (See Figure 1 – Figure 1 indicate the location of the study site in Burkina Faso!! – are you referring to Figure 2? – one of many unforced errors that permeate this manuscript). Leaves of *Prosopis africana* were the most frequently used plant part in the recipes formulation (72.35%) following by stem barks (15.35%) and root barks (12.30%). Moreover, the decoctions were the most employed mode of preparation (81.4%) followed by maceration (18.60%). Results also showed five modes of administration are used by the traditional healers of this region, which were: body bath, purgation, oral route, mouthwash and inhalation. The body bath (40.24%) was the most mode of administration, while and inhalation (7.2%) was the least employed method (Table 2).

Table 1: Mean (??) Distribution (%) of traditional healers by age (These age

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

categories were NOT specified in the Methodology –correct his oversight)

n=36

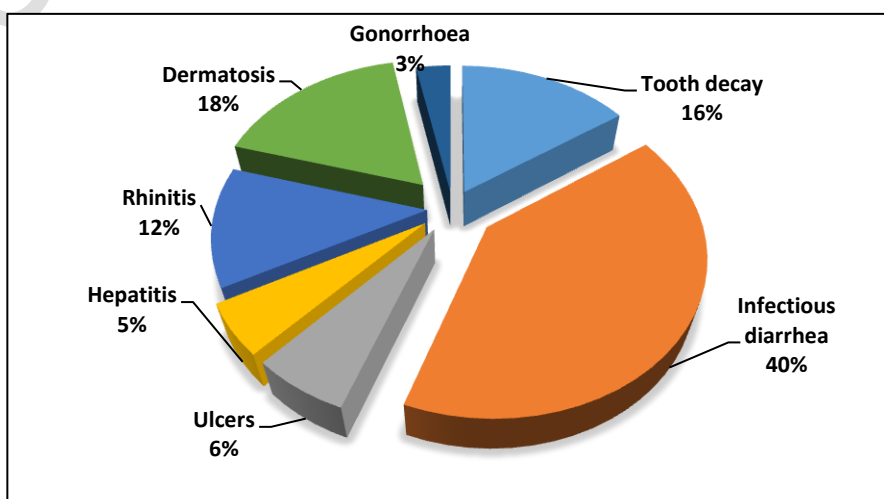


Figure 2: Infectious Diagram represented diseases treated given by traditional healers in province of Zounweogo.

Parts (%)			Formulation (%)		Administration route (%)				
Leaves	Stem barks	Root barks	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 2H: 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* PAO4 only necessary for Methodology section – remove all other occurrences outside methodology), *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218 (These ATCC designations stay to differentiate between these 2 *E. coli* strains) and (why is “and” also in italics? – one of many unforced errors that permeate this manuscript) *Staphylococcus aureus* ATCC 25923 only necessary for Methodology section – remove all other occurrences outside methodology). Results are presented in Table 3H and Table 4V, which gives the different values of the minimum inhibitory concentrations (MIC) and the inhibition diameters of the extracts respectively (See below). Ethanol – section 2.4 indicate methanol, not ethanol – one of many unforced errors that permeate this manuscript) extracts from leaves and stem barks had the best antibacterial effects. Indeed, the best minimum inhibitory concentrations were observed with the leaf ethanol – section 2.4 indicate methanol, not ethanol – one of many unforced errors that permeate this manuscript) extracts (0.39 mg/mL) and stem barks extract (0.78 mg/mL) on *Staphylococcus aureus* ATCC 25923. Root extract Root extracts (– one of many unforced errors that permeate this manuscript – has this manuscript been professionally proofread before its submission?) have been inactive on all strains. The (why no space after the sentence end period and the start of the next sentence? – one of many unforced errors that permeate this manuscript) best inhibition diameters were observed with leaves ethanol – section 2.4 indicate methanol, not ethanol – one of many unforced errors that permeate this manuscript) extract (13±10 mm, - why this comma? – one of many unforced errors that permeate this manuscript) – on *Escherichia coli* ATCC 25922 and *Escherichia coli* ATCC 35218. The smallest inhibition diameters were observed by root extracts on all strains (why is there not period after your sentences - – one of many unforced errors that permeate this manuscript). Nothing is stated about the efficacy of the Aqueous decoction and Aqueous maceration – correct this oversight!

Extracts MIC (mg/mL)								
Strain	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. aeruginosa</i> PAO1	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 III: Minimum inhibitory concentrations of extracts

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

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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 IV: Inhibition diameters (mm) of stem barks, leaves and root [barks](#) 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 (should this not be 3.3?) Phytochemical screening

Preliminary (nowhere in methodology section was it indicated that this was a preliminary screening) chemical screening results reported in Table V-5 revealed the presence of tannins in stem barks and leaves. Sterols and triterpenes, reducing compounds, and leucoanthocyanosides were documented in root barks, stem barks and leaves. Carotenoids and flavonoid glycosides were revealed in the leaves, while alkaloid salts were found presented in the root barks.

Table 5V: Preliminary Phytochemical screening

Solvents/Chemical groups	Samples of samples		
	Root barks	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 (write in English)	+	+	+
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 anthracénosides	+	nd	nd
Coumarin derivatives	nd	nd	nd
Glycosides of sterols and triterpenes	+	+	+
Aqueous extracts			
Alkaloids salts (see spelling)	+	nd	nd
Reducing compounds	+	+	+
Polyphénols (tanins)	nd	+	+
Saponosides	+	+	nd

nd : Not detected; + : Present

4. Discussion

4.1 Ethnobotanical survey

Aspects to be discussed:

- Age
- Gender/sex
- Infections treated
- Most frequently plant part
- Preparation mode
- Administration mode

4.2 Antibacterial activity

Aspects to be discussed:

Results presented in Table 3 and Table 4

3.3 Phytochemical screening Results presented in Table 3

- Root bark
- Stem bark
- Leaves

~~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 s~~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 – one of many unforced errors that permeate this manuscript) 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 – one of many unforced errors that permeate this manuscript) 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 revealed 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, leucoanthocyan, 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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