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Research Article

Contribution to the ethnobotanical and phytochemical study of *Zanthoxylum zanthoxyloides* (Lam.) Waterman (Rutaceae) from Mali

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Abstract

Zanthoxylum zanthoxyloides (Lam.) Waterman (Rutaceae) is a tree from 5 to 15 m high that grows spontaneously in West Africa. It is frequently used in Malian traditional medicine for the treatment of various ailments. Thus, the present work is part of the development of this plant through an ethnobotanical survey and a phytochemical study of its leaves, stems and roots collected in Yanfolila and Ouélessébougou. The ethnobotanical survey showed that this species is used in the treatment of various pathologies such as tooth decay, toothache, throat, stomach and headaches, sickle cell anemia, sterility, cardiovascular diseases, edema, childbirth pains, and other diseases, sexual impotence, malaria, bilharzia, diabetes, hemorrhoids, dermatosis, constipation, fever, eye infections, dysentery. In most cases the preparation is done by decoction. The phytochemical screening showed the presence of coumarins, sterols and terpenes in the three investigated organs from the two sites. The alkaloids have been found in the roots and in the stems; the mucilages in all parts except in the roots of Yanfolila. The tannins are present everywhere except in the roots and in the stems of Yanfolila; flavonoids are present everywhere except in the stems of Yanfolila. The richness of the powder of these different organs in mineral elements such as calcium, iron, magnesium, sodium, potassium and copper was also highlighted.

Keywords: *Zanthoxylum zanthoxyloides*, ethnobotanical survey, phytochemistry, mineral elements

INTRODUCTION

Since the ancient times, the plants have been used to relieve and cure diseases. In fact, their therapeutic properties are due to the presence of natural bioactive compounds. These compounds are accumulated in the different organs (leaves, flowers, fruits, stems, roots, barks, etc.)[1]. Currently, the development of microbial resistance to antibiotics and the toxicity of synthetic antioxidants have led researchers to draw from the plant world and particularly from medicinal plants in search of effective natural molecules[2]. Many studies have highlighted the presence of secondary metabolites with biological activities such as polyphenols, alkaloids, terpenes, etc..[1]. The African flora is known for its richness and includes thousands of plant species. Some of them have been scientifically studied and have resulted in drugs that can be used in primary health care according to the recommendations of the World Health Organization[3]. Mali has a rich and diverse flora. Among the medicinal plants that constitute its plant cover is *Zanthoxylum zanthoxyloides*[4]. It is a plant of great medicinal fame and owes its fame to its virtue as a painkiller. Indeed, it is nicknamed, "the aspirin of the poor". Well known by the traditional therapists, it is used to treat sickle cell crises, cancer, labor pains, hemorrhoids, rheumatism, snake bites and edema[5]. The roots of the plant are used internally against enteritis, dysentery, diarrhea, urethritis, as a vermifuge and externally on edema, hemorrhoids, abscesses, snake bites, yaws, leprous wounds, syphilitic wounds as well as rheumatic, arthritic pain among others[6]. The roots provide a warm, pungent and stupefying effect on the palate when chewed, and are commonly used in the treatment of gum pain, toothache and tooth decay. The root decoction is used as a mouthwash for sore throat[7].

From the roots of the plant, canthin-6-one, chelerythrine and berberine were isolated and identified. These alkaloids are responsible of the antimicrobial activity of this species[8]. The crude extracts of root bark showed anti-inflammatory, analgesic activities in rodents, antifungal in several small clinical trials, moderate *in vitro* antibacterial against a range of pathogenic bacteria, moderate *in vitro* antifungal, moderate antiviral against herpes simplex virus and antiprotozoal activity against Leishmania major[7]. An ethanol extract of root bark exhibited moderate antioxidant activities *in vitro*.

Dichloromethane and methanol extracts of root bark of this plant showed fungal activities against *Cladosporium cucumerimum*, a champion plant pathogen that particularly attacks members of the Cucurbitaceae family and *Candida albicans* a yeast causing local and systemic mycosis in immunocompromised individuals and bactericidal against *Bacillus subtilis* and *Streptococcus mutans* ATCC25175 [9].

Thus, the objective of this work is part of the valorization of *Z. zanthoxyloides* through an ethnobotanical survey followed by phytochemical study.

MATERIAL AND METHODS

Plant material

The plant material used was constituted of leaves, stems and roots of *Z. zanthoxyloides*. These organs were collected in Yanfolila (11°10'43 "N, 8°09'20 "W) and Ouélessébougou (12°00'00 "N, 7°55'00 "W). The identification of the species was carried out at the Department of Traditional Medicine (DMT) of Mali where a reference sample is kept in the herbarium under number 3800/DMT. The organs were dried in the shade at room temperature before being reduced to powder and then stored in the laboratory of Organic Chemistry and Natural Substances of the Faculty of Sciences and Techniques of the University of Sciences, Techniques and Technologies of Bamako (USTTB).

Ethnobotanical survey

For this study we chose three areas: Bamako, Kati and Ouélessébougou (Figure 1). The choice of these areas is based on the fact that a local survey was carried out among traditional practitioners and herbalists in relation to the areas where the plant is most prevalent, and the three areas mentioned above were recommended by the people interviewed.

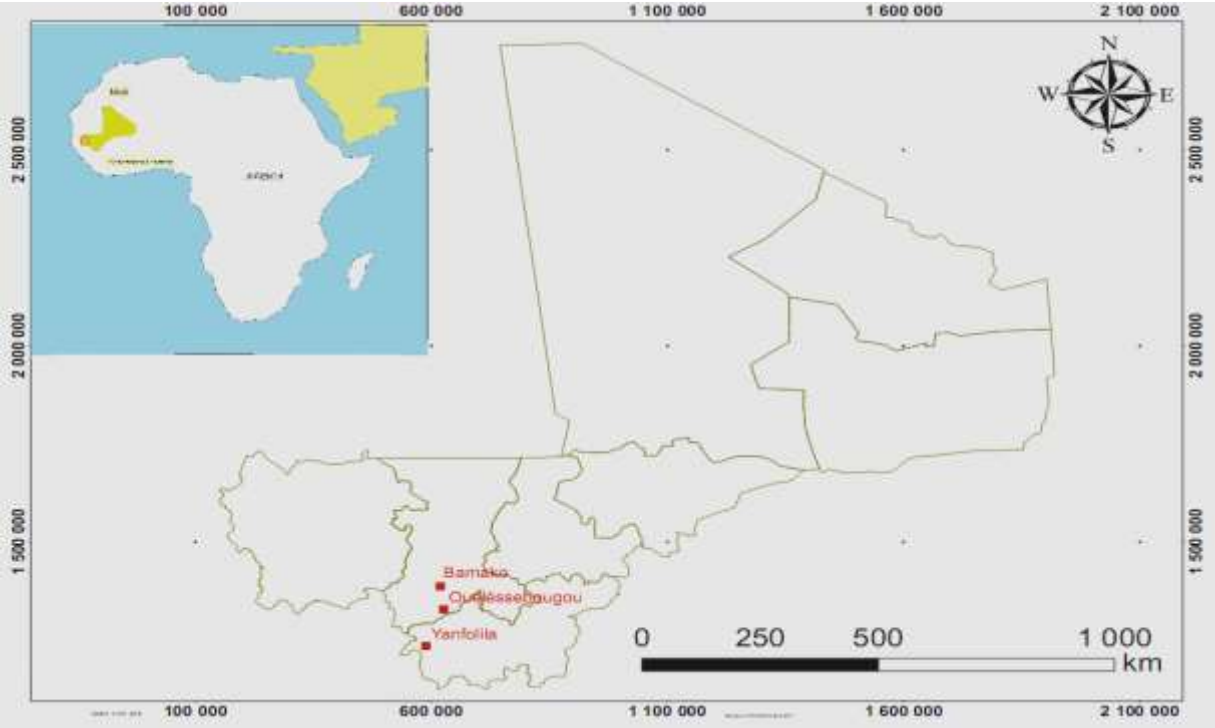

Figure 1: Map showing location of the study sites

We used a questionnaire as a survey tool[10]. These questions were intended to identify informations about the knowledge of the plant, its pharmacological properties and its mode of use among the traditherapists and herbalists. The survey period was from April to May 2018.

Phytochemical screening

The phytochemical screening of plant powders was carried out according to the method of Bruneton[11] and Harbone[12]. It consists in identifying the main chemical groups of a plant by tube characterization reactions with appropriate reagents. The identification of polyphenols was done by the ferric chloride ($FeCl_3$) reaction. Flavonoids were identified by the Shibata reagent or cyanidin reaction. Sterols and terpenes were detected by the Liebermann reaction. The tannins were detected by the Stiasny reagent. The search for saponosides was based on the measurement of the foam index. The reducing compounds were detected with Fehling liquor. The search for alkaloids was done using the general reagents for alkaloid characterization: Dragendorff, Bouchardât and Valser-Mayer reagents. The identification of cardiotonic heterosides is performed by three types of reagents: Baljet, Kedde and Raymond-Marthoud. The mucilages were identified by ethanol test. As for the coumarins, the revelation was carried out at UV 365 nm after addition of 25% ammonia.

Determination of mineral elements

The determination of mineral elements was performed using an atomic absorption spectrophotometer following the standard method of AOAC[13].

RESULTS

Data from survey

All the traditional health practitioners and herbalists in the three survey areas (Bamako, Kati and Ouélessébougou) declared that they knew the plant and its use in traditional medicine. This is show that the plant has a popular medicinal

use. The data from the survey of traditional health practitioners show that the different organs of this plant are used in the management of numerous pathologies and the recorded informations have been summarized in Tables I, II and III.

Table I: Utilizations in traditional medicine of the combination of leaves and stems of *Z. zanthoxyloides* (two bundles: 135 g)

Indications	Modes of use	Numbers of citation
Teethache	Mouthwash twice a day for 7 days with the decoctate.	12
Sore throats	Fumigation of the mouth twice a day with the decoctate for 3 days.	14
Dermatoses	2 applications/day during one week of the powder plaster associated with water.	1
Headaches	Fumigation and head bath with the decoction, twice a day for 4 days.	5
Malaria	Take a bath and drink 1 teacup (about 8 mL) of the decoction, twice a day for 1 week.	3
Bilharzia	Drink 1 teacup of the macerated 24 hours, 3 times/day until healing.	2
Constipation	Drink a glass of coffee on an empty stomach the macerated from 24 hours.	1
Fever	Take a bath and drink 1 teacup of the decoction, twice a day for 3 days.	1
Childbirth pains	Drink 1 tea glass of the decoction.	2
Cardiovascular diseases	Drink 1 teacup of the decoction, 2 times/day until the healing.	2
Sexual impotence	Drink 1 teacup of the decoction, 2 times/day until being healed.	3
Sterility	Drink 1 teacup of the decoction, 2 times/day until the healing.	3
Gastroenteritis	Drink 1 teacup of the decoction, 2 times/day until the healing.	1
Articular pains	Drink 1 teacup of the decoction, 2 times/day until the healing.	1
Hypertension	Drink 1 teacup of the decoction, 2 times/day until the healing.	1
Digestive candidiasis	Drink 1 teacup of the macerated of 24h, 2 times/day during 3 days.	2
Diarrhea	Drink 1 teacup of the decoction, 2 times/day until the healing.	2
Diseases related to Childhood Dentition	Take a bath and drink 1/2 teacup (about 40 mL) of the decoction, 2 times/day until the healing.	1
Syphilis	Drink 1 teacup of the macerated of 24h, 2 times/day until the healing.	1
Dysentery	Drink 1 teacup of the decoction, 3 times/day until the healing.	1
Anemia	Drink 1 teacup of the macerated, 2 times/day until the healing.	2

Table II: Utilizations in traditional medicine of the combination of the roots of Z. zanthoxyloides (One bundle: 127 g)

Indications	Modes of use	Numbers of citation
Tooth decay	Apply 1 pinch of powder, 2 times/day for 4 days	15
Sickle cell disease	Drink 1 teacup of the decoction, 2 times/day for 1 week	4
Oedema	2 applications/day during one week of the powder plaster, associated with water	3
Hemorrhoids	2 applications/day during one week of the powder plaster associated with water	4
Eye infections	Wash the eyes twice a day with the 24-hour macerate until the healing.	1
Intestinal worms	Drink 1 teacup of the 24-hour macerate on an empty stomach, twice a day for 7 days	3
Urinary incontinence	Put 3 pinches of two fingers of the powder, in a soup and drink it 2 days in a row	1
Gonorrhea	Drink 1 teacup of the macerated of 24 hours, 3 times/day for 3 days	2
Lack of appetite	Drink 1 teacup of the macerated of 24 hours, 3 times/day for 3 days	1

Table III: **Utilizations in traditional medicine** of the combination of leaves, stems and roots of *Z. zanthoxyloides* (three bundles)

Indications	Modes of use	Numbers of citation
Diabetes	Drink 1 teacup of the decoction, 2 times/day until the healing	2
Gingivitis	Mouthwash twice a day for 7 days with the decoction	2
Stomachaches	Drink 1 teacup of the decoction, 2 times/day during 4 days	11

Characterization tests

Phytochemical screening revealed the presence of numerous bioactive metabolites in the different parts of *Zanthoxylum zanthoxyloides* (Tableau IV).

Table IV : Résultats du screening Phytochimique de la poudre des trois organes

	Roots		Stems		Leaves	
Chemical groups	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Alkaloids	+	+	+	+	-	-
Polyphenols	-	+	+	+	+	+
Tannins	-	-	-	+	+	+
Flavonoids	+	+	+	+	+	+
Saponosides	-	-	-	-	-	-
Mucilages	-	-	+	+	+	+
Anthracenic derivatives	-	-	-	-	-	-
Cardiotonic heterosides	-	-	-	-	-	-
Sterols and terpenes	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Reducing compounds	-	-	-	-	-	-

*Site 1 : Yanfolila, Site 2: Ouélésséboungou +: Presence, - : Absence

Levels of mineral elements

The results of the determination of mineral elements in the leaves, stems and roots of *Z. zanthoxyloides* showed the richness of this plant in mineral elements especially, calcium, iron, magnesium, sodium, potassium and copper (Figures 2, 3 and 4). The highest levels were recorded with the calcium in the three studied organs.

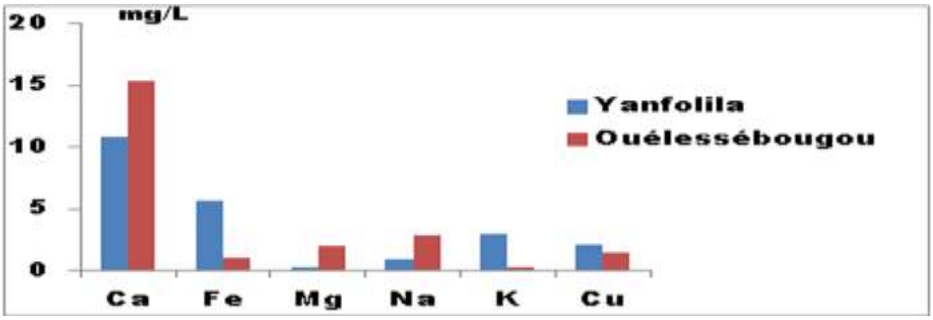

Figure 2: Levels of mineral elements in the roots per site

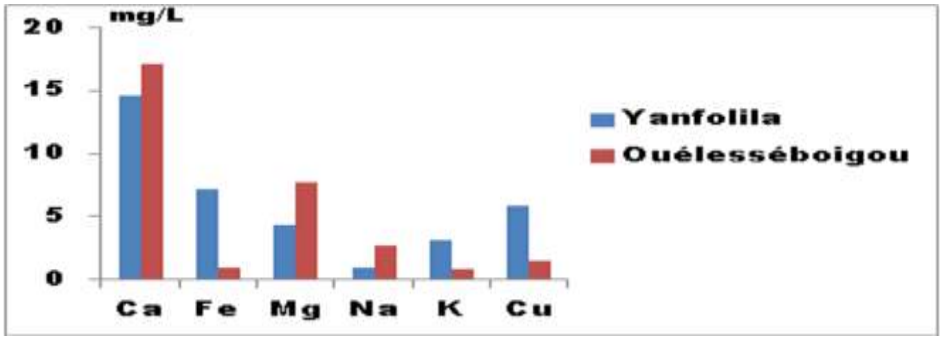

Figure 3: Levels of mineral elements in the stems per site

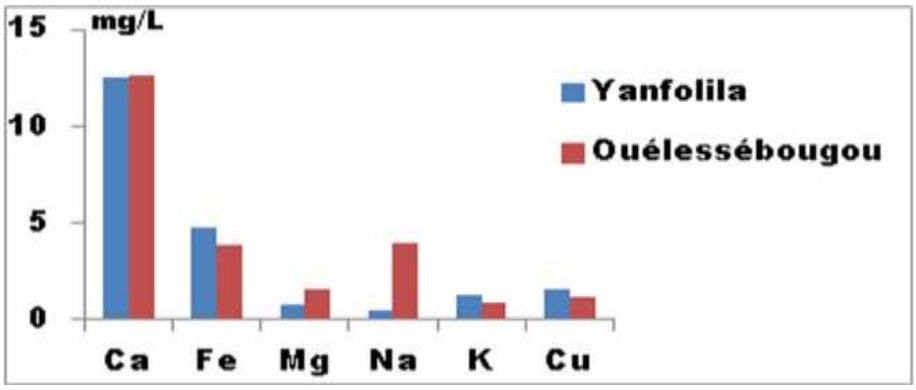

Figure 4: Levels of mineral elements in the leaves per site

DISCUSSION

Sterols and terpenes that possess usually important biological activities could justify the use of *Z zanthoxyloides* by traditional health practitioners for the treatment of malaria and pernicious attacks[14,15]. The coumarins, known for their anti-edematous properties, have also immunostimulating and cytotoxic properpties[11]. Traditionally, the coumarins are used to facilitate urinary and digestive elimination functions. This fact could justify the use of *Z zanthoxyloides* roots by the health practitioners to combat oedema, urinary incontinence, gastroenteritis and stomach aches. The alkaloids have antimicrobial and antimalarial activities and low cytotoxicity, which would justify the use of *Z zanthoxyloides* roots by traditional therapists to fight tooth decay, gingivitis[16]. Because of their richness in tannins, the traditional health practitioners use the leaves and stems to combat treat diarrhoea and dysentery. Indeed, tannins have antibacterial and anti-diarrhoeal activities[9].

From the ethnobotanical survey, it was found that thirty-three (33) diseases can be treated by *Z. zanthoxyloides*. The main cited diseases were: tooth decay, sore throat, teeth, stomach and headache, hemorrhoid. A study conducted by [7] in 2011 showed that the plant is known for the same pharmacological properties.

The phytochemical screening revealed the presence of alkaloids, polyphenols (tannins, flavonoids), coumarins, mucilages, sterols and terpenes. The flavonoids, coumarins, sterols and terpenes are present in the three organs of both sites. The saponosides, anthracene derivatives, cardiotonic heterosides and reducing compounds are totally absent in the three organs of both sites. The alkaloids have been found in the roots and stems. The tannins and mucilages are absent in the roots of both sites. The flavonoids have antioxidant and anti-inflammatory activities and play a positive role in the treatment of cardiovascular diseases. They are also known for their antiviral, antimicrobial, and antitumor activities[17,18]. This fact could explain the use of the plant's roots by the health traditherapiss to fight intestinal worms, cardiovascular diseases. The sterols and terpenes with their important biological activities could justify the use of *Z. zanthoxyloides* by the traditional health practitioners to treat malaria and pernicious attacks[13,14]. The coumarins, known for their anti-edematous properties, have been the subject of clinical studies in the patients with advanced cancers. They are immunostimulating and have cytotoxic activity[11]. Traditionally, the coumarins are used to facilitate urinary and digestive elimination functions. That could justify the use of *Z. zanthoxyloides* roots by health tradithérapeutes to fight against edema, urinary incontinence, gastroenteritis and stomach aches. The alkaloids have antimicrobial and antimalarial activity and low cytotoxicity, which would justify the use of the roots of *Z. zanthoxyloides* by traditional healers to fight against dental caries, gingivitis[16]. Due to their richness in tannins, the leaves and stems are used by traditional health practitioners to fight against diarrhea and dysentery. Indeed, the tannins have antibacterial and anti-diarrheal activities[9].

The results of the Atomic Absorption Spectrometry analysis revealed the presence of many mineral elements such as calcium, iron, magnesium, sodium, potassium and copper in the plant powder of the three organs harvested at each site. The calcium was the predominant element in all three organs from both sites, but its highest levels were recorded in the organs from the Ouélessébougou site. The calcium plays a crucial role in the proper functioning of our body, our cells, nerves, muscles, glands, and skeleton, but also our blood vessels. This could explain the use of the plant by traditional therapists to treat tooth decay. Iron is the second dominant element in the three organs of the two sites, but its highest contents were obtained in the organs from Yanfolila site. Its presence could explain the use of the plant to fight anemia by traditional healers. The level of magnesium is remarkable in the stems. This ion improves the sensitivity of the organs to insulin[19]. This could explain the use of the plant in the treatment of diabetes and cardiovascular diseases by traditional practitioners.

Sodium levels are important in leaves from Ouélessébougou. Recent experimental and clinical studies have shown major effects of sodium intake on endothelial function and potentially very important interactions between sodium intake and the immune system[20]. This may explain the use of the plant in the treatment of diabetes and cardiovascular disease by traditherapists.

The potassium is present in all three organs at both sites. This ion is the main cation inside the cells, plays a role in the maintenance of cellular osmolarity and acid-base balance, as well as in the transmission of nerve stimulation and the regulation of cardiac and muscular functions. It has also recently been shown to have an antihypertensive effect by promoting sodium excretion[21]. Its presence could explain the use of the species by traditherapists to combat constipation, high blood pressure and cardiovascular diseases.

In 2011[7], in the Netherlands, Matu obtained the same mineral elements in the pericarp of the fruit of *Z. zanthoxyloides*. Mineral elements are essential elements to better fight against certain diseases. Their deficiencies can have negative impact on human health[22].

CONCLUSION

The ethnobotanical survey carried out among Malian traditional practitioners and herbalists, allowed us to know

the abundant use of the plant by the rural populations in order to treat numerous common diseases.

The phytochemical screening of the plant allowed to identify the main secondary metabolites present. Thus, we identified the presence of alkaloids, polyphenolic compounds (flavonoids, tannins), coumarins, mucilages, sterols and terpenes. Most of these compounds have interesting biological activities that would justify the abundant and varied use of *Z. zanthoxyloides* in folk medicine. The species harvested from both sites contains the same chemical groups. Atomic Absorption Spectrometry analysis detected the presence of many mineral elements such as calcium, iron, magnesium, sodium, potassium and copper in the plant powder of the three organs of the two sites at different levels. These mineral elements are essential to better fight against certain diseases. These obtained data justify the place of *Z. zanthoxyloides* as a prised medicinal species by local communities in the African pharmacopoeia. In perspectives, to make bioguided studies in order to elucidate the secondary metabolites active on pathogens would be interesting.

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