**In vitro** Antisalmonellal and Antioxidant Activities of Leaves Extracts of *Tectona grandis* L. F. (Verbenaceae)

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Authors’ contributions

This work was carried out in collaboration among all authors. Author GTK did the biological assays, performed the statistical analysis and wrote the first draft of the manuscript. Author DG designed and supervised the study. Authors RST and SPCF managed the analyses and help in manuscript writing. Authors LCNF, NK and SEE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

**Aim:** Study aimed to evaluate the **in vitro** antisalmonellal and antioxidant properties of *Tectona grandis*, a medicinal plant commonly used in traditional Cameroonian medicine for the treatment of typhoid fever.

**Study Design:** **In vitro** Antimicrobial and antioxidant analyses of plant extract using established protocols.

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1. INTRODUCTION

The incidence of salmonellosis caused by *Salmonella* which cause damage both in humans and animals has increased considerably in recent decades [1, 2]. According to the World Health Organization (WHO), approximately 21 million cases of disease and approximately 161,000 deaths due to typhoid fever occur each year worldwide [3]. In Cameroon, the number of cases increased from about 124,526 to 154,103 between 2015 and 2016 [4]. This disease remains a challenge for health authorities in developing countries and especially in sub-Saharan Africa because of the precarious hygiene and sanitation conditions [5]. In animals, the most dangerous bacteria which are *Salmonella enterica* serotype Typhimurium and serotype Enteritidis, significantly inhibit their development and are a real reservoir of human contamination due to risk pose a to consumers by food products of animal origin contaminated by subclinically infected animals [1,6].

Enterobacteriaceae of the genus *Salmonella* thwart the free radicals produced against them by macrophages by expressing the oxyR gene, responsible for the accumulation of free radicals in the body during their pathogenicity [7]. An abnormal high level of free radicals in the body causes damage to biological molecules and express cytotoxic and mutagenic characteristics of metabolites released during lipid oxidation [8]. The inhibition of these radicals is therefore of paramount importance in the preservation of the body's homeostasis. In addition, antimicrobial resistance and the proven toxicity of some synthetic products [9] is a major problem worldwide and a handicap for salmonellosis control [10], hence the need to find new treatment.

*Tectona grandis* belonging to the family Verbenaceae is found mainly in India and tropical countries under the name "teak". This plant contains many secondary metabolites [11] that could justify its traditional use as an anti-inflammatory, laxative, astringent and analgesic [12]. In Cameroon, it is traditionally used in the Department of Moungo (Loum) to treat diabetes and many bacterial infections including typhoid fever. Thus, the objective of this work was to evaluate the antisalmonellal and antioxidant activities of the hydroethanolic, ethanolic and aqueous extracts of *T. Grandis*.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Tectona grandis* were harvested in Loum (Moungo Division, Littoral region of Cameroon) in August 2018. The plant was identified at the National Herbarium of Cameroon (Yaoundé by Dr. TCHIENGUE Basthelemy (Botanist)) in comparison with the reference sample kept under number 18580/SRF Cam (D. Dang Botanical Collection No. 160 Ref.).

2.2 Preparation of Extracts

The leaves of *T. grandis* were harvested, dried out in the sun (about 25°C) and crushed.
obtained powder was used for the preparation of ethanolic extract (95% ethanol), hydroethanolic extracts (70% ethanol, 50% ethanol, 30% ethanol) and aqueous (infusion, decoction, maceration) extracts. The preparation of these extracts was made by maceration of 100 g of powder for 48 hours in one liter (1 L) of each solvent (ethanol, 70% ethanol, 50% ethanol and 30% ethanol) while stirring twice each day. This mixture (solvent and extract) was subsequently filtered using Whatman N°1 paper. The filtrate obtained was evaporated using a rotary evaporator (Büchi R200) for the ethanol solvent, and 70% and 50% ethanol solvent; then placed in the ventilated oven (Memmert) set at 40°C until the solvent has evaporated completely. The filtrate obtained from the ethanol solvent 30% was placed directly in the ventilated oven set at 45°C until the solvent has evaporated completely. The preparation of the aqueous extracts (infusion and decoction) was made following the methods proposed by Duke [13].

- **Macerated extract**: It was obtained by maceration of 50 g of powder in 500 ml of distilled water for 48 hours, shaking three times a day. Subsequently, the mixture was filtered with Whatman N°1 paper and finally dried in an oven (Memmert) set at 45°C.

- **Infused extract**: A mass of 50 g of powder was mixed with 500 ml of distilled water previously boiled and the mixture was allowed to infusion for 15 minutes and then filtered with Whatman N°1 paper. The filtrate obtained was dried in an oven (Memmert) set at 45°C.

- **Decocted extract**: It was obtained by introducing 50 g of powder into 500 ml of distilled water and bringing the mixture to boil for 15 minutes. After cooling, the mixture was filtered with Whatman N°1 paper and the filtrate was dried in a ventilator oven (Memmert) set at 40°C.

### 2.3 Bacterial Material

The microorganisms used were bacteria of the genus *Salmonella*, consisting of a strain of *Salmonella enterica* serotype Typhi collection “American Type Culture Collection” (ATCC 6539) and isolates of *Salmonella enterica* serotype Typhi, serotype Enteritidis and serotype Typhimurium, all from Bacteriology laboratory of the Centre Pasteur of Yaoundé (Cameroon). These isolates were stored in the Microbiology and Antimicrobial Substrate in a glycerol/Mueller-Hinton broth (MHB) mixture (1:1) at -4°C and activation was performed with the streak technique on *Salmonella/Shigella* agar (SSA).

### 2.4 *In vitro* Evaluation of the Antisalmonellal Activity of *Tectona grandis* Leaf Extracts

MIC and MBC values of the different samples were determined by microdilution using INT colorimetric assay as previously described Mativandilela, et al. [14]. Briefly, the samples were dissolved in 5% dimethyl-sulfoxide (DMSO)/Mueller Hinton Broth (MHB) and serially diluted twofold (in a 96-well microplate). Then, 100 μL of inoculum (1.5 x 10^8 CFU/mL) prepared in MHB was added in each well. Ciprofloxacin and Oxytetracyclin were used as reference drug and the well containing the vehicle (DMSO 2.5%) as control. The plates were then covered with a sterile plate sealer and gently shaked to mix the contents of the wells. After 18 h of incubation at 37°C, the MIC value of each sample, defined as the lowest sample concentration that completely inhibited bacteria growth, was detected following addition of 40 μL INT (0.2 mg/mL) and incubation at 37°C for 30 min. Viable bacteria reduced the yellow dye to pink. For each extract, three columns were made and the revelation was made on two columns. The third was used to determine minimum bactericidal concentrations. This test was performed three times.

The MBC value was determined by adding 50 μL aliquots of the preparations, which did not show any growth after incubation during MIC assays, to 150 μL of MHB. Then, these preparations were incubated at 37°C for 48 h. The MBC was regarded as the lowest concentration of samples, which did not produce a color change after addition of INT. Each assay was performed in three independent tests in triplicate. In case there were differences, the MIC or MBC values were taken as the most frequently occurring values.

### 2.5 *In vitro* Antioxidant Activity of *Tectona grandis* Leaf Extracts

#### 2.5.1 Hydrogen peroxide scavenging capacity assay

The ability of the *T. grandis* extracts to scavenge hydrogen peroxide was determined according to the method described by Ruch, et al. [15]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (200, 100, 50, 25 and 12.5 μg/mL) in distilled water were added to a hydrogen peroxide
solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both T. grandis extracts and standard compounds were calculated:

\[
\text{% Scavenged } = \frac{\text{Absorbance of control} - \text{Absorbance of Extract}}{\text{Absorbance of control}} \times 100
\]

### 2.5.2 DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical reduction assay

This test is based on the reduction of free violet-coloured 1,1-diphenyl-2-picrylhydrazyl (DPPH,) radical to a stable yellow derivative in the presence of antioxidant compounds. The antiradical activities by the method of reducing DPPH- extracts of Tectona grandis were evaluated using L-ascorbic acid as control [16]. 100 μL extract (2000 μg/mL) were introduced into the tubes of the first two lines. Then 100 μL of methanol were introduced into all the tubes from the second line followed by successive dilutions in series of factor 2 at this level. Finally, 900 μL of a methanolic solution of DPPH (20 mg/L) were added in the first three columns and 900 μL of pure methanol in the last column. Five (5) final extract concentrations of 200; 100; 50; 25 and 12.5 μg/mL are obtained. After incubation for 30 minutes at room temperature in the dark, the optical densities of the contents of each tube were obtained with a spectrophotometer at 517 nm. Each sample was tested at the five concentrations and in triplicate. The optical densities were converted into inhibition percentages according to the following formula:

\[
\text{% inhibition } = \frac{\text{Absorbance of DPPH} - \text{Absorbance of Extract}}{\text{Absorbance of DPPH}} \times 100
\]

### 2.5.3 FRAP (Ferric Reducing Antioxidant Power) assay

The reducing power of iron (Fe3+) in Tectona grandis extracts was determined according to the method described by Padmaja, et al. [17]. To do this, one millilitre of extract at different concentrations (200; 100; 50; 25 and 12.5 μg/mL) was mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a 1% potassium ferricyanide solution K3Fe(CN)6. The assembly was incubated in a water bath at 50°C for 20 min; then 2.5 mL of 10% trichloroacetic acid was added to stop the reaction and the tubes were centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of supernatant was combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% FeCl3 methanolic solution. The absorbance of the reaction medium was read at 700 nm against a similarly prepared control, replacing the extract with distilled water. The positive control was represented by a solution of a standard antioxidant, Vitamin C (Vit C), whose absorbance was measured under the same conditions as the samples. An increase in absorbance corresponds to an increase in the reducing power of the extracts tested [18].

### 2.5.4 Hydroxyl radical scavenging activity assay

The activity of trapping hydroxyl radicals was determined by the method of Selvakumar, et al. [19] using the Fenton reaction. For this purpose, 60 μL of iron chloride (FeCl3) (1.0 mM) were mixed with 90 μL of 1,10-phenanthroline (1 mM), subsequently 2.4 mL of phosphate buffer (0.2 M, pH 7.4) was added. A volume of 150 μL of H2O2 (0.17 M) and 1.5 mL of extracts at different concentrations (from 12.5 to 200 μg/mL) were mixed. Addition of hydrogen peroxide (H2O2) to the reaction mixture made it possible to initiate the reaction, the mixture was incubated for 5 minutes at room temperature. After incubation, the optical density of the mixture was read at 560 nm. The positive control was represented by a solution of a standard antioxidant, butylhydroxytoluene (BHT). The optical densities were converted into percentages of hydroxyl radical scavenging activity of the extracts tested according to the formula below:

\[
\text{% inhibition } = \frac{\text{Absorbance of control} - \text{Absorbance of Extract}}{\text{Absorbance of control}} \times 100
\]

### 2.5.5 Total phenol content determination

The total phenol content of the various extracts was determined by the spectrophotometric method using the Folin-Ciocalteu reagent as described by Ramde-Tiendrebeogo, et al. [20]. The Folin-Ciocalteu reagent is a yellow acid consisting of a mixture of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PW12O40). It is reduced, during the oxidation of phenols to a mixture of blue oxides of tungsten (W6O23) and molybdenum (Mo7O23). The blue coloration produced has a maximum absorption at 760 nm. The reaction mixture in this test consisted of 0.02 ml extract (2 mg/mL), 0.2 ml 2N Folin-Ciocalteu reagent and 0.4 ml 20% sodium carbonate solution. The mixture was stirred and
incubated in a water bath at 40°C for 20 minutes and then, the absorbance was measured at 760 nm. The extract was replaced by distilled water in the control tubes. A calibration curve was plotted using gallic acid (0-2 mg/mL); the results were expressed in milligrams equivalent gallic acid per milligram of extract. Each sample was detected in triplicate.

2.5.6 Total flavonoids content determination

The evaluation of total flavonoids was performed using the aluminum trichloride method [17]. 100 μL extract (2 mg/ml) was mixed with 1.49 ml distilled water and 30 μL NaNO₂ (5%). After 5 minutes of incubation at room temperature, 30 μL of AlCl₃ (10%) were added. After further 6 min incubation, 200 μL NaOH (0.1 M) and 240 μL distilled water were added. After homogenization, the absorbance was measured at 510 nm and converted to milligram equivalent catechin per gram of extract from the catechin calibration curve. Each sample was detected in triplicate.

2.6 Phytochemical Investigations of Tectona grandis leaf extracts

The major phytochemical classes such as triterpenes (Liebermann-Burchard test), sterols (Salkowski’s test), alkaloids (Mayer’s test), polyphenols (ferric chloride test), flavonoids (aluminum chloride test), anthraquinones (Borntrager’s test), Anthocyanin, saponins (foam test), and tannins (gelatin test) present in leaves extracts of Tectona grandis were investigated as previously described by Harbone [21].

2.7 Statistical Analysis

The results were expressed as mean ± Standard deviation. Statistical analysis was carried out using Statistical Package for Social Sciences Software program (SPSS 20.0). Statistical analysis of data was performed by one way analysis of variance (ANOVA), followed by Waller-Duncan test. P values < 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Antisalmonellial activity in vitro

The activity of the different extracts of Tectona grandis on the isolates and bacterial strains studied are presented in Table 1. It can be seen from this table that all plant extracts have antibacterial activity that varies from one bacterial species to another. The antibacterial activities of the various extracts range from 64 μg/mL to 1024 μg/mL. Ethanol extracts, 70% ethanol and aqueous extract (decoked) had the highest spectrum of activity with MIC ≤ 128 μg/mL against the pathogens tested. Other extracts presented MIC ≥256 μg/mL.

3.1.2 In vitro antioxidant activities

3.1.2.1 Determination of scavenging activity against hydrogen peroxide

Fig. 1 shows the hydrogen peroxide (H₂O₂) trapping activity of T. grandis leaf extracts. The extracts caused a strong inhibition of hydrogen peroxide at all concentrations. The aqueous decoction presented the highest percentage of inhibition (77.83%) followed by ethanolic extract (72.91%) compared to vitamin C (70.20%) at concentrations ≤ 25 μg/mL. Above 50 μg/ml, the reference molecule (vitamin C) had the highest percentage of inhibition (90.50%). At the 200 μg/mL concentration, the trapping percentages were higher for 70% ethanol extracts (81.82%), ethanolic extracts (80.54%) and 50% ethanol extracts (79.39%) compared to the other extracts.

3.1.2.2 Effects of Tectona grandis extracts on DPPH free radical scavenging activity

The trapping activity of DPPH radicals of different extracts of T. grandis was evaluated and the results are presented in Fig. 2. These results shows that the percentage of inhibition of the 50% ethanol extract was the most active followed by the ethanolic extract, 70% ethanol extract and the decoction, although it remains all below the control (vitamin C). However, aqueous macerate followed by aqueous infusion and 30% ethanol extract had the lowest inhibition percentages at all concentrations. Concentrations that trap 50% of DPPH (IC₅₀) (Table 2) reveal that the IC₅₀ of the 50% ethanol extract was the lowest compared to vitamin C. However, aqueous extracts and 30% ethanol extract showed significantly elevated IC₅₀ (p<0.05) compared to vitamin C.

3.1.2.3 Ferric-Reducing antioxidant Power (FRAP) assay of Tectona grandis extracts

The potential of extracts to reduce ferric iron (oxidized form) to ferrous iron (reduced form) (Fe³⁺→Fe²⁺) at different concentrations is shown.
in Fig. 3. It appears from this Figure that only the 70% ethanol extract has a higher reducing power than vitamin C from 12.5 to 50 µg/mL. From 100 µg/mL, vitamin C takes over followed by ethanolic extract. Aqueous extracts with the lowest reducing power of iron.

3.1.2.4 Hydroxyl radical (OH\textsuperscript{-}) scavenging activities

Fig. 4 shows the results of the inhibitory potential of \textit{T. grandis} extracts at different concentrations. Analysis of this Figure shows that compared to the control (BHT), the inhibitory potential of the extracts is significantly low. However, ethanol extract has the highest percentage of inhibition followed by ethanol extract 50% and ethanol extract 70% comparatively. Aqueous extracts and 30% ethanol extract with the lowest inhibitory potential compared to BHT and other extracts.

3.1.2.5 Total phenols content of \textit{Tectona grandis} extracts

Phenol levels in \textit{T. grandis} extracts were determined using the calibration curve for phenolic compounds and the results are presented in Fig. 5. Analysis of this Figure shows that the 50% ethanol extract is the most concentrated in phenolic compounds, and its content is significantly (p<0.05) higher than that of all other extracts. Aqueous macerate is the least concentrated extract (p<0.05) in phenolic compounds followed by aqueous infusion compared to other extracts.

Fig. 1. Effects of different concentrations of \textit{Tectona grandis} extracts on Hydrogen peroxide scavenging

Fig. 2. Anti-radical effects of \textit{T. grandis} extracts grown on DPPH at different concentrations
Table 1. MIC, MBC and CMB/CMI of the different extracts and fractions of *Tectona grandis* on the different isolates and bacterial strains

<table>
<thead>
<tr>
<th>Extracts of <em>T. grandis</em></th>
<th>Strain/ isolates</th>
<th>STS</th>
<th>ST</th>
<th>SE</th>
<th>STM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract</td>
<td>MIC (µg/mL)</td>
<td>64</td>
<td>64</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>MBC (µg/mL)</td>
<td>512</td>
<td>128</td>
<td>512</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>MBC/MIC</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ethanol extract (70%)</td>
<td>MIC (µg/mL)</td>
<td>128</td>
<td>64</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>MBC (µg/mL)</td>
<td>512</td>
<td>128</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>MBC/MIC</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Ethanol extract (50%)</td>
<td>MIC (µg/mL)</td>
<td>256</td>
<td>256</td>
<td>512</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>MBC (µg/mL)</td>
<td>1024</td>
<td>256</td>
<td>-</td>
<td>512</td>
</tr>
<tr>
<td></td>
<td>MBC/MIC</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Ethanol extract (30%)</td>
<td>MIC (µg/mL)</td>
<td>1024</td>
<td>256</td>
<td>-</td>
<td>1024</td>
</tr>
<tr>
<td></td>
<td>MBC (µg/mL)</td>
<td>-</td>
<td>1024</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MBC/MIC</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Aqueous Decocted extract</td>
<td>MIC (µg/mL)</td>
<td>64</td>
<td>64</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>MBC (µg/mL)</td>
<td>512</td>
<td>256</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>MBC/MIC</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Aqueous Infusion extract</td>
<td>MIC (µg/mL)</td>
<td>256</td>
<td>128</td>
<td>1024</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>MBC (µg/mL)</td>
<td>1024</td>
<td>512</td>
<td>-</td>
<td>512</td>
</tr>
<tr>
<td></td>
<td>MBC/MIC</td>
<td>4</td>
<td>4</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Aqueous Macerate extract</td>
<td>MIC (µg/mL)</td>
<td>-</td>
<td>1024</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MBC (µg/mL)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MBC/MIC</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Ciprofloxacin</td>
<td>MIC (µg/mL)</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
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<tr>
<td></td>
<td>MBC (µg/mL)</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MBC/MIC</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Oxytetracyclin</td>
<td>MIC (µg/mL)</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>MBC (µg/mL)</td>
<td>64</td>
<td>32</td>
<td>32</td>
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<tr>
<td></td>
<td>MBC/MIC</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
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</table>

<sup>-</sup> > 1024 µg/mL. STs: *Salmonella enterica* serotype Typhi strain ATCC6539; ST: *Salmonella enterica* serotype Typhii; SE: *Salmonella enterica* serotype Enteritidis; STM: *Salmonella enterica* serotype Typhimurium; MIC (µg/mL): Minimum Inhibitory Concentration, MBC (µg/mL): Minimum Bactericidal Concentrations

Table 2. IC<sub>50</sub> values of DPPH of *Tectona grandis* extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>10.44±1.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol (70%)</td>
<td>8.70±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol (50%)</td>
<td>7.10±0.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol (30%)</td>
<td>17.35±0.81&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aqueous Decocted</td>
<td>15.70±2.26&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aqueous infusion</td>
<td>23.26±4.70&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aqueous macerate</td>
<td>31.72±4.34&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>8.48±0.01&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d,e,f,g,h</sup>: Figures with the same letter are not significantly different at the 5% threshold

3.1.2.6 Total flavonoids content of *Tectona grandis* extracts

The flavonoids contents of *T. grandis* extracts were determined using the flavonoid calibration curve and the results are presented in Fig. 6. Analysis of this Figure shows that 50% ethanol extract and ethanolic extract are the most concentrated in flavonoids compounds, and their content is significantly (p<0.05) higher than all other extracts despite the fact that 50% ethanol extract has a slightly higher rate than the ethanolic extract. Macerated and aqueous infused being the least concentrated (p<0.05) in flavonoids compounds compared to other extracts.

3.1.3 Phytochemical screening of *Tectona grandis* extracts

Several groups of compounds were detected in different extracts grown from the phytochemical test and the results are presented in Table 3. It appears that flavonoids, phenols, anthocyanins, sterols and triterpenes were present in all extracts tested. However, anthraquinones were detected only in aqueous extracts and 30% ethanol extract, while tannins were present only in infused and macerated.
Fig. 3. Reducing power of iron of *Tectona grandis* extracts

![Graph showing reducing power of iron](image)

Fig. 4. Percentage hydroxyl radical scavenging activities of *T. grandis* leaf extracts

![Graph showing percentage hydroxyl radical scavenging activities](image)

Fig. 5. Total phenol content of *T. grandis* extracts

*Figures with the same letter are not significantly different at the 5% threshold. The values in the figure are presented as averages ± standard deviation of 3 repetitions.*
Table 3. Main groups of compounds present in the plant extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Alkaloids</th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Sterols</th>
<th>Triterpenes</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Anthocyanine</th>
<th>Anthraquinons</th>
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<tbody>
<tr>
<td>Ethanolic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol (70%)</td>
<td>+</td>
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3.2 Discussion

3.2.1 In vitro antisalmonellal activities

The antisalmonellal activities of \( T. \) grandis extracts vary according to solvent systems and strains and isolates. This could be attributed to the differences in phytochemical compositions observed between extracts. Thus, the differences in chemical composition of the extracts may be due to the plant species and/or extraction solvent system used. The leaves of \( T. \) grandis contain phenols, alkaloids, flavonoids, triterpenes, saponins and anthraquinones. These results corroborate those of Asif [11] who showed the presence of alkaloids, tannins, flavonoids and saponins in the extracts of the stems of this plant. Ethanolic extract, 70% ethanol extract and aqueous decoction were found to be more active (MIC \( \leq 128 \mu g/ml \) on strain and the three isolates tested. The others extracts were significantly active and moderately active (MIC >128 \( \mu g/ml \)). The ethanol extract can therefore be classified as highly active on \( Salmonella \) enterica serotype Typhi ATCC6539 strain and \( Salmonella \) enterica serotype Typhi and Typhimurium isolates and significantly active on \( Salmonella \) enterica serotype Enteritidis. The aqueous decoction is highly active only on \( Salmonella \) enterica serotype Typhi ATCC6539 strain and \( Salmonella \) enterica serotype Typhi isolate. According to Tamokou, et al. [22], a plant extract is considered highly active if the MIC < 100 µg/mL; significantly active when 100 ≤ MIC ≤ 512 µg/mL; moderately active when 512 ≤ MIC ≤ 2048µg/mL; slightly active if MIC > 2048 µg/ml and not active when MIC > 10 mg/kg. These activities would justify the traditional use of \( T. \) grandis against typhoid fever. These results support those of Prakash, et al. [23], Bitchagno, et al. [24] and Lanka and Parimala [25] who showed similar activities with other bacterial strains. The difference in activity observed between the different germs tested may result in their transferable genetic variabilities of resistance between strains [26]. According to the Gatsing and Adoga [26] scale, substances are considered bacteriostatic agents when the MBC/MIC ratio is > 4 and bactericidal agents when the MBC/MIC ratio is ≤ 4. Thus, the 70% ethanol extract is the only bactericidal extract on all germs tested.

3.2.2 In vitro antioxidant activities

Reactive oxygen species (ROS) responsible for oxidative stress are one of the most important factors in the pathogenesis of various diseases [27]. Thus, finding extracts that combine antibacterial activity and antioxidant activities are necessary for a better therapy. This is why several antioxidant tests have been carried out. According to Alam, et al. [28], it is necessary and recommended to use more than one method to evaluate the antioxidant activity of a given substance because of the complexity and diversity of oxidation processes.

Hydrogen peroxide (\( H_2O_2 \)) plays a key role in the pathogenesis of many diseases [27]. In the
presence of metal ions, it is transformed into more toxic hydroxyl radicals that can act as a mediator in cellular damage. It can also produce singlet oxygen by reacting with a superoxide anion or with hypochlorous acid (HOCl) or chloramines in living systems. Hydrogen peroxide can degrade some hemoproteins, such as hemoglobin, to release Ferric ions [28]. Hydrogen peroxide is involved in the inactivation of different enzymes by the oxidation of essential thiol groups [29]. Thus, the measurement of $H_2O_2$ trapping activity is an important element for antioxidant evaluation. In this study, the trapping activity of aqueous decocted is more effective than all others extracts and standard (vitamin C) at concentrations $\leq 25 \mu g/ml$ at the concentrations of 200 $\mu g/ml$, 70% ethanol extract, ethanolic extract and 50% ethanol extract have present the goodness activities. Thus, since the elimination of $H_2O_2$ is very important for antioxidant defence in the body, *T. grandis* leaf extracts can be used for this purpose. These results corroborate those of Ghaisas, et al. [30] and Pooja, et al. [31] who had already shown similar hydrogen peroxide trapping activities with the methanolic extract of *T. grandis*.

The results obtained with the various antioxidant tests performed (DPPH', OH' and FRAP) reveal ethanolic extract, 70% ethanol and 50% ethanol most active extracts. At the same time, these extracts have shown a high content of phenolic and flavonoids compounds, which indicate that the more phenolic compounds are in a plant, the better its antioxidant activity. According to Cheng, et al. [32], the high content of phenols and particularly flavonoids, whose antioxidant potential is recognized, is at the origin of the antioxidant activity of the extracts. This could also be due to the presence of the other different types of secondary metabolites found in the plant or their synergies. The IC$_{50}$ values of the antiradical activity (DPPH') of the *T. grandis* extracts shows that they are good antioxidants because the majority of their IC$_{50}$ range from 7.10 to 15.70 $\mu g/mL$ except for aqueous infused and macerate which showed IC$_{50}$ of 23.26 and 31.72 $\mu g/mL$ respectively. 50% ethanol extract (7.10 $\mu g/mL$) having a much better activity than vitamin C (8.48 $\mu g/mL$). Indeed, according to Souri, et al. [33], the antioxidant potential of a plant is high when the IC$_{50}$ < 20 $\mu g/mL$; moderate when 20 $\mu g/mL$ ≤ IC$_{50}$ ≤ 75 $\mu g/mL$ and low when IC$_{50}$ > 75 $\mu g/mL$. On the basis of this scale, we can classify the *T. grandis* extracts into two groups: moderate activity for aqueous infused and macerated extracts and high activity for the rest of the extracts. These results are in agreement with those of Kushwah, et al. [34], Prakash, et al. [23] who had obtained similar antioxidant activities with methanol extracts of *T. grandis*. However, these studies are in agreement with those of Kodjio, et al. [35], Djoueudam, et al. [36] and Sokoudjou, et al. [2] who showed the mixed anti-salmonelal and antioxidant activity of some medicinal plants.

4. CONCLUSION

Based on the above data, it is concluded that the leaf extracts of *Tectona grandis* plant have interesting mixed anti-salmonelal and antioxidant activities that can be used for the effective management of salmonellosis. They also have a high hydrogen peroxide trapping capacity. Further studies are needed to evaluate the in vivo therapeutic effect of the most active extracts and their antioxidant potential in vivo.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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