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Evaluation of Antiradical and Antibacterial Activities of Hydroethanolic Extract of *Spondias mombin* Leaves from Benin

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Abstract: *Spondias mombin* is a plant species traditionally used in Benin in the treatment of many infections such as *salmonellosis* and urinary tract infections. In order to provide a scientific basis for this use, this work aims the biological and chemical valorization of *S. mombin*. After the phytochemical screening of the hydroalcoolic extract, the contents of phenolic compounds were evaluated and the antibacterial and anti-free radical activities were determined. The results obtained reveal, the presence of several metabolites such as tannins, flavonoid, coumarins, reducing compounds and anthraquinones known for their variable biological activities. The contents of total phenolic compounds (40.68 mg Eq GA/g) and flavonoids (228.40 mg Eq Q/g) are very interesting. The anti-radical activity of this extract is very high with an IC_{50}<0.007 mg/mL compared to that of ascorbic acid (2.84 mg / ml). An interesting antibacterial activity was noted on the resistant strains of *Streptococcus D, Staphylococcus aureus, Klebsiella pneumoniae* and *Citrobacter Sp*. These good antioxidant and antimicrobial properties could be related to the presence of secondary metabolites and confirm the traditional use of *S. mombin* as traditional antioxidant and antibacterial agent.
Keywords: antibacterial properties, anti-free radical properties, secondary metabolites, *Spondias mombin*

I. INTRODUCTION

Spondias Mombin, Prunier mombin (French), Java plum (English) or akikontin (fon) is a plant species native to the tropical Americas, widespread throughout Africa and tropical Asia and found in all regions of Benin [1]. It is a deciduous tree up to 25 m tall with a trunk exceeding 50 cm in diameter and a deeply cracked bark, prickly when young. The branches are low and the branches glabrous. The leaves are alternate, once pinnate with a strange terminal leaflet. The fruits are ovoid or ellipsoid, orange to yellow or brown and in clusters of 1 to 20 fruits [2]. It is used commonly for food and medicinal purposes. Concerning it biological activities, the antibacterial and antifungal activity of bark, root and leaf extracts of *Spondias mombin* on a broad spectrum of beta-lactamase-producing *enterobacteria* has been demonstrated [3, 4]. Their hypotensive [5], lipid-lowering and abortive activity, as well as their richness in tannins, saponins, flavonoids, sterols, quinine, mono and sesquiterpenes, triterpenes, steroids, phenylpropanoid glycosides and cinnamic derivatives have been reported [6, 7]. It has also been reported that, the aqueous, methanolic and ethanolic extracts of the leaves of *Spondias mombin* have antihelminthic properties [8-10]; anxiolytics properties [11]; antiviral properties on Coxsackie and Herpes simplex viruses [12]; pronounced antibacterial properties against *Bacillus cereus*, *Streptococcus pyogenes* and *Mycobacterium fortuitum*; molluscicide properties against snail *Biomphalaria glabrata* [13] and anti-diabetic properties [14]. Its fruits have shown their effectiveness in the treatment of infertility [15]. The present study aims to contribute to the medicinal valorization of *Spondias mombin*, by the identification of its secondary metabolites, as well as the evaluation of the anti-free radical and antibacterial properties of its leaves hydroethanolic extract.

Photo 1 : *Spondias mombin*

II. MATERIALS AND METHODS

2.1. Plant material

The leaves of *Spondias mombin* were collected in July 2019 in the city of Abomey-Calavi in the Atlantic department of Benin.

2.2. Bacterial strains

The bacterial strains used are multiresistant clinical bacterial strains. These are *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus D* and *Citrobacter spp*.

2.3. Method

2.3.1. Pre-treatment of plant material

The leaves of *Spondias mombin* were collected and dried at room temperature in the laboratory (25°C-30°C) until their masses stabilized and then reduced into powder.

2.3.2. Selection of bacterial strains

Each bacterial strain was cultured on Miller Hunter agar and incubated in an oven at 37°C for 24 hours. After the culture of bacteria, the bacterial inocula were prepared in Miller Hunter agar using a colony isolated from each bacteria.

2.3.3. Identification of secondary metabolites of the hydroethanolic extract of *S. mombin*

Secondary metabolites have been identified by coloration and precipitation reactions specific to each family of metabolites [16-18].

Preparation of the hydroethanolic extract of *S. mombin*

The technique used to prepare the extracts is that of maceration. The hydro-ethanolic extract of *Spondias mombin* was prepared at room temperature by mixing 300 grams of powder with 800 milliliters of 70% ethanol for 24 hours. After filtration, the extracts were evaporated at mild temperature (40°C) using a rotary evaporator of the Heidolph type and then dried in an oven at 40°C [19]. The extract obtained was kept in the refrigerator until use.

Determination of phenolic compounds of the hydroethanolic extract of *S. mombin*

The determination of total phenolic compounds was made by the Folin-Ciocalteu reagent [20, 21]. The aluminum trichloride (AlCl3) method has been used to quantify total flavonoids [19, 21], while the determination of condensed tannins was carried out by the sulfuric vanillin method [19, 22].

2.3.4. Evaluation of the antibacterial activity of the hydroethanolic extract of *S. mombin*

Antibacterial activity was determined by the micro-dilution method in microplates and petri dishes [23-25].

Evaluation of the sensitivity of bacteria to the hydroethanolic extract of *S. mombin*

The sensitivity test of the bacterial strains to the hydroethanolic extract of *Spondias mombin*, consisted of adding 100 µl of bacterial inoculum to 100 µl of concentrated extract at 20 mg/ml. Then The microplate was, incubated for 24 hours at 37°C. 40 µl of an aqueous solution of Iodonitrotetrazolium (INT) concentrated to 0.2 mg were added to each well followed by an incubation time of 30 minutes. The appearance of a pink or red colour in a well indicates the inactivity of the extract. The tests were carried out in triplicate.

Determination of Minimum Inhibitory Concentrations (MIC)

The MICs were determined on five multidrug resistant strains, *Escherichia coli, Staphylococcus aureus, Enterococcus D, Citrobacter sp* and *klebsiella pneumoniae* using a concentration range of 5 mg/ml; 2.5 mg/ml; 1.25 mg/ml; 0.625 mg/ml; 0.312 mg/ml; 0.156 mg/ml; 0.078 mg/ml and 0.039 mg/ml.

Assessment of anti-free radical activity

The anti-free radical activity of *Spondias mombin* was evaluated by the 2,2-diphenyl-1-picrylhydrazyle (DPPH) method, based on the measurement of the free radical scavenging of a DPPH solution which results in the disappearance of the purple color of it. To do this, the tanks were left in the dark for one hour and the absorbance measured at 517 nm [20, 26, 27]. The percentage of inhibition of the DPPH radical translating the anti-free radical activity of the extracts was determined according to equation 1 below:

Equation (1) $P(\%) = \frac{(Ab - Ae)}{Ab} * 100$

With P: percentage of trapping; Ab: absorbance of the blank, Ae: Absorbance of the sample.

III. RESULTS AND DISCUSSION

3.1. Identification of secondary metabolites of the hydroethanolic extract of the leaves of *S. mombin*

Table 1 shows the secondary metabolites present in the *S. mombin* leaves. We note the presence of tannins, flavonoids, coumarins, reducing compounds and anthraquinones.

The samples from Nigeria revealed the presence of the current secondary metabolites, as well as other secondary metabolites such as saponins, alkaloids, phenols, oxalates, phytates and cyanogenic glycosides [7, 28, 29]. The differences observed could be attributed to particularities of harvesting area such as type of soil and climatic conditions.

Table 1: Secondary metabolites of the leaves of *S. mombin*

Secondary metabolites	***Spondias mombin***
Tannins	+
Cachectic tannins	-
Gallic tannins	+
Flavonoids	+
Anthocyanins	-
Leuco-anthocyanins	-
Saponins	-
Mucillages	-
Reducing compounds	+
Alkaloids	-
Coumarins	+
Cyanogenic derivatives	-
Anthraquinones	+
Quinones	-

(+): Positive (-): Negative

The presence of tannins could well explained the use of *S. mombin* for its antimicrobial properties since ellagitannins geraniin, galloylgeraniin, didehydroellagitannin, isolated from this plant species have revealed a pronounced antiviral activity against Coxsackie and Herpes simplex viruses [12].

3.2. Content of phenolic compounds in the hydroethanolic extract of the leaves of *S. mombin*

The content of phenolic compounds (total phenolic compounds and total flavonoids) of the hydroalcoholic extract of *S. mombin* is summarized in Table 2. It appears from Table 2 that the hydroalcoholic extract of *S. mombin* contains 40, 68 mg Eq GA/g of total phenolic compounds and 228.40 mg Eq Q/g of total flavonoids.

Table 2: Content of total phenolic compounds

Secondary metabolites	***Spondias monbin***
Total phenolic compounds	40, 68 mg Eq GA/g
Total Flavonoids	228.40 mg Eq Q/g

The sample analysed by Nworu et al., (2007) [29] revealed the presence of saponins, flavonoids, tannins, glycosides, resins, protein, and triterpenes. The one characterized by Njoku et al., (2007) [28] showed the presence of tannins (3.82%), saponins (7.60%), flavonoids (3.00%) alkaloids (6.00%) and phenols (1.00%) whereas Igwe et al., (2010) [7] identified secondary metabolites such as saponins (4.80 ± 0.35%), alkaloids (3.40 ± 0.10%), flavonoids (2.80 ± 0.36%), tannins (1.47 ± 0.06%), oxalates (0.92 ± 0.09%), phytates (1.73 ± 0.19%) and cyanogenic glycosides (0.01 ± 0.00%). The differences observed could be attributed to particularities of harvesting area.

3.3. Anti-free radical activity of the hydroethanolic extract of the leaves of *S. mombin*

Figure 1 shows the free radical trapping rate as a function of the concentrations, of the hydroethanolic extract of *S. mombin*, while Table 2 summarizes the 50% inhibition concentrations of the DPPH (IC_{50}) radicals.

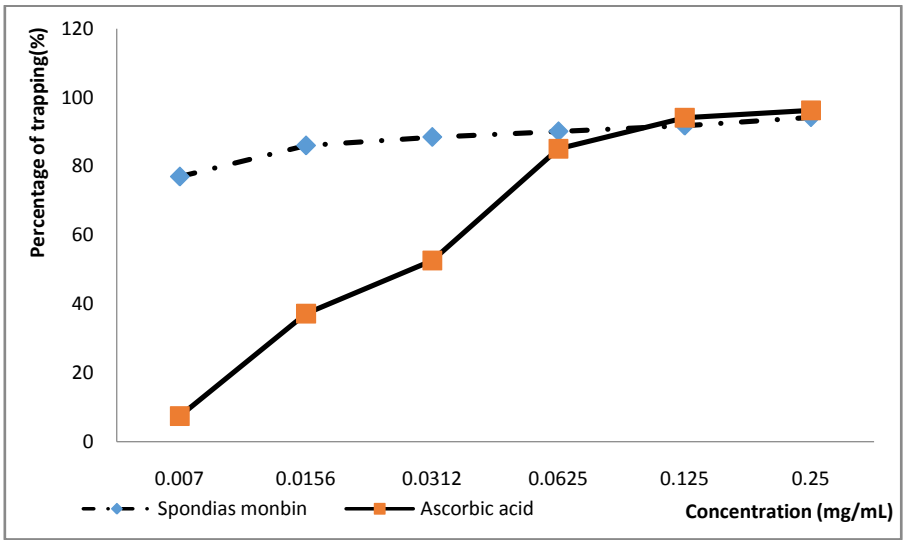

Figure 1: Anti-free radical activity of *Spondias mombin* compared to that of ascorbic acid

Table 2: 50% inhibition concentration of DPPH radicals)

Extracts	***Spondias mombin***	**Ascorbic acid**
IC_{50} (mg/ml)	<0.007	2.84

It appears from Figure 1 that the inhibition of the DPPH radical by *S. mombin* is dose dependent [30]. The lower is the IC_{50}, the greater is the antioxidant power of the extract. Thus the very low IC_{50} of *S. mombin* (<0.007 mg/ml) reflects its very high antioxidant power. The anti-free radical potential of low dose *Spondias mombin* (<0.125 mg/ml) is very high and significantly higher than that of ascorbic acid. It is comparable to that of ascorbic acid at higher concentrations (>0.125 mg/ml). The antioxidant power of medicinal plants being in relation to their contents in total phenolic compounds, flavonoids and tannins [31, 32], the presence of these compounds in the hydroalcoholic extract of *S mombin*, could explain its strong antioxidant power.

3.4. Antibacterial activity of the hydroethanolic extract of the leaves of *Spondias mombin*

Table 3 reports the sensitivity of the bacterial strains studied, to the hydroalcoholic extract of *S. mombin*, while Table 4 summarizes the Minimum Inhibitory Concentrations (MIC) of the extract. It appears from Table 3 that the five resistant bacterial strains studied are sensitive to the ethanolic extract of *S. mombin*.

Table 3: Activity of the extracts on the various bacterial species

	Streptocoque D	*Staphylococcus aureus*	*Klebsiella Pneumoniae*	*Citrobacter Sp*	*E. coli*
Spondias mombin	+	+	+	+	+

(+): inhibition (-): No inhibition

The inhibitory concentrations obtained vary from 625 µg/ml to 2250 µg/ml. The most sensitive strain to the hydroalcoholic extract of *S. mombin* is *Streptococcus D* while *E. coli* is the bacterial strain least sensitive to this extract.

Table 4: Determination of the MIC

MIC (µg/ml)	*Streptocoque D*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Citrobacter Sp*	*E. coli*
S. mombin	625	1125	1125	1125	2250

The antibacterial activity of *S. Mombin* could be linked to the presence of phenolic compounds (flavonoids and tannins), since their antibacterial properties have already been reported [12].

IV. CONCLUSION

The present study dealt with the evaluation of the anti-radical and antibacterial activities of the hydroethanolic extract of the leaves of *S. mombin* collected in Benin. Results obtained revealed the possibility to use this plant species as an alternative in the treatment of several infections due to resistant strains such as *Streptocoque D, Staphylococcus aureus, Klebsiella pneumoniae* and *Citrobacter Sp.* Its richness in secondary metabolites could well explained its good antioxidant properties and confirm the traditional use of *S. mombin* as traditional antioxidant and antibacterial agent.

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