HPLC Standardization of Herbal Drugs and Evaluation of their Antimicrobial Properties: Studies of Leaves and Flowers of *Senna italica* Mill. Grown in Benin (Western Africa)

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Abstract

This study aims to evaluate the phytochemistry and antimicrobial activities of the leaves and flowers of *Senna italica* Mill.

Qualitative phytochemical screening was done with method based on differential coloration and precipitation. Thin Layer Chromatography was used for characterization of sennosides in aqueous and hydro-ethanolic extract of leaves and flowers of *S. italica*. Then HPLC was used for quantification of sennosides. The antifungal activity of the extracts was evaluated on *Candida albicans* and *Trichophyton rubrum* by incorporation method. Well diffusion technique, coupled with the microdilution determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (CMB) was used for antibacterial testing.

The results of study showed the presence of large groups of secondary metabolites in the leaves and flowers of *S. italica* including tannins, gallic tannins, flavonoids, steroids and some glycosides. TLC test showed that leaves and flowers of *S. italica* contain sennosides A, B, C and D. HPLC test showed that the content of the sennosides of flowers is higher than that of the dried leaves in the sun. According to daily dose of sennosides recommended, 20 mg to 30 mg as laxative, this dose can be attained with 0.718 to 0.862g of leaf powder or 1.390 to 1.668g of *S. italica* flower powder. The extracts have no antifungal activity but the hydroethanolic extracts of both leaves and flowers of *S. italica* have bactericidal activity on strains of *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *E. coli* clinical strain.

This quantification of sennosides is a standardization of the herbal tea of *S. italica* and will limit these possible side effects. This plant is a good candidate for the development of improved traditional medicine.

**Keywords**: *Senna italica*; Sennosides; HPLC; Antifungal and Antibacterial Activity; Benin

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**Introduction**

In Africa, traditional medicine is a source of knowledge, philosophy and cosmogony still largely untapped. It offers effective and accessible treatment options for pathologies prevalent in communities. It is also a national cultural heritage and serves as a link between people and their culture on the one hand and between people and their history on the other [1]. Traditional medicine is defined as "the sum total of the knowledge, skill and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" [2-4].

Resolution AFR/RC50/R3 of 31 August 2000 of WHO encourages African countries to develop regional strategies on traditional medicine to undertake research on medicinal plants and to promote their optimal uses in delivery health care systems [2]. The implementation of these strategies will allow the production of Drug based on local raw materials [1].

In Benin, several plants traditionally used against various diseases are researched through ethnobotanical, biochemical and clinical trials [5,6]. Among these plants is *Senna italica* Mill, a specie of *Senna* highly grown in the localities of Agoue in the department of Mono in Benin. The specie is used for its laxative properties. Its therapeutic effect appears after 8 to 12 hours of time and is due to sennosides [7]. Nevertheless, in pregnant women, *S. italica* can cause abortion because of its mimetic effect of oxytocin on the uterus [8]. *S. italica* represents a major source of income for the populations of Agoue. Its production extends over several hectares in Agoue. Traders come from several countries in the sub-region (Togo, Nigeria, Ghana, Côte d’Ivoire and Gabon) to buy supplies at lower cost.

The interest of the local and sub-regional traders for this plant as well as its frequent use has aroused the curiosity of the leader of a missionary religious group in Agoue locality. Back from her mission in Agoue, the missionary approached the Laboratory of Pharmacology and Toxicology of Faculty of Health Sciences, University of Abomey-Calavi in Benin for scientific advice on the local use of *S. italica* At this occasion, it was noticed that the tea made from this plant was not standardized in Benin and the variation of *S. italica* cultivated in Benin, in medicinal uses, in toxicity and in content of active ingredients was unclear. Scientific data on the phytochemistry and antimicrobial properties of *S. italica* are insufficient for optimal recovery of this resource. Investigations have shown that the laxative effect of *Senna italica* Mill. is due to hydroxyanthracenic derivatives and specifically to sennosides [7]. But, the concentration of this category of secondary metabolite present in *S. italica* cultivated in Benin, was not known. It was also isolated from the aerial parts of *S. italica* Ten types of flavonoids including tamarixetine (3-rutinoside-7-rhamnoside), beta-sitosterol, stigmasterol, alpha-amyrin, 1,5- dihydroxy-3-methyl anthraquinone and anthraquinone [9]. However, more accurate quantitative data on antimicrobial activities about leaves and flowers of *S. italica* are not sufficiently documented.

**Aim of the Study**

The aim of this study was to contribute to the standardization of herbal teas made from leaves or flowers of *S. italica* cultivated in Benin for a more rational use by high performance liquid chromatography (HPLC), an efficient method for the analysis of herbal drugs and herbal preparations. In addition, it was to evaluate the antimicrobial properties of the extracts of the leaves and flowers of this same plant.

**Materials and Methods**

**Plant sampling and obtaining powders**

The leaves and flowers of *S. italica* were harvested at Agoué in Benin. The identification was made at the National Herbarium of Benin with the number AA6648/HNB. After harvest, the plant drugs were divided into two parts. Part was dried in the sun. The other part was dried in the shade in a room where the temperature varied between 27 and 29°C. The temperature control was done with an atmospheric thermometer COMBIMAL brand. The drying lasted two weeks. Dry herbal drugs were sprayed and then powders are kept in small bags away from the sun and moisture.

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Extraction

The powder was extracted by maceration and decoction. By decoction, the method of Angaman [10] modified was used. Thus, 60g of powder of each vegetable drug was added 0.8 liter of distilled water. The mixture was boiled for 30 minutes and then filtered. By maceration, it was used the technique of Zirihi [11], where 60g of powder of each sample were extracted with 0.8 liter of mixture: 70% ethanol 95° and 30% distilled water for 72h with intermittent agitation.

Each extraction operation was repeated twice on the residue remaining after the first extraction. The extracts were filtered with hydrophilic cotton and Whatman paper giving eight extracts (hydro ethanolic extract of leaves, hydro ethanolic extracts of flowers, aqueous extract of leaves, aqueous extract of flowers, according to the mode of drying of vegetable drugs). Each filtrate was brought to the Memmert® oven at 39°C until dry extracts were obtained. The yield (R in %) of each extraction was calculated as follows:

\[ R(\%) = \frac{\text{mass of extract (g)}}{\text{mass of test portion}} \times 100. \]

Phytochemical screening

Qualitative tests

Two qualitative tests were carried out: colorimetric tests on leaf and flowers powders of Senna italica Mill., sun-dried according to the Tamene and Endale [12] method for the detection of different phytochemicals groups; and by thin layer chromatography on the extracts according to Aktar, et al. [13] for the identification of sennosides. But here, the revelation was made with a UV lamp.

Quantitative test: High performance liquid chromatography (HPLC)

The sennoside dosage by HPLC is a more specific and accurate modern method. In this study, sennosides were dosed by the method of Bozzi, et al [14]. This is an external standardization method and thus allows calculating the sennoside content in concentration or percentage mass. PURSENIDE® 20 mg tablet from the NOVARTIS laboratory was used as control.

Preparation of control range

One tablet of PURSENIDE® 20 mg was dissolved in 5 mL of methanol giving a solution of 4 mg/mL. The mixture was centrifuged and then 25 μl (0.1 mg/mL of sennosides) of the supernate was taken. It was followed by half-way dilutions until the range of eight levels of concentrations (100 μg/mL, 50 μg/mL, 0.78 μg/mL) was obtained. In addition, 20 μL of each concentration were injected three times into HPLC in order to determine the average of the area under curve of each concentration and to draw the most accurate standardization curve possible. The flow of injection was 1 mL/min at 25°C. Detection was made at 290 nm UV.

Sample preparation

Primary solutions at 1 mg/mL were prepared by dissolving 1 mg of each dry extract in 1 mL of methanol. In total, eight primary solutions were prepared. It had followed a dilution to the tenth of each of them. Table 1 below summarizes the elution gradient used to determine the sennosides of our extracts.

Assessment of antimicrobial activities

The antifungal activity of the extracts was evaluated on Candida albicans and Trichophyton rubrum isolated and identified from superficial lesions in the microbiology department of the National Hospital and University Center Hubert Koutoukou Maga (CNHU/HKM) of Cotonou. The antibacterial activity was performed on Staphylococcus aureus ATCC 25923 (reference strain), Escherichia coli ATCC 25922 (reference strain), Pseudomonas aeruginosa and E. coli (Clinical strains). These bacterial strains were obtained at the Research Unit in Applied Microbiology and Pharmacology of Natural Substances, University of Abomey-Calavi.
Re-isolation of microbial strains

Strains of *Candida albicans* and *Trichophyton rubrum* were transplanted on Sabouraud agar then incubated in an oven at 27°C for 48 hours (*Candida albicans*) and 15 days (*Trichophyton rubrum*) in order to obtain young cultures and isolated colonies. The different bacterial strains were subcultured on Mueller-Hinton Agar medium (MHA) then incubated in an oven at 37°C for 24 hours to obtain a young culture and isolated colonies. Isolated colonies were used to prepare the inoculum.

**Antifungal activity**

**Preparation of culture medium and extract incorporation**

The tests were carried out on Sabouraud agar. The hot culture medium was divided into a series of 13 test tubes numbered from 1 to 13. Tube 1 contains 20 ml of the culture medium and tubes 2 through 13 contain 10 ml. The extract was incorporated into the agar according to the double dilution method in inclined tubes. The series of tubes thus contains concentrations of the extract ranging from 100 mg/mL to 0.098 mg/mL. The tubes were sterilized by autoclaving at 121°C for 15 minutes and then slanted such that upon solidification, the agar forms a slope inside the tube [15].

**Determination of CMF (minimal fungicidal concentration) and MIC (minimum inhibitory concentration)**

CMF is the minimum concentration of extract corresponding to the absence of colonies after seeding. As for the MIC, an extract inhibits 50% growth of microorganisms studied. It is determined graphically after having made the curve of antifongigrams.

**Candida albicans culture**

Using a sterile loop, a young colony (48 h) of *C. albicans* was collected and homogenized in 2.5 mL of sterile distilled water to obtain a 0.5 Mac Farland suspension [16]. The suspension was diluted to one-tenth (1/10). The culture of germs on the prepared media was carried out by inoculation of 10 μl. The tubes were then incubated at 27°C in an oven for 48 hours [15].

**Trichophyton rubrum culture**

An inoculum of $10^5$ germs/mL was prepared by taken a young yeast colony from a sterile loop in a tube containing 2.5 mL of sterilized distilled water and adjusting with the Malassez cell (100 mL). The culture of germs on the prepared media was carried out by injecting 10 μL [15]. The tubes were then incubated at 27°C for 13 days.

**Calculation of the survival (S)**

Direct counting of colonies with the naked eye assessed survival. Growth in the 11 experimental tubes was evaluated as a calculated percentage relative to 100% survival in the growth control tube [15]. $S = n/N \times 100$; $n$ is the number of colonies in the experimental tube; $N$ is the number of colonies in the control tube.

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**Table 1: Elution gradient used for the determination of sennosides.**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Eluent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%A (H$_2$O/ACN 90:10) (AA; 0.1%)</td>
</tr>
<tr>
<td>00</td>
<td>95</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>25</td>
<td>85</td>
</tr>
<tr>
<td>35</td>
<td>75</td>
</tr>
<tr>
<td>40</td>
<td>95</td>
</tr>
</tbody>
</table>

**Citation:** Alidehou Jerrold Agbankpe, *et al.* “HPLC Standardization of Herbal Drugs and Evaluation of their Antimicrobial Properties: Studies of Leaves and Flowers of *Senna italica* Mill. Grown in Benin (Western Africa).” *EC Pharmacology and Toxicology* 8.2 (2020): 01-17.
Antibacterial activity

Preparation of inoculum and extracts

A pure 24 hours colony of each bacteria strain was emulsified in 5 ml of Mueller-Hinton Broth (MHB) and adjusted to 0.5 McFarland.

The aqueous and hydro-ethanolic extracts of leaves and flowers were reconstituted in distilled water at a concentration of 60 mg/mL. The prepared solutions were sterilized by filtration using filter-syringes on 0.22 μm Millipore membrane. The sterility of the stock solutions was verified by culturing aliquots of each solution on Mueller Hinton II media and incubated at 37°C for 24 to 48 hours.

Susceptibility tests

Each inoculum was seeded by swab on Petri dishes containing Mueller Hinton II agar. With sterile Pasteur pipette tip, 5 wells of 6 mm diameter were dug out. Then, using a tip and a micropipette, 50 μL of each extract was transferred to the wells. A well containing sterile distilled water served as negative control. The petri dishes were kept at ambient temperature for 30 min to 1h for a pre-diffusion of the substances before being incubated at 37°C for 24h [16]. Meanwhile, swab of each inoculum was cultured onto MH II plates and reference antibiotic disks were used as positive controls. The tests were repeated three times and the antibacterial activity of the extracts was determined by measuring the inhibition zone diameters around each well (Table 2).

<table>
<thead>
<tr>
<th>Diameter of the inhibition zone (Δ)</th>
<th>Degree of sensitivity of the germ</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ &lt;7 mm</td>
<td>Resistant</td>
<td>-</td>
</tr>
<tr>
<td>7 mm ≤ Δ &lt; 8 mm</td>
<td>Sensitive</td>
<td>+</td>
</tr>
<tr>
<td>8 mm ≤ Δ &lt; 9 mm</td>
<td>Moderately sensitive</td>
<td>++</td>
</tr>
<tr>
<td>Δ ≥ 9 mm</td>
<td>Very sensitive</td>
<td>+++</td>
</tr>
</tbody>
</table>

Table 2: Standard values used to interpret the results of the susceptibility tests the plant extracts [17,18].

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

This study step was performed using 96 well plates method described by Agbankpe., et al [16]. 100 μL of the stock solution of each extract was added to 50 μL of different bacterial suspensions in a liquid medium containing 100 μL of Mueller-Hinton broth. Positive and negative controls were prepared and respectively made of, 100 μL of MH broth + 50 μL of bacterial suspension and 100 μL of MH broth + 100 μL of the stock solutions of the extracts being tested. The micro-plates were covered with parafilm paper and incubated at 37°C for 24 hours. The MIC was estimated with naked eyes compared to the controls and each well was cultured on MH II agar and incubated at 37°C for 24 hours for determination of the MBC. The MBC is the smallest concentration of extract at which no bacteria colony can be observed. The antibiotic power (ap) of each extract was thereafter calculated with the formula CMB/CMI.

Results

The highest yields are those of aqueous extracts. Sun drying does not seem to significantly influence these yields (Table 3). The qualitative and quantitative estimation of the chemical compounds of the various plant and extracted drugs can better help to evaluate the levels of yield.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Sun drying (yield in %)</th>
<th>Room drying (yield in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves Aqueous extract</td>
<td>22.9</td>
<td>22.28</td>
</tr>
<tr>
<td>Flowers Aqueous extract</td>
<td>16.5</td>
<td>16.07</td>
</tr>
<tr>
<td>Leaves hydro-ethanolic extract</td>
<td>18.37</td>
<td>13.67</td>
</tr>
<tr>
<td>Flowers Hydro-ethanolic extract</td>
<td>11.78</td>
<td>11.67</td>
</tr>
</tbody>
</table>

Table 3: Yield (%) of extractions.
Phytochemical screening

Qualitative tests

Alkaloids, catechin tannins, cardiotonics, cyanogenic derivatives, saponosides are absent in leaves and flowers. The leaves contain the flavone while the flowers contain the flavonol. Discrepancy was observed for Triterpenes between leaves and flowers. Only the leaves contain the Triterpenes (Table 4).

<table>
<thead>
<tr>
<th>Family</th>
<th>Classes</th>
<th>S. italica (sun drying)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>Nitrogen compounds</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Quinoid derivatives</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiotonics</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cyanogenic derivatives</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponoside (IM)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reductor compounds</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mucilage</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenolic compounds</td>
<td>Tanins</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tanins catechic</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Tanins gallic</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>Flavone</td>
</tr>
<tr>
<td></td>
<td>anthocyanes</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Leuco-anthocyanes</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Anthracene free</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>O-heterosides</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>C-heterosides</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 4: Results of colorimetric tests.

IM: Moss Index; +: Present; -: Absent; +/-: In Trace.

Characterization of sennosides by thin layer chromatography

Figure 1 summarizes results thin layer chromatography. For the witness, six spots were observed, four of which were identified as those of sennosides while the other two are unidentified. The first four spots (from top to bottom) appeared in lemon yellow form with the UV lamp thus testifying to the presence of sennosides. According to Aktar, et al. [13], the migration of sennosides on a CCM plate gives bands in the following order: sennoside C, sennoside D, sennoside A and sennoside B. four lemon-yellow spots were also observed for S. italica leaves and flowers. Referring to the spotlights, the leaves and flowers of S. italica contain sennosides A, B, C and D. In addition, the retention factors of these compounds were 0.9; 0.8; 0.7 and 0.6, respectively (Figure 1).

HPLC quantitative test

The calibration curve established with a concentration range of 100 µg/ mL to 0.78 µg/mL following a serial dilution and three series of injections at each level of the concentration range of the extracts are as follows (Figure 2).
Figure 1: Chromatographic profile (CCM) of sennosides.

P: Control; F1: Aqueous Extract of the Leaves; F2: Aqueous Extract of the Flowers; f3: Hydro-Ethanolic Extract of the Leaves and f4: Hydro-Ethanolic Extract of the Flowers of Senna italica Mill.

Figure 2: Sennoside dosage in control (PURSENIDE) by HPLC (external calibration).

The correlation coefficient of the linear regression line of the calibration curve was $R^2 = 0.998 > 0.99$, which indicates a good correlation between the measured absorbance and the sennoside concentrations.

The purpose of this dosage was not to specify each type of sennoside, the chromatographic profiles obtained are those of a set of sennosides. This justifies the broad aspect of the chromatographic peaks obtained (Figure 3 to 11).

**Figure 3:** Chromatogram of the control (PURSENIDE).

**Figure 4:** Chromatogram of the Hydro ethanolic extract of Senna italica flowers (sun drying).
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**Figure 5:** Chromatogram of the aqueous extract of *Senna italica* flowers (sun drying).

**Figure 6:** Chromatogram of the aqueous extract of *Senna italica* leaves (sun drying).

**Figure 7:** Chromatogram of the Hydro ethanolic extract of Senna italica leaves (sun drying).

**Figure 8:** Chromatogram of the aqueous extract of Senna italica leaves (room drying).

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HPLC Standardization of Herbal Drugs and Evaluation of their Antimicrobial Properties: Studies of Leaves and Flowers of *Senna italica* Mill. Grown in Benin (Western Africa)

Figure 9: Chromatogram of the hydro ethanolic extract of *Senna italica* leaves (room drying).

Figure 10: Chromatogram of the aqueous extract of *S. italica* flowers (room drying).

The data from these chromatograms are summarized in the tables below (Table 5 and 6). Table 5 shows that the content of the sennosides of flowers is higher than that of the dried leaves in the sun. Although sennosides are soluble as well as hot water as alcohol, the hydroalcohol mixture allows better extraction of sennosides compared to hot water taken isolated as an extraction solvent. From table 6, we find that the sennoside content of the leaves is higher than that of dried flowers under the same conditions (27 - 29°C) away from the sun. In addition, the ethanol/water mixture allows a better extraction of sennosides compared to hot water.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time of retention in min</th>
<th>Area under curve in mUA</th>
<th>Quantity of sennosides in μg/mL (for 1 mg of extract)</th>
<th>Quantity of sennosides (mg) for 100 mg of DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faq</td>
<td>16.95</td>
<td>1.3</td>
<td>3.414</td>
<td>0.078</td>
</tr>
<tr>
<td>Flaq</td>
<td>16.94</td>
<td>10.507</td>
<td>109.011</td>
<td>1.198</td>
</tr>
<tr>
<td>Feh</td>
<td>16.92</td>
<td>0.536</td>
<td>5.559</td>
<td>0.102</td>
</tr>
<tr>
<td>Fleh</td>
<td>17.02</td>
<td>12.040</td>
<td>124.922</td>
<td>1.472</td>
</tr>
</tbody>
</table>

Table 5: Results of Sennoside Determination of Sun-dried Vegetable Drugs (DV).

Faq: Aqueous Extract of Leaves; Flaq: Aqueous Extract of Flowers; Feh: Hydroethanolic Extract of Leaves; Fleh: Hydroethanolic Extract of Flowers; DV: Vegetable Drug; mUA: Milli Arbitrary Unit.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time of retention in min</th>
<th>Area under curve in mUA</th>
<th>Quantity of sennosides in μg/mL (for 1 mg of extract)</th>
<th>Quantity of sennosides (mg) for 100 mg of DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faq</td>
<td>16.80</td>
<td>8.782</td>
<td>91.123</td>
<td>2.030</td>
</tr>
<tr>
<td>Flaq</td>
<td>16.86</td>
<td>4.615</td>
<td>47.879</td>
<td>0.769</td>
</tr>
<tr>
<td>Feh</td>
<td>16.90</td>
<td>24.524</td>
<td>254.453</td>
<td>3.478</td>
</tr>
<tr>
<td>Fleh</td>
<td>16.95</td>
<td>11.445</td>
<td>118.747</td>
<td>1.386</td>
</tr>
</tbody>
</table>

Table 6: Results of Sennoside Determination of Dried vegetable Drugs in room.

Faq: Aqueous Extract of Leaves; Flaq: Aqueous Extract of Flowers; Feh: Hydroethanolic Extract of Leaves; Fleh: Hydroethanolic Extract of Flowers; DV: Vegetable Drug; mUA: Milli Arbitrary Unit.
Antifungal activity of extracts

The evaluation of antifungal activities was done in vitro and focused on clinical strains (isolated from superficial lesions) of *Candida albicans* and *Trichophyton rubrum*. Survival is 100% for all extracts at all concentrations on *C. albicans* and *T. rubrum*. The extracts therefore have no antifungal activity on these two strains. Figure 12 confirms this result. Under these conditions, it is not possible to determine the minimum fungicidal concentrations of the various extract.

![Figure 11: Chromatogram of the Hydro ethanolic extract of S. italica flowers (room drying).](image)

Antibacterial activity of extracts

Susceptibility tests

The results of the susceptibility tests are summarized in table 7. The diameters of the zones of inhibition recorded vary from 07 to 09 mm for aqueous extracts and from 12 to 16 mm for hydro-ethanolic extracts. Indeed, zones of inhibition of hydro-ethanolic extract of leaves of *S. italica* on strains of *E. coli* (clinical strain), *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 are respectively 10 mm, 12 mm and 13 mm. However, according to Tsiririnrindravo., et al. [17] and Agbankpe., et al. [16], a bacterial strain is said to be very sensitive to an extract if this extract induces an inhibition zone greater than or equal to 09 mm in diameter. The three above-mentioned bacterial strains are very sensitive to the hydroethanolic extract of *S. italica* leaves. This sensitivity is even more pronounced with hydroethanolic extract of *S. italica* flowers on the three bacterial strains mentioned above. In the latter case, the zones of inhibition are 12 mm, 13 mm and 16 mm respectively for *E. coli*, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. As for *P. aeruginosa*, this bacterial strain has not shown a low sensitivity to hydroethanolic extract of the leaves of *S. italica*. Therefore, the organisms studied (with the exception of *P. aeruginosa*) proved to be very sensitive to all our hydro-ethanolic extracts (Table 7).

<table>
<thead>
<tr>
<th>Strains</th>
<th>Inhibition zone diameters (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fₗₐq (3 mg)</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 25923</td>
<td>07</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>(-)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>(-)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>(-)</td>
</tr>
</tbody>
</table>

Table 7: Inhibition zone diameters of extracts on bacterial strains.

*ATCC: American Type Culture Collection; S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; P. aeruginosa: Pseudomonas aeruginosa; F: Leaves of Senna italica; Fl: Flowers of Senna italica; aq: Aqueous Extracts; eh: Hydro-Ethanol Extracts; (-): No Inhibition.*
Minimal inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and antibiotic powers (a.p) of *S. italica* extracts on the strains tested

Hydro-ethanolic extracts are more active on bacterial strains than the aqueous extracts. *E. coli* is more sensitive to hydro-ethanolic extracts of leaves and flowers of *S. italica* than the hydro-ethanolic extracts of leaves. Compared to reference organisms, clinical strains (isolates) are less sensitive to our extracts. *S. aureus* ATCC 25923 was quite sensitive to aqueous extract of leaves and aqueous extract of flowers. The ratio CMB/CMI, reflects the antibiotic power of our extracts [19]. Hydro-ethanolic extracts of both leaves and flowers of *Senna italica* Mill. have bactericidal power on *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *E. coli* (clinical) strains. As for the aqueous extract of *Senna italica* Mill. flowers, it has a bacteriostatic power on both strains of *E. coli* (Table 8).

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC</th>
<th>MBC</th>
<th>a.p</th>
<th>MIC</th>
<th>MBC</th>
<th>a.p</th>
<th>MIC</th>
<th>MBC</th>
<th>a.p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> ATCC 25923</td>
<td>3.750</td>
<td>7.500</td>
<td>2*</td>
<td>1.875</td>
<td>3.750</td>
<td>2*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>1.875</td>
<td>3.750</td>
<td>2*</td>
<td>0.234</td>
<td>0.234</td>
<td>1*</td>
<td>1.875</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>3.750</td>
<td>15</td>
<td>4</td>
<td>1.875</td>
<td>3.750</td>
<td>2*</td>
<td>1.875</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.500</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 8: MIC, MBC and antibiotic power (a.p) of *S. italica* extracts on the strains tested.

ATCC: American Type Culture Collection; *S. aureus*: Staphylococcus aureus; *E. coli*: Escherichia coli; *P. aeruginosa*: Pseudomonas aeruginosa; F: Leaves of *Senna italica*; Fl: Flowers of *Senna italica*; aq: Aqueous Extracts; eh: Hydro-Ethanolic Extracts; (-): No Inhibition; a.p. With * = bactericidal power; a.p. without * = Bacteriostatic Power.

Discussion

The choice of leaves and flowers of *S. italica* is explained by the fact that it is these different parts that are the most used according to the herbalists. However, the use of leaves and fruits is more common than that of flowers [5,20]. The use of this plant by natives was often either a decoction or hydro-ethanolic maceration. Consequently, these two extraction techniques were used in the framework of our research. Of course, we also consider the physico-chemical properties of sennosides.

We have not objectified alkaloids in the vegetable drugs of *S. italica*. This result is contrary to that of Dabai, *et al.* in Nigeria in 2012 [21], which showed alkaloids in the leaves and roots of *S. italica*. This discrepancy may be related to the fact that plant drugs have been harvested in different regions and at different times because environmental factors can influence the chemical composition of plants. Quinone and anthracene derivatives were found in the leaves of *S. italica*, Both in our study and that of Barbosa, *et al.* in 2004 [22]. The presence of flavonoids observed in our study in the leaves of *S. italica* was also reported by El Sayed, *et al.* [9].

The similarity between the chromatogram of the control (mixture of sennosides) and that of the extracts analyzed testifies to the fact that our extracts are authentic extracts of *Senna*. This similarity was also noted in spectral data.

Drying away from the sun and humidity at a temperature between 20 and 40°C allows having a very high content of sennosides present in the leaves of *S. italica* compared to sun drying. The use of leaves *S. italica* as a herbal drug is more beneficial (especially for the producer in terms of production yield) than that of flowers because the sennoside content of leaves exceeds that of flowers. In addition, during the rainy season and during watering, the flowers fall and therefore constitute a loss for the producer, which is not the case of leaves. On the other hand, such a high content of leaf sennosides can be explained by the fact that the harvest was done firstly in the early morning (thus at the time when there is an increase in the sennoside content) [20] and at the moment when the plant was in full bloom (thus a significant synthesis of secondary metabolites) [6] on the other hand. *Senna* leaves are the most commonly used natural laxatives.
Indeed, the standardized extracts of *Senna* have a laxative activity higher by about 30% than that caused by an equivalent quantity of pure sennosides because of a synergy with the accompanying substances whose mucilage also known for their laxative activity [6]. Several species of *Senna* are grown on a large scale in several countries such as Southern India for *Cassia angustifolia* and the Nile Valley for *Cassia senna*. The recommended quantity of sennosides daily is approximately 25 to 30 mg [6]. With *Senna* grown at Agoue in the department of Mono in Benin, this quantity can be reached with 0.718 to 0.862g of leaf powder or 1.390 to 1.668g of *S. italica* flower powder. These quantities are similar to those of *Cassia angustifolia* (0.75g of leaf powder [6]) to obtain the same laxative effect.

*S. italica*, a plant known locally as “Agoue gbe” is widely cultivated in the localities of Agoue (Mono department in Benin) where it represents a major source of income for many homes. This plant is well consumed locally as herbal tea for constipation self-treatment. It is very rich in bioactive substances responsible for its laxative effect or purgative. These substances are essentially the anthracene heterosides and in particular, the sennosides that were quantified in present study using HPLC-DAD technique. This quantification allowed to notice that both the leaves and the flowers of this plant are rich in sennosides and to have a clear idea of the dose to ingest of its herbal tea to have the desired therapeutic effect.

The results of *in vitro* antifungal tests showed that at concentrations ranging from 100 to 0.098 mg/mL, aqueous and hydro-ethanolic extracts of leaves and flowers of *S. italica* were neither active on *C. albicans* nor *T. rubrum* strains. These results are contrary to those found by Lemli., et al. [23] who found that *Cassia* derivatives are active on *C. albicans*. On the other hand, these results are similar to those of Somchit., et al. [24] who found that strains of *C. albicans* are resistant to the aqueous and hydro-ethanolic extracts of *Cassia alata* leaves (although this was a *Cassia* derivative) while the same microorganism is sensitive to aqueous extracts and hydro-ethanolic bark of *C. alata*.

These differences could be explained by the fact that the (microbiological) activities of plants are linked to the presence of secondary metabolites. The concentration of these secondary metabolites in a plant varies according to the harvest period and the harvesting site. In addition, the microbiological activity of a plant varies according to the vegetable drug used on the one hand and the extraction solvent on the other hand [25].

All bacterial strains used in this study (with the exception of *P. aeruginosa*) were very sensitive to hydro-ethanolic extracts. Therefore, the ethanol/water mixture is a good pair of solvents for the extraction of antibacterial bioactive compounds. Our results confirm the antimicrobial activity of extracts of *Senna italica* Mill. reported by Masoko., et al. [26] and by Dabai., et al. [21] on *S. aureus*, *E. coli* and *P. aeruginosa* strains. Antimicrobial activity of these extracts could be explained by the presence in the plant of phenolic compounds including flavonoids and tannins [19,27,28]. As for the more pronounced antimicrobial activity of the hydro-ethanolic extract of the flowers, it could probably be explained by the presence of the triterpenes [28] in *S. italica* flowers in addition to phenolic compounds. About flavonoids, several types with antimicrobial properties have been isolated from *S. italica*. These include tamarixetin, beta-sitosterol, stigmasterol and alpha-amyrin isolated from the aerial parts of *S. italica* by El sayed., et al [9].

The antimicrobial activity of leaves extracts of *S. italica* reported by Mosoko., et al. on *E. coli* [26] was more pronounced on this germ with a MIC of 0.16 mg/mL compared to the MIC found in our study of 0.234 mg/mL. This difference may be because Mosoko., et al. used an acetone extract while ours is hydro-ethanol.

The size of inhibition diameters of antibiotics compared to those of our extracts can be explained by the fact that these antibiotics are pure chemical substances while our extracts are mixtures of phytochemicals. In our study, we noticed that the hydro-ethanolic extract of *S. italica* flowers is more active on the studied germs than the hydro-ethanolic extract of the leaves of this same plant.

**Conclusion**

The leaves and flowers of *S. italica* are rich in sennosides, a molecule responsible for its laxative effect. Quantifying this class of molecule gives a clear idea of the dose of herbal tea *S. italica* to ingest to have the desired therapeutic effect. This approach to standardization of herbal tea of *S. italica* can limit these possible side effects (including abdominal pain) and prevent abuse that can lead to hypokalemia. In addition, this study notes that the ethanol/water mixture allows a better extraction of the bioactive substances of this plant. In addition,
drying the leaves away from the sun is more advantageous compared to a drying made locally in the sun. Additional studies could lead to the development of an improved traditional medicine from \textit{S. italica}.

\section*{Bibliography}


HPLC Standardization of Herbal Drugs and Evaluation of their Antimicrobial Properties: Studies of Leaves and Flowers of *Senna italica* Mill. Grown in Benin (Western Africa)


