

Original Article

Phytochemical Study and Anticancer Activity of Root Extracts of *Zanthoxylum zanthoxyloides* (Lam.) Waterman (Rutaceae), Collected in Mali

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Abstract - *Zanthoxylum Zanthoxyloides* (Lam.) Waterman, commonly known by its vernacular name “Wo”, is a medicinal plant of the Rutaceae family widely used in Malian traditional medicine for the treatment of several diseases, including cancer. The aim of this study was to evaluate the anticancer activity of root extracts of *Zanthoxylum zanthoxyloides* collected from two sites in Mali (Yanfolila and Ouélessébougou). Ethyl acetate and aqueous extracts were prepared from powdered roots. Chemical constituents were characterized using standard tube reaction tests. The anticancer activity of the extracts was evaluated on the MCF-7 human breast cancer cell line using the resazurin assay. Phytochemical screening revealed the presence of alkaloids, coumarins, flavonoids, sterols, and terpenes. The ethyl acetate extracts from Ouélessébougou and Yanfolila exhibited anticancer activity with IC_{50} values of 37.28 $\mu\text{g/mL}$ and 47.10 $\mu\text{g/mL}$, respectively. The ethyl acetate extract from Ouélessébougou showed the highest activity. These results are nevertheless clearly better than those of tamoxifen (with an $IC_{50} = 4.425 \mu\text{g/mL}$) and [5-Fluorouracil] (with an IC_{50} of 0.525 $\mu\text{g/mL}$) tested on the same cell line in the lab under the same conditions. The anticancer activity observed is the first study on extracts of *Z. zanthoxyloides*, a medicinal plant collected in Mali. This activity could be due to a synergistic action of the chemical components. The anticancer activity of the extracts could be attributed to a synergistic action of their chemical constituents. Further investigations are needed to isolate and identify the compounds responsible for this activity.

Keywords - *Zanthoxylum zanthoxyloides*, Anticancer activity, Mali.

1. Introduction

For centuries, both humans and animals have relied extensively on plants for food and medicinal needs. Across all continents, the use of medicinal plants to treat various diseases is an ancestral practice rooted in cultural and religious values and transmitted from generation to generation. The tropical environment, of which Africa is a part, possesses remarkable biological diversity [1].

Medicinal plants, therefore, play an important role in traditional healthcare systems worldwide, particularly in regions where access to modern medicines remains limited [2]. Plants have adapted to their environments by developing a wide range of molecules with remarkable physicochemical properties. Although these compounds are essential for plant survival, humans have long exploited them in various fields such as pharmaceuticals, cosmetics, and agrochemicals, among others [3]. Cancer remains one of the leading causes of death worldwide. In response to this, research is increasingly turning to nature to find ways to limit the side

effects of cancer treatments. It continues to be a major global health challenge, driving the constant search for effective and less toxic treatments. Among the avenues being explored, medicinal plants play an important role thanks to their bioactive compounds that can inhibit tumor growth, trigger cancer cell apoptosis, or boost immune defenses. Current research highlights the promising potential of anticancer plants. Plants contain a wide variety of molecules with antitumor properties:

- Alkaloids: vinblastine, vincristine (from *Catharanthus roseus*), used in chemotherapy;
- Polyphenols: curcumin (turmeric), resveratrol (grape), quercetin (onions, apples);
- Terpenes: taxol (Pacific yew), boswellic acids (boswellia);
- Flavonoids: luteolin, apigenin, which inhibit cell proliferation;
- Saponins: modulate immunity and cell death.



These compounds work through several mechanisms: triggering apoptosis, blocking angiogenesis, adjusting cell signaling pathways, and neutralizing free radicals.

Several plants are the focus of intensive studies for their potential anticancer properties:

- *Curcuma longa* (curcumin): anti-inflammatory and anticancer activity in various cell models (breast, colon, prostate)
- *Camellia sinensis* (green tea): catechins that inhibit tumor growth and promote cancer cell death
- *Taxus brevifolia* (Pacific yew): source of paclitaxel, a major chemotherapy drug
- *Viscum album* (mistletoe): used alongside conventional treatments, boosts the immune response
- *Withania somnifera* (ashwagandha): triggers apoptosis and reduces proliferation in some cancers [4].

Among the many medicinal plants available worldwide, our attention was focused on *Zanthoxylum zanthoxyloides*. It is a tree that grows naturally in West Africa, reaching 5 to 15 m in height, with a straight trunk, often short and branched, and a rounded crown. The trunk, branches, and midrib of the leaflets bear large conical, recurved, and sharp claw-shaped spines, reaching up to 1 cm in length, brown in color with a lighter base. This evergreen shrub flowers from October to March, while its fruits ripen from February to April [5]. The roots, measuring 2 to 4 cm in diameter, have yellow wood. The root bark, yellow-brown in color, is relatively thin (1 to 2 mm) and marked with bright yellow spots. The taste of the root bark, more intense than that of the stem bark, is aromatic, bitter, and peppery. Chewing the roots causes numbness of the tongue [5]. Several studies have been conducted on different species of the genus *Zanthoxylum* to isolate compounds with antitumor activity. These include

berberine, fagaronine, nitidine, 5-methoxycanthin-6-one, 4-methylthiocanthin-6-one, acronycine, and skimmianine [6].

However, no study has yet been conducted on the anticancer activity of ethyl acetate and aqueous extracts of *Zanthoxylum zanthoxyloides* collected in Mali. The present study aims to evaluate the anticancer activity of ethyl acetate and aqueous extracts obtained from root powder on the proliferation of MCF-7 human breast cancer cells.

2. Material and Methods

2.1. Material

2.1.1. Plant Material

The plant material used in this study consisted of roots of *Zanthoxylum Zanthoxyloides* (Lam.) Waterman. They were collected from their natural habitat in Yanfolila, a town located in the Sikasso region (3rd administrative region of Mali), and in Ouélessébougou, a town located in the Koulikoro region (2nd administrative region of Mali). The collection was carried out with the assistance of a traditional healer.

The species was identified by a forestry engineer at the Institute of Research in Traditional Medicine and Traditional Pharmacopoeia (INRMPT), formerly the Department of Traditional Medicine (DMT) of Mali, as *Zanthoxylum Zanthoxyloides* (Lam.) Waterman. A voucher specimen is deposited in the herbarium of the same institute under reference number 3800/DMT.

The plant organs were shade-dried at room temperature for two weeks in the Laboratory of Organic Chemistry and Natural Substances of the Faculty of Science and Technology, University of Sciences, Techniques and Technologies of Bamako (USTTB). They were then ground into powder using a mill and stored in cloth bags, protected from light and moisture, for subsequent analyses.

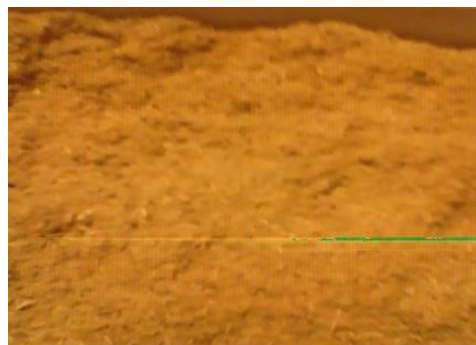
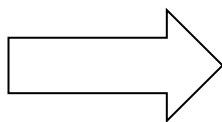


Fig. 1 Whole root and root powder of *Zanthoxylum Zanthoxyloides*

2.2. Cancer Cells

The antitumor assays were conducted on the MCF-7 human breast cancer cell line, the only one that was available at the Laboratory of Pharmaceutical Plant and Fungal

Sciences of the ECREIN research team (*MicroEnvironnement CellulaiRE Immuno-modulation et Nutrition*) at the Faculty of Pharmacy, University of Auvergne.



Fig. 2 MCF-7 cancer cells in culture medium

2.3. Tested Products

The tested products consisted of ethyl acetate and aqueous root extracts of *Zanthoxylum Zanthoxyloides* collected from the Yanfolila and Ouëlessébougou sites.

3. Methods

3.1. Preparation of Extracts

Since plant material often contains significant amounts of fats, waxes, terpenes, pigments, and other lipophilic substances that may interfere with the extraction process, particularly by inducing emulsion formation, a preliminary delipidation step was carried out. Two hundred grams (200 g) of powdered plant material were treated with hexane (500 mL) under continuous mechanical stirring at room temperature for 24 h using a SKIL-type metallic stirrer.

The mixture was then filtered under vacuum, and the plant material was subjected to three successive macerations in methanol (1 L) for 24 h each. After filtration, the methanol was evaporated to dryness under reduced pressure at 50 °C using a rotary evaporator (Büchi Rotavapor R-151) to obtain the dry methanolic extract, which was collected in a glass flask and stored under refrigeration until use. The dry methanolic extract was then redissolved in methanol, hexane, and water. The resulting mixture was subjected to a second delipidation step with hexane, repeated three times. Ethyl acetate was subsequently added to the mixture. After phase separation, ethyl acetate and aqueous fractions were obtained.

These two fractions were concentrated to dryness under reduced pressure at 50 °C using a rotary evaporator (Büchi Rotavapor R-151) to obtain the dry ethyl acetate and aqueous extracts, which were collected in glass flasks and stored under refrigeration until further use.

3.2. Phytochemical Screening

Colorimetric tube reactions using specific reagents were carried out to identify the major chemical groups present in the ethyl acetate and aqueous root extracts.

3.3. Flavonoids

One milliliter (1 mL) of each extract was treated with a few drops of concentrated HCl. A few milligrams of magnesium turnings were then added. The appearance of a pinkish-red coloration confirmed the presence of flavonoids [7].

3.4. Tannins

To 1 mL of each extract, 2 to 3 drops of 1% FeCl₃ solution were added. After a few minutes of incubation, the appearance of a dark blue or green coloration indicated the presence of tannins [8].

3.5. Alkaloids

To 2 mL of each extract, 5 mL of 1% HCl was added. The mixture was incubated in a water bath. Each extract was then divided into two portions: Mayer's reagent was added to the first portion, and Wagner's reagent to the second. The formation of white and brown precipitates, respectively, indicated the presence of alkaloids [9].

3.6. Terpenoids

One milliliter (1 mL) of each extract was treated with 0.4 mL of concentrated H₂SO₄. The appearance of two phases and a brown coloration at the interface revealed the presence of terpenoids [10].

3.7. Reducing Sugars

To 5 mL of each extract, 1 mL of Fehling's solution (A+B) was added, and the mixture was heated in a water bath for 5 minutes. The appearance of a brick-red precipitate indicated a positive test [11].

3.8. Coumarins

Five milliliters (5 mL) of ether extract obtained after 24 h of maceration were evaporated in open air and then redissolved in 2 mL of hot water. The solution was divided into two test tubes. The presence of coumarins was detected by adding 0.5 mL of 25% NH₄OH to one of the tubes and observing under UV light at 366 nm. An intense blue

fluorescence in the treated tube indicated the presence of coumarins [7].

3.9. Mucilages

The formation of flocculent precipitates after mixing 1 mL of 10% decoction with 5 mL of absolute ethanol indicated the presence of mucilages [5].

3.10. Cardiotonic Glycosides

A chloroform phase was prepared from 1 mL of extract powder to which 10 mL of 60% ethanol and 5 mL of 10% lead acetate solution were added. After heating the mixture for 10 minutes in a boiling water bath, 10 mL of chloroform was added, and the mixture was shaken. The organic phase was separated and divided into three test tubes. The contents of each tube were evaporated to dryness in a boiling water bath. The residue in each tube was redissolved in 0.4 mL of isopropanol.

To the first tube, 1 mL of Baljet reagent was added; to the second, 1 mL of Kedde reagent; and to the third, 1 mL of Raymond-Marthoud reagent. Five drops of a 5% KOH solution in ethanol were then added to each tube.

The development, after 10 minutes, of an orange coloration in the first tube, a violet-red coloration in the second, and a transient violet coloration in the third indicated the presence of cardiotonic glycosides [7].

3.11. Anthracene Derivatives

A chloroform extract was first prepared from 1 mL of extract and 10 mL of chloroform, followed by heating for 3 minutes in a water bath. The solution was filtered while hot. The residue exhausted by chloroform was used to detect glycosidic forms (*O*-glycosides and *C*-glycosides).

3.12. Free Anthracene Derivatives

Were identified by adding 1 mL of 50% diluted ammonia to 1 mL of chloroform extract, followed by agitation. The appearance of a more or less red coloration indicated their presence.

3.13. O-Glycosides

Were characterized by adding 10 mL of distilled water and 1 mL of concentrated HCl to the residue from the chloroform-extracted extract. The mixture was heated for 15 minutes in a water bath, cooled under running water, and filtered. Five milliliters of chloroform were added to 5 mL of the filtrate. After shaking, the organic phase was separated and treated with 1 mL of 50% diluted ammonia. The appearance of a more or less intense red coloration indicated their presence.

3.14. C-glycosides

Were characterized by adding 10 mL of distilled water and 1 mL of 10% FeCl₃ to the aqueous phase previously

obtained during *O*-glycoside testing. The mixture was heated for 30 minutes in a water bath and cooled under running water. After extraction with 5 mL of chloroform, 1 mL of 50% diluted ammonia was added to the organic phase. The appearance of a more or less intense red coloration indicated their presence [11].

3.15. Saponins

One milliliter (1 mL) of each extract was mixed with 2 mL of hot distilled water. The mixture was shaken for 15 seconds and allowed to stand for 15 minutes. The presence of persistent foam exceeding 1 cm in height confirmed the presence of saponins [12].

3.16. Anticancer Activity

The anticancer activity was evaluated using the resazurin assay.

MCF-7 cells were seeded into 96-well plates at a concentration of 50,000 cells/mL. Two hundred microliters (200 µL) of culture medium were added to each well, corresponding to 10,000 cells per well. The cells were then incubated at 37°C in an atmosphere containing 5% CO₂.

After 24 hours, the cells were exposed to different extract concentrations: 10 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL. At the same time, for each extract, a DMSO control column (the extract's solvent) and a doxorubicin column as a positive control were set up. After 72 hours of incubation, the culture medium was replaced with a resazurin solution (25 µg/mL), which is reduced by metabolically active cells into fluorescent resorufin, measurable at 590 nm.

Fluorescence intensity is proportional to the number of viable cells. Fluorescence was measured using a Fluoroskan plate reader with the Résa-96 program at 590 nm.

If no inhibition or only weak inhibition of cell proliferation was observed during this initial screening, the extract study was discontinued. Conversely, if cytotoxic activity was observed, a second screening was performed using refined concentration ranges.

Once the optimal screening concentrations were identified, three independent experiments were conducted under the same conditions to validate the results.

For each extract, the 50% inhibitory concentration (IC₅₀) was calculated.

Results are presented as mean ± SEM (standard error of the mean).

Statistical analysis was performed using the paired Student's *t*-test (N = 3).

4. Results and Discussion

4.1. Results

4.1.1. Chemical Constituents

Table 1. List of the different chemical families identified in the root extracts of *Zanthoxylum zanthoxyloides*

Chemical Constituents	Ethyl Acetate Extract		Aqueous extract	
	Site 1	Site 2	Site 1	Site 2
Alkaloïds	+	+	-	-
Flavonoïds	+	+	-	-
Tannins	-	-	-	-
Saponins	-	-	-	-
Mucilages	-	-	-	-
Anthracene Derivatives	-	-	-	-
Hétérosides cardiotoniques	-	-	-	-
Terpenoids	+	+	-	-
Coumarines	-	-	+	+
Reducing Sugars	-	-	-	-

Site 1 : Yanfolila

Site 2 : Ouélessébougou

+: Attendance

-: Absence

4.2. Anticancer Activity

Table 2. Percentage Inhibition of MCF-7 Cancer Cell Proliferation as a Function of the Concentrations of the Ethyl Acetate Extract from the Yanfolila Site

Concentrations (µg/mL)		40	45	50	55	60	65	70	75	CI ₅₀
Inhibition rate (%)	N 1	15.38	12.78	22.25	16.89	27.21	40.15	73.63	90.39	66.43
	N 2	49.41	64.21	77.84	84.76	87.93	89.16	91.85	90.34	40.18
	N 3	44.49	55.54	72.90	80.67	87.19	89.16	91.85	90.34	42.44
Average		36.43	44.14	57.66	60.77	67.44	73.75	85.78	90.82	47.10
SEM		10.62	15.89	17.76	21.97	20.12	16.82	6.08	0.46	

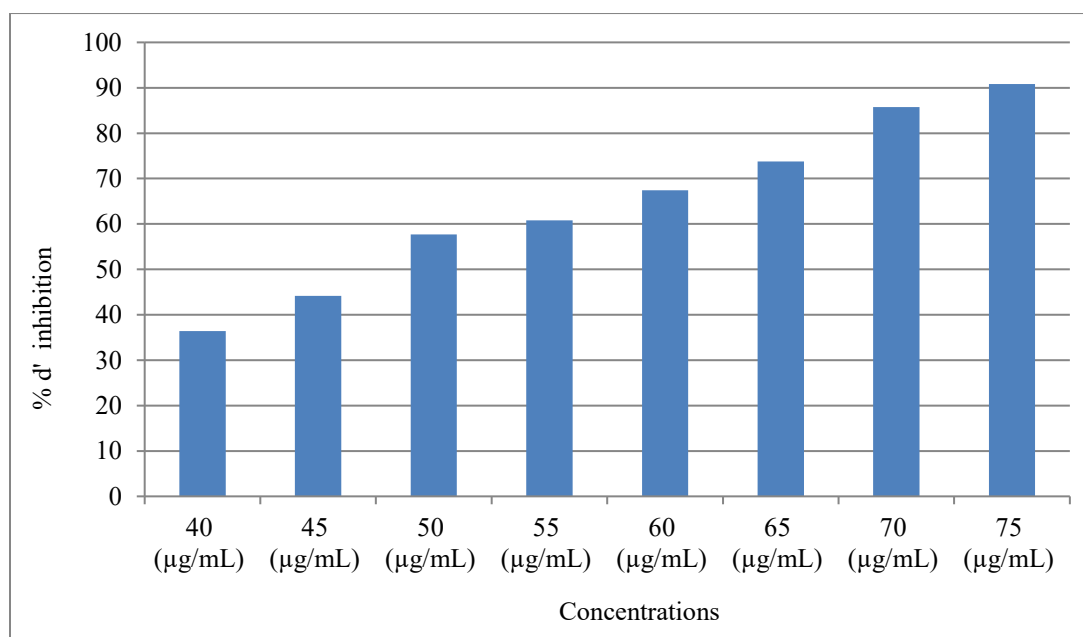
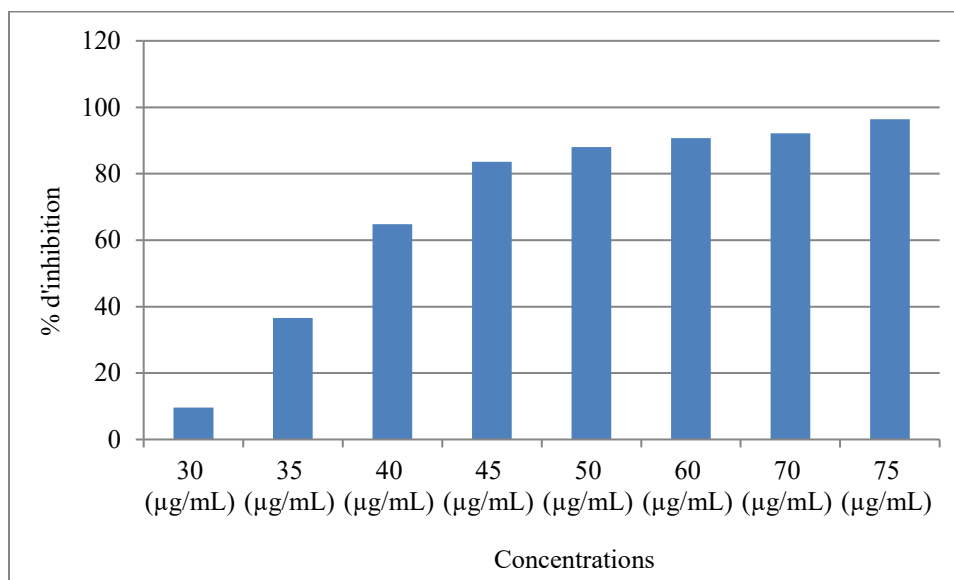


Fig. 3 Percentage Inhibition of MCF-7 Cancer Cell Proliferation as a Function of the Concentrations of the Ethyl Acetate Extract from the Yanfolila Site (average $\pm$ SEM Paired t-test on 3N)

Table 3. Percentage Inhibition of MCF-7 Cancer Cell Proliferation as a Function of the Concentrations of the Ethyl Acetate Extract from the Ouélessébougou Site

Concentrations ($\mu\text{g/mL}$)		30	35	40	45	50	60	70	75	CI ₅₀
Inhibition rate (%)	N 1	14.56	41.44	59.39	81.86	88.15	92.08	93.56	95.07	57.74
	N 2	4.37	33.68	66.59	82.21	84.19	86.34	87.58	96.68	37.39
	N 3	9.86	34.74	68.52	86.64	91.80	93.81	95.47	97.50	37.17
Average		9.60	36.62	64.83	83.57	88.04	90.75	92.20	96.42	37.28
SEM		2.19	2.10	2.62	1.35	2.20	1.55	1.26	0.65	

**Fig. 3 Percentage Inhibition of MCF-7 Cancer Cell Proliferation as a Function of the Concentrations of the Ethyl Acetate Extract from the Ouélessébougou Site (average $\pm$ SEM Paired t-test on 3N)**

5. Discussion

Alkaloids, flavonoids, terpenoids, and coumarins were identified in the extracts from both collection sites. Alkaloids are known for their potent pharmacological effects, particularly their anticancer activities [13].

Flavonoids have also demonstrated anticancer properties through their ability to inhibit cancer cell growth and induce apoptosis (programmed cell death) [14].

Coumarins are recognized for their anticoagulant and anti-inflammatory effects [6]. Sterols contribute to cholesterol reduction and exhibit anti-inflammatory and anticancer properties [15]. Terpenes possess anti-inflammatory, antiviral, and anticancer activities [16].

The ethyl acetate root extracts of *Zanthoxylum zanthoxyloides* from both sites exhibited anticancer activity. It is noteworthy that the ethyl acetate extract from Ouélessébougou, with an IC₅₀ value of 37.28 $\mu\text{g/mL}$, showed greater activity than the ethyl acetate extract from Yanfolila, which had an IC₅₀ value of 47.10 $\mu\text{g/mL}$. This activity may be attributed to the presence of alkaloids and flavonoids in the extracts.

In contrast, the aqueous extracts from both sites showed no significant effect on the proliferation of MCF-7 cells under our experimental conditions.

Our findings are consistent with those reported by other researchers. An *in vitro* study demonstrated that *Zanthoxylum zanthoxyloides* extracts exhibited cytotoxic activity against several cancer cell lines.

The extracts induced apoptosis and inhibited cancer cell proliferation. Similarly, alkaloids isolated from *Zanthoxylum zanthoxyloides* showed inhibitory effects on cancer cell growth, suggesting their therapeutic potential.

Berberine, fagaronine, nitidine, 5-methoxycanthin-6-one, 4-methylthiocanthin-6-one, acronycine, and skimmianine, compounds isolated from *Zanthoxylum zanthoxyloides* by Odebiyi and Sofowora [17], have also been reported to exhibit antitumor activity.

Extracts of *E. senegalensis* were tested on five cancer cell lines. The dichloromethane and ethyl acetate fractions showed interesting cytotoxic activity [18].

This study represents the first evaluation of the anticancer activity of ethyl acetate and aqueous extracts of *Zanthoxylum zanthoxyloides* of Malian origin.

Conclusion and Perspectives

This study demonstrated that *Zanthoxylum zanthoxyloides* is a potential natural resource with many facets, offering interesting paths for developing new cancer treatments. It is necessary to deepen the understanding of

their mechanisms of action and try to optimize their clinical use. This convergence between herbal medicine and oncology opens encouraging prospects in the fight against cancer.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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