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Research Paper

Preliminary Phytochemical Screening and Antimicrobial Activity of the Hydroethanolic Extract of the Fruits of *Solanum torvum* (Swartz) (Solanaceae) Use as Vegetable in Togo

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Abstract

The fruits of *Solanum torvum* (Swartz), a vegetable-fruit, are used in traditional medicine in Togo in the treatment of infectious diseases and as an anti-anemic. This study then focused on the antimicrobial activity assessment of the hydroethanolic extract of these fruits in the interest of contributing to the valorization of this Togolese flora's species. A hydroethanolic extraction (50 % - 50 %: v/v) was performed followed by preliminary phytochemical tests. Antimicrobial activity was determined on fourteen bacterial strains using the agar diffusion method. Qualitative phytochemical screening revealed the presence of alkaloids, reducing compounds, tannins, cardiac glycosides, flavonoids, coumarins, triterpenes, saponins, total carbohydrates and free quinones. The extract was active on the reference strains of *S. aureus*, *E. coli*, *S. pneumoniae* and *P. aeruginosa*. However, this activity was only observed on clinical strains of *S. pneumoniae* and *P. aeruginosa*. The extract showed MICs of 25 and 50 mg/ml and BMCs of 50 and 100 mg/ml respectively for *S. pneumoniae* and *P. aeruginosa*. The MBC/MIC ratio for these two strains was 2. These fruits would then have bacteriostatic activity on *S. pneumoniae* and *P. aeruginosa*. The antibacterial properties of the extract on these germs could justify the use of this plant in traditional medicine for the treatment of certain bacterial infections.

Keywords: *Solanum torvum*, fruits, phytochemical compounds, antimicrobial activity

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INTRODUCTION

The use of antibiotics in the treatment of bacterial infections is currently limited by the development of antibiotic resistance in bacteria. This bacterial resistance is becoming a growing concern with the proliferation of multi-drug resistant strains such as ESBLs and MRSA, especially in hospitals¹. Antimicrobial resistance then became a public health issue and a real concern for the scientific communities. The search for new natural antibacterial agents based on medicinal plants, used in the treatment of infectious diseases then became essential².

Among the plants used in antibiotic therapy are *Solanum torvum* (Swartz) (Solanaceae), a plant native to Central and South America. The species is cultivated as a vegetable in West Africa, Central Africa, South and East Asia. Indeed, *Solanum torvum* (Swartz) is an edible plant whose fruits are an ingredient in the preparation of certain meals. In addition, the seeds and roots are used in traditional

medicine to treat infections and other diseases such as malaria and anemia³. In Togo, the fruits of *Solanum torvum* are used as a fruiting vegetable and are also used in the treatment of anemia. It is therefore necessary to valorize this species in health promotion. It is in this context that this study fits, with the objective to contributing to the valorization of *Solanum torvum* (Swartz), through qualitative phytochemical screening and evaluation of the antimicrobial activity of the hydroethanolic extract of its fruits.

MATERIALS AND METHODS

Study framework

This study was carried out at the Laboratory of Process Engineering and Natural Resources (LAGEPREN) of the Faculty of Sciences of the University of Lomé and at the Laboratory of Medical Bacteriology of the National Institute of Hygiene (INH) of Lomé.

Material

Plant material

Fruits of *Solanum torvum* (Swartz) (Solanaceae) constituted the main plant material of this study. They were harvested in the prefecture of Agoe, at Légbassito (Lomé-Togo), then dried for one week at room temperature and ground to powder using a laboratory mill.

Microbiological material

The microorganisms used for the antimicrobial test were made up of reference and clinical strains isolated in the medical bacteriology laboratory of the National Institute of Hygiene (INH) in Lomé. They are *Salmonella typhi mirium* ATCC 14028, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 18331, *Shigella flexneiri* ATCC, *Pseudomonas aeruginosa* ATCC 27853 and *Streptococcus pneumoniae* ATCC 49619. These are strains recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for susceptibility studies. These strains were selected based on their medical importance and frequency in infectious diseases.

Methods

Preparation of the extract

Three hundred grams (300 g) of *Solanum torvum* (Swartz) fruit powder was macerated for 72 hours in a hydroethanolic solvent of proportion (50 % - 50 %: v/v). After maceration, the solution was filtered through a filter paper. The filtrate obtained was evaporated dry under vacuum using a Büchi type rotary evaporator whose water bath temperature was set at 45 °C. The dry extract was weighed and stored in a small glass bottle, labelled and protected from light in the refrigerator for use in the various tests.

Calculation of extraction yield

Yield is the amount of extract obtained from a plant material. It is expressed as a percentage in relation to the dry matter (vegetable powder) and has been calculated according to the following formula: $R (\%) = M_1 \times 100 / M_0$

R: Extract yield expressed as a percentage (%)

M_1 : mass of the extract obtained (g)

M_0 : mass of vegetable powder (g)

Preliminary phytochemical screening

Phytochemical screening is a set of qualitative and quantitative tests that allow the presence and quantification of biomolecules such as: alkaloids; flavonoids; tannins; total polyphenols; saponins; carbohydrates; coumarins and triterpenes contained in a plant organ to be detected and quantified. The detection of the presence of these secondary metabolites was carried out either by precipitation reactions and/or coloring of the reaction medium and their quantification by UV-Visible spectrophotometric assays.

Alkaloids research

The detection of alkaloids in the extract was carried out qualitatively following the different methods described by previous studies⁴.

➤ Dragendorff Test

An aqueous extract solution was treated with a few drops of Dragendorff's reagent (potassium iodide and bismuth

solution). The formation of red precipitate indicates the presence of alkaloids.

➤ Mayer Test

A few drops of Mayer reagent (potassium iodide and mercury) were added to an aqueous solution of the extract. The formation of a precipitate of yellow coloration reveals the presence of alkaloids.

➤ Wagner Test

When a few drops of Wagner reagent are introduced into an aqueous solution containing extract, the formation of a reddish-brown precipitate indicates the presence of alkaloids.

Saponin research

➤ Foam Test

According to the experimental protocol, 0.5 g of extract was diluted in 2 ml of distilled water contained in a graduated test tube with a total volume of 15 ml. After shaking the solution for 15 seconds, the solution was left to stand for 15 minutes. The formation of a persistent foam layer at least 1 cm thick shows that saponins are present in the samples studied⁵.

Research on reducing compounds

➤ Fehling test

The extract was dissolved in 5 ml of distilled water, then hydrolyzed with diluted hydrochloric acid (HCl) and neutralized with an alkaline solution. After the addition of Fehling's liqueur (reagent A: copper sulfate and reagent B: potassium hydroxide), the mixture was stirred well and then heated in a test tube with a Bunsen burner flame until a red precipitate appeared indicating the presence of reducing compounds⁶.

Tannin research

➤ Reaction with ferric chloride 1 %

To 1 ml of aqueous extract solution, 2 ml of water and 1-2 drops of 1 % ferric chloride reagent were added. The formation of a blue, blue-black or black coloration indicates the presence of gallic tannins; while the formation of a green or dark green coloration corresponds to the presence of catechic tannins⁷.

➤ Reaction with 10 % lead acetate

To 3 ml of aqueous extract was added 1 ml of 10 % lead acetate. The appearance of a whitish or brownish precipitate indicates the presence of tannins in the extract⁴.

➤ Reaction with ammoniacal copper sulfate

A volume of 2 ml of copper sulfate and 2 drops of ammonia are added to 2 ml of the extract. The presence of tannins in the extract is indicated by a blue-green precipitate.

Flavonoids research

Flavonoids were detected after mixing an extract with a few drops of a sodium hydroxide solution (NaOH: 1 %). The formation of an intense yellow color becoming colorless with the addition of diluted hydrochloric acid, corresponds to a positive test⁶.

Research of phytosterols or triterpenes

The extract was treated with a few drops of sulphuric acid (1 M), then shaken and left to rest. The appearance of a golden-yellow color indicates the presence of triterpenes⁴.

Searching for coumarins

A volume of 2 ml of the extract solution was introduced into a test tube to which 0.5 ml of a 10 % NaOH solution was added. The mixture was heated in a water bath to boiling. After cooling, the tubes containing the heated solutions were observed at 365 nm with a UV lamp. A fluorescent yellow coloration means that coumarins are present in the samples⁴⁻⁶.

Identification of total carbohydrates

To 1 ml aqueous extract was added 500 μ L of α -naphthol dissolved in ethanol. After mixing, 1 ml of concentrated sulfuric acid is slowly added to the wall of the inclined specimen without mixing to form a layer. A positive reaction is indicated by the appearance of a purple ring at the interface between the acid and the sugar solution⁸.

Cardiac glycoside research

A volume of 2 ml of chloroform is added to 1 ml of the aqueous solution of *S. torvum* powder, the appearance of a reddish-brown coloration after the addition of sulfuric acid (H_2SO_4) indicates the presence of cardiac glycosides⁷.

Evaluation of antimicrobial activity of the hydroethanolic extract of *Solanum torvum* fruits

➤ Strain treatment

The collected strains are grown on appropriate media (Chapman for *S. aureus*, Mac conkey for *E. coli*, Sabouraud for *C. albicans*, Hecktoen for *Shigella flexneirii* and *Salmonella typhimurium*, Fresh Blood Gelose for *Streptococcus pneumoniae* and Chocolate Gelose for *Klebsiella pneumoniae*) and preserved. These strains will be transplanted on agar medium without inhibitor (nutritive agar) following the recommendations of the French Society of Microbiology (SFM).

➤ Inoculum preparation

The microorganisms were cultured in Muller Hinton broth for 18 to 20 hours so that they were in the exponential growth phase. Each culture was then suspended in a saline solution of sodium chloride (0.9 % NaCl) at a turbidity equivalent to that of the 0.5 standard on the Mac Farland scale. This suspension, whose OD at 625 nm should be between 0.08 and 0.10, corresponds to approximately 1 to 2.106 CFU/ml and will be used as inoculum for the tests.

➤ Preparing the test solution

The extract is dissolved in distilled water at a concentration of 100 mg/ml. The sterility of this solution was verified by inoculating an aliquot on Mueller Hinton agar and incubating at 37 °C for 24 hours.

➤ Culture medium preparation

The dehydrated Muller Hinton agar medium (MH) was suspended in distilled water at 36 g/l and then heated in a water bath until completely dissolved. The pH was adjusted to 7.3 ± 0.1 and then the medium was autoclaved at 121 °C for 15 minutes. It was then cooled to 45 - 50 °C and poured into sterile 90 mm diameter Petri dishes so that the thickness is 4 mm.

➤ Antimicrobial testing

• Agar diffusion methods

Muller Hinton agars were inoculated by flooding. After drying the plates, the agar is perforated at 6 points of equal distance with a sterile tip cut so that the diameter is 6 mm. The cavities thus formed are filled with 50 μ l of the test

solution. The plates are incubated in an incubator at 37 °C for 24 hours. The bactericidal action is manifested by the formation of a halo around the wells. The results were read by measuring the diameters of the inhibition zones. The test was carried out twice and an average was made on the two determinations⁹.

➤ Determination of MIC and WBC by the dilution method

The MIC and WBC were determined for the extracts that were active on germs during the liquid diffusion test.

- Determination of the minimum inhibitory concentration

The minimum inhibitory concentration is determined by the macrodilution tube method. MH broth was used to prepare serial dilutions at half concentrations ranging from 50 to 0.390 mg/ml. 10 μ L of the inoculum was added to each tube containing 500 μ L of test solutions. Tubes without inoculum were considered as negative controls. All these tubes were incubated at 37 °C for 24 hours. The first tube in the series that did not show any visible sign of culture was considered to be MIC⁹.

- Determination of the minimum bactericidal concentration

For the BMC determination, an oese was taken from the tubes that showed no visible culture during the MIC determination and seeded on nutrient agar for bacteria and Sabouraud for *Candida*.

After 24 hours incubation, the lowest concentration of the extract that did not give any colonies is considered as the MBC. In order to determine whether the observed antimicrobial effect is bactericidal or bacteriostatic, the BMC/MIC ratio was performed. A BMC/MIC ratio greater than 1 is considered bacteriostatic and a BMC/MIC ratio less than or equal to 1 is considered bactericidal. Finally, when this ratio is greater than or equal to 16, the activity is non-existent⁹.

Statistical analysis

All results were entered into the EXCEL 2016 spreadsheet program and processed using Graph Pad Prism version 8.4.3 statistical software. The significance level was set at 5 % ($p < 0.05$). This methodology with the material involved resulted in results that were discussed and conclusions reached.

RESULTS

Yield of extraction

Table 1 shows the extraction yield and physical characteristics of the hydroethanolic extract of the fruits of *Solanum torvum*.

Table 1: Physical appearance and extraction yield of *S. torvum*

Color	Physical Appearance	Yield	Solvent
Brown	Sticky paste	18,46 %	Water-ethanol (50 %-50 %: v/v)

Results of the preliminary phytochemical screening

The qualitative phytochemical tests carried out on the hydroethanol extract revealed the existence of a variety of secondary metabolites (Table 2).

Qualitative phytochemical tests (Table 2) revealed thus the presence of alkaloids, reducing compounds, tannins, cardiac glycosides, flavonoids, coumarins, triterpenes, saponins, total carbohydrates and free quinones in the hydroethanol extract of *Solanum torvum*.

Results of antimicrobial tests

Antimicrobial test results are recorded in tables 3 and 4.

Table 2: Result of the preliminary phytochemical screening of *S. torvum*

Phytochemical compounds	Results
Alkaloids	+
Reducing compounds	+
Tannins	+
Cardiac glucosides	+
Flavonoids	+
Coumarines	+
Triterpenes	+
Saponines	+
Total carbohydrates	+
Free quinones	+

Presence : +

Table 3: Results of *S. torvum* activity on reference strains

Strains	Inhibition diameter (mm)		Sensitivity		Effects	
	ATB/ATF	ST	MIC	MBC	MBC/MIC	
<i>S. aureus</i>	14.60 ± 0.10	9.00 ± 0.15	IND	IND	IND	IND
<i>C. albicans</i>	15.50 ± 0.10	0.00 ± 0.00	IND	IND	IND	IND
<i>S. flexneiri</i>	13.25 ± 0.15	0.00 ± 0.00	IND	IND	IND	IND
<i>S. typhimurium</i>	13.80 ± 0.16	0.00 ± 0.00	IND	IND	IND	IND
<i>E. coli</i>	13.10 ± 0.15	8.10 ± 0.15	IND	IND	IND	IND
<i>S. pneumoniae</i>	13.50 ± 0.10	14.29 ± 0.01	25	50	2	Bacteriostatics
<i>P. aeruginosa</i>	14.15 ± 0.15	13.64 ± 0.14	50	100	2	Bacteriostatics

ATB: Antibiotic (Gentamycin 10 µg); ATF: Antifungal (Nystatin 100 UI); ST: *Solanum torvum* 100 mg/ml; MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration; IND: indeterminate

Table 4: Activity results of *S. torvum* on clinical strains

Strains	Inhibition diameter (mm)		Sensitivity		Effects	
	ATB/ATF	ST	CMI	CMB	CMB/CMI	
<i>S. aureus</i>	14.10 ± 0.40	0.00 ± 0.00	IND	IND	IND	IND
<i>C. albicans</i>	15.10 ± 0.10	0.00 ± 0.00	IND	IND	IND	IND
<i>S. flexneiri</i>	13.25 ± 0.10	0.00 ± 0.00	IND	IND	IND	IND
<i>S. typhimurium</i>	13.70 ± 0.11	0.00 ± 0.00	IND	IND	IND	IND
<i>E. coli</i>	13.10 ± 0.15	0.00 ± 0.00	IND	IND	IND	IND
<i>S. pneumoniae</i>	13.25 ± 0.20	14.00 ± 0.10	25	50	2	Bacteriostatics
<i>P. aeruginosa</i>	14.00 ± 0.20	13.50 ± 0.10	50	100	2	Bacteriostatics

ATB: Antibiotic (Gentamycin 10 µg); ATF: Antifungal (Nystatin 100 UI); ST: *Solanum torvum* 100 mg/ml MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration; IND: indeterminate

DISCUSSION

Extraction yield

The yield of hydroethanolic extraction of the fruits of *S. torvum* obtained in the present study (18.46 %) is within the range of those reported by Okou *et al.*¹⁰ and Kanga *et al.*¹¹. In fact, with a hydroethanolic extraction, considering a proportion of 70 % alcohol, these authors reported a yield of 40 % and with an aqueous extraction, they obtained a yield of 10 %. This variation in the extraction yield depending on the nature of the solvent is justified by the solubility of the bioactive molecules in them. Considering the hydroethanolic

extraction carried out in the present study (50 % - 50 %: v/v), in comparison with those of Kanga *et al.*¹¹ (70 % - 30 %: v/v), it can say that the biochemical compounds of the extract would be more hydrophobic than hydrophilic. This is confirmed by the 10 % yield obtained with the aqueous extraction which is low compared to 18.46 % obtained in this study and even lower compared to 40 % obtained with 70 % ethanol by Kanga *et al.*¹¹. These yields can also be explained by the extraction method, the variety of the plant species considered and environmental conditions such as climate and soil type which influence the nature of the biochemical compounds and therefore the yield of

extraction. These intrinsic and environmental factors can justify the extraction yields reported by Okou *et al.*¹⁰ which were 7 % and 2 % respectively with 70 % and 100 % ethanol as solvent and 37.6 % with the aqueous extract.

Qualitative phytochemical screening

Phytochemical screening revealed the presence of numerous compounds. This justifies the use of the fruits of *Solanum torvum* in many conditions in traditional medicine¹². Our results are close to those obtained by Kanga *et al.*¹¹ who also reported the presence of polyphenols, tannins, flavonoids, saponins and alkaloids. Chah *et al.*¹³ also reported similar results with the presence of alkaloids, flavonoids, saponins, tannins and glycosides.

Antimicrobial activity

The hydroethanolic extract of *solanum torvum* fruits was active on reference strains of *S. aureus*, *E. coli*, *S. pneumoniae*, *P. aeruginosa*, with inhibition diameters of 9.00 ± 0.15 ; 8.10 ± 0.15 ; 14.29 ± 0.01 and 13.64 ± 0.14 , respectively. However, activity was only observed in clinical strains of *S. pneumoniae*, *P. aeruginosa* with inhibition diameters of 14.00 ± 0.10 and 13.50 ± 0.10 , respectively. A similar result was obtained by Chah *et al.*¹³ with a methanolic extract of the fruits of *Solanum torvum*.

MIC and MBC were determined for the strains on which the extract was active by the liquid dilution method. Results showed MICs of 25 and 50 mg/ml and MBCs of 50 and 100 mg/ml respectively for *S. pneumoniae* and *P. aeruginosa*. This result is consistent with that obtained by Shah *et al.*¹³ with an indeterminate MIC for *E. coli* and a MIC of 0.3125 mg/ml for *P. aeruginosa*. On the other hand, no effect was observed on *S. aureus* and *S. typhimurium* strains, contrary to what was reported by Chah *et al.*¹³. This difference could be explained by the fact that the solvent used in our study for extraction is a mixture of water and ethanol, whereas Chah *et al.*¹³ used methanol only, the extraction of phytoelements would depend on the type of solvent.

The CMB/CMI ratio carried out with the results on germs showed that the fruit extract of *Solanum torvum* analyzed would have bacteriostatic activity on *S. pneumoniae* and *P. aeruginosa* (CMB/CMI = 2).

Although the CMB/CMI ratio is greater than 1 and considered bacteriostatic, it is not greater than or equal to 16.0. Therefore, the hydroethanolic extract of *solanum torvum* fruits analyzed can be considered effective against *S. pneumoniae* and *P. aeruginosa*. This antimicrobial capacity could be explained by the presence of antimicrobial compounds in the fruits of this plant. Indeed, many pharmacologically bioactive compounds such as alkaloids, flavonoids, tannins, anthraquinones and phenolic compounds are involved in the antibacterial activities of many plants¹⁴.

CONCLUSION

The study of the hydroethanol extract of the fruits of *Solanum torvum* (Swartz) (Solanaceae) found the presence of numerous bioactive compounds. The extract was active on certain strains of bacteria, justifying its use in traditional medicine for the treatment of numerous infections and other pathologies. It can therefore be used in the development of improved traditional medicines, in a context of resistance of bacterial strains against conventional antibiotics.

Conflicts of interest: None

Authors' Contributions: Conceived and designed the experiments: MM, AT, KE and AAL; Performed the experiments: MM, EKD, SF, AT, KE; Analyzed the data: AT, KE and MH; Contributed reagents/materials/analysis tools: MM, EKD, NKM and BB; Supervision of all analyzes: BB; Paper writing: MM, EKD, SF, AT, KE and MH.

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