ISSN 2462-2052 | eISSN 2600-8718 *Journal of Science and Mathematics Letters*
DOI: https://doi.org/10.37134/jsml.vol12.1.12.2024 *Volume 12, Issue 1, 102-110, 2024*

Research Article

Antibacterial and Antioxidant Activities of Fruits of *Solanum hirtum* Vahl. from Venezuela

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Received: 1 November 2023; **Accepted:** 24 December 2023; **Published:** 5 January 2024

ABSTRACT

Solanum hirtum (Solanaceae) has been used in traditional medicine to heal several illnesses, it's worked as an antiseptic for sore throat and other diseases. The present study describes the phytochemical profiles, antibacterial, and antioxidant properties of *S. hirtum* fruits. The phytochemicals from the dried fruits were extracted using a maceration technique with methanol and the flash column chromatography with *n*-hexane, dichloromethane, and methanol was the selected means to fraction the extract. The phytochemical screening was implemented using standard chemical tests. The antibacterial and antioxidant activities were screened using the disc diffusion method, and analyzing the radical scavenging activity using 2,2´-diphenyl-1-picryhydrazyl (DPPH), respectively, whilst the total phenolic content was measured using the Folin-Ciocalteu method. The phytochemical analysis results revealed the presence of terpenoids, steroids, phenols, flavonoids, and alkaloids. Antibacterial activity results showed the dichloromethane fraction exhibited activity against *S. aureus* (7.0 mm) and *K. pneumoniae* (9.0 mm) and methanolic fraction against *P. aeruginosa* (7.0 mm), *S. aureus* (8.0 mm) and *K. pneumoniae* (9.0 mm). In the DPPH scavenging assay, the IC_{50} value of the extract was 268.66±0.01 μg/mL. *S. hirtum* showed dose-dependent DPPH radicals scavenging activity. The total phenolic content (in gallic acid equivalents, GAE) was 18.54±0.30 mg GAE/g and the flavonoids content (in quercetin equivalents, QE) was 5.14±0.10 mg QE/g. *Solanum* genus is enriched with a diverse class of chemical compounds based on the literature survey and in our study, the resulting bioactivities are basically due to a mixture of phytochemicals possessed in the extracts or may be due to the synergistic activity of various constituents held by this plant species.

Keywords: *Solanum*, *Solanum hirtum*, Antibacterial activity, DPPH, Antioxidant activity.

1. INTRODUCTION

The Solanaceae family comprises about 90 genera and 3.000 species, which are widely distributed in the world. They are a rich source of active secondary metabolites (Coletto et al., 2004). Within this family, the genus *Solanum* is the largest and most complex with more than

2000 species distributed across subtropical and tropical regions of Asia, Africa, non-arid Africa, Americas, Australia, and India (Kaunda and Zhang, 2019). In Venezuelan flora, the Solanaceae are very well represented and form a group of plants of relative importance due to their floristic diversity, consisting of 32 genera and around 215 species (Benítez de Rojas, 1994).

A series of pharmacological studies have been carried out to verify and validate the traditional medicinal applications of many plants in this genus. Numerous species of *Solanum* are known to possess a variety of biological activities including antimycotic (González et al., 2004), antibacterial (Doss et al., 209), antiviral (Yousafa et al., 2013), anti-inflammatory (Anosike et al., 2012), antidepressant (Mohan et al., 2013), antihypertensive (Ibarrola et al, 2022), molluscicidal (El-Sherbini et al., 2009), anticarcinogenic (Fekry et al., 2019) and cytotoxic properties (Ikeda et al., 2003). Previous phytochemical investigations on *Solanum* species led to the identification of steroidal saponins, steroidal alkaloids, terpenes, flavonoids, lignans, sterols, phenolic comopunds, coumarins, amongst other compounds (Kaunda and Zhang, 2019; Elizalde-Romero et al., 2021).

Solanum hirtum Vahl. (Figure 1) is a suffrutescent perennial native to northern South America and Mexico. The species belongs to the subgenus Leptostemonium, section Lasiocarpa. All species in this section of the genus are andromonoecious, but the number and proportion of staminate flowers varies among the species (Diggle, 1991; 1993). Since no reports have been published on the qualitative phytochemical analysis of the crude extracts and biologic activity of this plant. The phytochemical screening, antioxidant, and antibacterial effects of *S. hirtum* fruit extract are being reported in this research.

Figure 1. *Solanum hirtum* Vahl. (Salinas, 2012)

2. MATERIALS AND METHODS

2.1. Plant material

The fruits of *Solanum hirtum* Vahl. were collected in the Municipality Urdaneta, Sector Quebrada de Cuevas, Trujillo-Venezuela. Voucher specimen N° MG001 was deposited at the Faculty of Pharmacy and Bioanalysis Herbarium, University of Los Andes (MERF Herbarium).

2.2. Extraction of plant material

S. hirtum fruits were dried at 40°C during 72 h and grinded afterwards. The grinded material (300 g) was extracted at room temperature with methanol for a period of two days with periodic daily stirring, and the extract was finally filtered, the solvent was removed under

reduced pressure. The dry extract was later kept in tightly stoppered bottles in a refrigerator until required for further analysis.

2.3. Fractionation of crude extracts

The crude methanolic extract of *S. hirtum* fruits (10 g) was subjected to flash chromatography over silica gel. The column was eluted in order with *n*-hexane (SHFH, 0.27 g), dichloromethane (SHFD, 0.24 g) and methanol (SHFM, 5.3 g) to obtain 3 fractions.

2.4. Qualitative phytochemical analysis

The phytochemical analysis of *n*-hexane, dichloromethane, and methanol fractions of the fruits of *S. hirtum* consisted in verifying the presence of secondary metabolites using the following standard methods according to literature reports (Banu and Cathrine, 2015).

Alkaloids test. Test extract was acidified with 1% hydrochloric acid (HCl) and it was then treated with a few drops of Mayer, Wagner, and Dragendroff's reagents separately in different test tubes. A creamy white (Mayer), reddish brown (Wagner), or orange brown (Dragendroff's) precipitate indicates the presence of alkaloids.

Flavonoids test. The extract was dissolved in ethanol (5 mL) and few fragments of magnesium ribbon and concentrated hydrochloric acid were added (drop wise). If any pink to crimson colour developed, the presence of flavonol glucosides was inferred.

Phenols test. The extract was dissolved in distilled water (5 mL). A few drops of neutral 5% ferric chloride solution were added to this. A dark green colour indicated the presence of phenolic compounds.

Tannins test. The extract was dissolved in distilled water (5 mL) and 1% solution of gelatin containing 10% NaCl (2 mL) was added to it. The white precipitate indicated the presence of tannins.

Terpenoids test. The extract was dissolved in chloroform and acetic anhydride (1 mL) was added. Then, 1 or 2 drops of concentrated sulphuric acid were slowly added along the sides of the test tube. A reddish-brown colouration at the interface represented the existence of terpenoids.

Saponins test. The extract was diluted with distilled water and then shaken vigorously for a few minutes. The formation of stable foam that persisted on heating was taken as an indication for the presence of saponins.

Coumarins test. The extract was diluted in ethanol and ammonium hydroxide solution (1 mL) was added. The appearance of yellow fluorescence under UV light (λ_{max} 365 nm) confirmed the presence of coumarins.

Quinones test. The extract was dissolved in ethanol followed by the addition of concentrated sulfuric acid. The formation of red-coloured solution was indicative of the existence of quinones.

Anthraquinones test. The extract was dissolved in ethanol, then chloroform (3 mL) were added, and it was shaken after that, the chloroform layer was separated and 10% ammonia solution was added to it. Pink or slight red colour on the upper part of the aqueous layer confirmed the presence of anthraquinones.

2.5. Antibacterial activity

The antibacterial activity was determined by the agar diffusion method with discs (CLSI, 2017). The following microorganisms were tested: *Staphylococcus aureus* (ATCC 27922), *Enterococcus faecalis* (ATCC 27922), *Klebsiella pneumoniae* (ATCC 23357), *Escherichia coli*

(ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), as reference strains provided by the Department of Microbiology and Parasitology of the Faculty of Pharmacy and Bioanalysis of the Universidad de Los Andes. An 18 h culture of each microorganism was used in 2.5 mL Müeller-Hinton (MH) broth at 37°C. The bacterial inoculum was adjusted with saline physiological solution to the Mc Farland Turbidity Standard N° 0.5 (10^{6-8} CFU/mL). Each inoculum was spread with a swab on the surface of a plate containing Müeller-Hinton agar and then a disc of filter paper (6 mm diameter) previously impregnated with 10 µL of the compound dissolved in DMSO was placed on the surface at a 1 mg/mL concentration of each extract. A disc impregnated with DMSO was included as a negative control. In addition, the standard disc of the reference antibiotic was placed as a positive control for each of the microorganisms (Piperacillin® 100 µg/disc for *E. coli*, *K. pneumonia* and *P. aeruginosa*; Erythromycin® 15 µg/disc for *S. aureus*; Ampicillin® 10 µg/disc for *E. faecalis*). After having placed the discs on the Petri plates, they were refrigerated at 4°C for 24 h (Velasco et al., 2007). The reading of the inhibition halos was performed at 24 h and it was measured (mm) around the disc. All antibacterial activity trials were done twice.

2.6. Antioxidant activity

The free radical scavenging activity of the crude extract was determined using a stable free radical. The DPPH solution was prepared at a concentration of 6×10^{-2} mM of DPPH in ethanol. During the assays, 1 mL of the fraction SHFM (1000 µg/mL) was mixed with 3 mL DPPH solution. Simultaneously, a positive control (ascorbic acid) was prepared. The mixture was incubated at room temperature for 30 min and later read on a Spectronic Genesys TM 10 Bio reader plate at 517 nm. All measurements were carried out in triplicate. The *n*-hexane (SHFH) and dichloromethane (SHFD) fractions were not evaluated due to low solubility in ethanol. The IC_{50} value was calculated based on the percentage of inhibition of each concentration, namely, 500, 250, 125, 62.5, 31.25, and 15.62 µg/mL. The percentage of inhibition (y) for each concentration (x), the points (x and y) are plotted on the coordinate plane, then the line equation $y = ax + b$ is determined by calculating using linear regression where a and b are constants, x is the concentration sample (ppm), and y is the percentage of inhibition (%). Antioxidant activity is expressed by IC_{50}, namely, the sample's concentration that can reduce 50% of DPPH radicals (Akar et al., 2017; Villano et al., 2007).

2.7. Total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu's reagent as described by Bouslamti et al. (2022) with slight modifications. An aliquot of 50 µL fraction SHFM was pooled with 250 µL of the Folin-Ciocalteu reagent (10%). After 4 min, 250 µL mL of a 7.5% sodium carbonate (Na_2CO_3) solution were added. Before estimating the absorbance at 760 nm, the combination was prepared by reacting at room temperature for 2 h. Milligrams of gallic acid equivalents (mg GAE/g extract) were used to express the results. All experiments were performed in triplicate.

2.8. Total flavonoids content

The aluminum chloride colorimetric method was used to determine the total flavonoid content (Rojas et al., 2015). We prepared 1 mL of extract and 2 mL of 2% aluminium chloride in methanol. The absorbance at 420 nm has been measured after 30 min incubation, and quercetin equivalents per gram of extract (mg QE/g extract) are used to represent the estimated flavonoid content. All experiments were performed in triplicate.

2.9. Statistical analysis

The mean and standard deviation of triplicates of plant extracts were calculated using Microsoft Excel. To evaluate their significance ($p<0.05$), all results were subjected to a 5%-point Tukey's test for one-way analysis (ANOVA) (Bewick et al., 2004).

3. RESULTS AND DISCUSSION

3.1. Qualitative preliminary phytochemical analysis

In the present study, the phytochemical screening test of fruit fractions of *S. hirtum* revealed the presence of steroids, terpenenoids, phenols, flavonoids and alkaloids, as shown in Table 1.

Table 1. Preliminary qualitative phytochemical analysis of *S. hirtum*

Class of compounds	SHFH	SHFD	SHFM
Steroid	+	+	+
Terpenoid	+	+	+
Phenols	-	-	+
Flavonoids	-	-	+
Coumarins	-	-	-
Quinones	-	-	-
Alkaloids	+	+	+
Saponins	-	-	-
Anthraquinones	-	.-	-
Tannins	-	-	-

+: present. -: absently. SHFH: *n*-hexane fraction. SHFD: dichloromethane fraction. SHFM: methanolic fraction

The results showed that the dichloromethane and methanol fractions contained more bioactive compounds compared to the *n*-hexane fraction. Coumarins, quinones, saponins, anthraquinones, and tannins were absent in all extracts tested. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra, 1993). Steroids have been reported to have antibacterial properties and they are essential compounds, especially because of their relationship with compounds such as sex hormones (Okwu, 2001). The phenolic compounds are one of the largest groups of plant metabolites. They possess biological properties as antioxidants that protect cells from oxidative stress and eliminate free radicals by donating hydrogen atoms. The action of phenolics as neuroprotective compounds, fungicides, bactericides, their anti-atherosclerosis effects, and anticancinogenic activity are well documented. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Several workers have reported the analgesic, antispasmodic, and antibacterial properties of alkaloids (Thirumurugan et al., 2018). The results obtained in this study suggest that the identified phytochemical compounds may be the bioactive constituents of *S. hirtum*.

3.2. Antibacterial activity

The fractions of *S. hirtum* were tested against five bacterial pathogens using the agar diffusion method. The results of the activity are presented in Table 2.

Table 2. Antibacterial activity of *S. hirtum*

Sample	Inhibition zone diameter ± SD (mm)[c]				
	E. faecalis	***S. aureus***	***P. aeruginosa***	***K. pneumoniae***	***E. coli***
SHFD	-	7.0 ± 0.0	-	9.0 ± 0.0	-
SHFM	-	8.0 ± 0.0	7.0 ± 0.0	9.0 ± 0.0	-
[a]Eritromicina®15 µg	ND	26.0 ± 0.0	ND	ND	ND
[a]Piperacilina®100 µg	ND	ND	32.0 ± 0.0	18.0 ± 0.0	21.0 ± 0.0
[a]Ampicilina®10µg	17.0 ± 0.0	ND	ND	ND	ND
[b]DMSO	-	-	-	-	-

[a]Positive control. [b]Negative control. SHFD: dichloromethane fraction. SHFM: methanolic fraction. DMSO: dimethyl sulfoxide. [c]Inhibition zone diameter (mm) including diameter of the disc 6 mm. -: inactive. ND: undetermined. The values are the average of three separate measurements of the inhibition halos (mm) ± Standard Deviation (SD) of the tested extracts. All results are statistically significant (p value < 0.05)

The results proved that the hexane extract was not soluble in dimethyl sulfoxide, so it was not possible to determine the antibacterial activity under the experimental conditions established in the study. Dichloromethane fraction (SHFD) exhibited activity against *S. aureus* (7.0 mm) and *K. pneumoniae* (9.0 mm). Methanol fractions (SHFM) have antibacterial activity against *P. aeruginosa* (7.0 mm), *S. aureus* (8.0 mm) and *K. pneumoniae* (9.0 mm) and no inhibition was found in *E. faecalis* and *E. coli*. The antibacterial activity of the active fractions might be due to the presence of terpenes, steroids, flavonoids, phenols, and alkaloids that are known to have antibacterial properties (Twaij and Hasan, 2022). To our knowledge, no detailed information is available on the antibacterial activities of *S. hirtum* in the literature.

3.3. Antioxidant activity

The antioxidant activity depends on the presence of bioactive components that can quench peroxyl radicals or inhibit the oxidation reaction of organic materials. The most current research on antioxidant action focuses on flavonoids, tannins, and other phenolic constituents (Sulekha et al., 2009). The inhibition of DPPH radical scavenging assay of *S. hirtum* fruits is tabulated in Table 3 and Figure 2

Table 3. Antioxidant activity of *S. hirtum*

Sample	Concentrations (µg/mL)	Inhibition±SD (%)	IC_{50}±SD (µg/mL)
Ascorbic acid	12.5	20.67±0.47	
	25	37.09±0.43	
	50	69.02±0.18	35.20±0.01
	75	96.36±0.26	
	100	97.08±0.07	
SHFM	15.62	32.00±0.14	
	31.25	34.42±0.17	
	62.5	37.93±0.33	268.66±0.01
	125	42.83±0.14	
	250	50.81±0.19	
	500	66.18±0.14	

SHFM: methanolic fraction; IC_{50}: Concentration that can reduce 50% of DPPH radicals; Values are the mean of three separate measurements of the percentage inhibition (%) and medium antioxidant activity (IC_{50} express µg/mL); ± Standard Deviation (SD) of the extracts tested; All results are statistically significant (p value < 0.05)

These results indicated medium antioxidant activity for SHFM (IC_{50} value 268.660.01 µg/mL). The category as antioxidants based on DPPH assay was divided in: strong, medium, and weak with the IC_{50} <50, 50-100, 100-250, and 250-500 µg/mL, respectively (Kato et al.,

2009). *S. hirtum* fruits showed dose-dependent DPPH radicals scavenging activity. The decrease in DPPH absorbance caused by antioxidants is due to the reaction between antioxidant molecules and the radical, which results in the removal of the radical by hydrogen donation. The antioxidant activity of *S. hirtum* fruits might be attributed to the presence of phytochemicals such as flavonoids and phenolic compounds. Flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties (Muruhan et al., 2013).

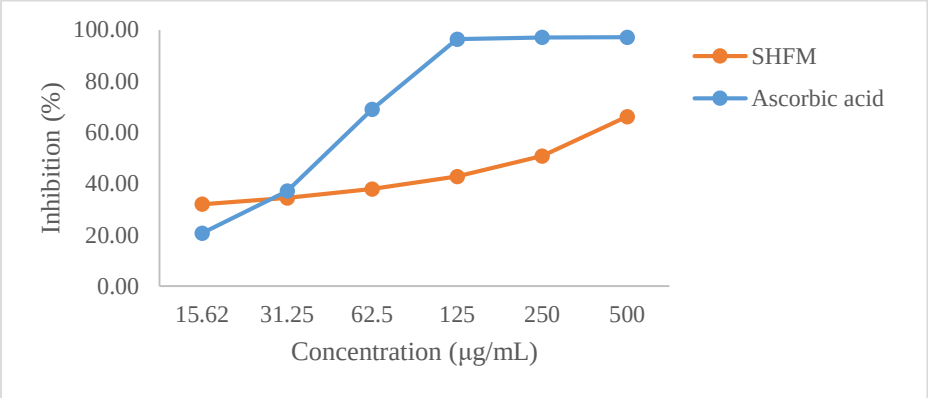

Figure 2. Inhibition of DPPH radical scavenging assay of *S. hirtum*

3.4. Total phenolics and flavonoids

Phenolic compound are one of the major constituents as primary antioxidants or free radical terminators, flavonoids are the most diverse and widespread group of natural compounds and probably the most important phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Therefore, the content of flavonoid and phenolics was determined in the extract. The total phenolic content (TPC) was expressed as mg gallic acid equivalent (GAE) per g extract. The calibration curve for gallic acid and the regression equations, $y = 0.0285x + 0.0224$, R^2=0.9989 shows that the TPC for SHFM fraction was equal to 18.54±0.30 mg GAE/g. The total flavonoids content (TFC) was expressed as quercetin equivalents per gram of extract (mg QE/g extract). The calibration curve for quercetin and the regression equation, $y = 0.0023x + 0.0294$, R^2: 0.995 show that TFC for SHFM fraction was equal to 5.14±0.10 mg QE/g. The methanolic fraction was the only one evaluated because the hexane and dichloromethane fractions gave negative results for phenolic compounds in the phytochemical screening. Phenolic compounds have received a lot of attention as potential natural antioxidants due to their ability to act as both efficient radical scavengers and metal chelators. The result indicates an association between antioxidant activity and phenolic compounds, suggesting that phenolic compounds are probably responsible for the antioxidant activities of *S. hirtum*.

4. CONCLUSION

The qualitative analyses of phytoconstituents of *Solanum hirtum* fruits were performed in this research. The methanolic fraction exhibits the maximum number of phytochemicals compared to other fractions, which may be due to the polar nature of the solvents. The methanolic fraction of the plant showed a medium antioxidant activity (IC_{50} value 268.66±0.01 µg/mL), low total phenolic content (18.54±0.30 mg GAE/g), low flavonoid content (5.14±0.10 mg QE/g) and antibacterial activity against *P. aeruginosa* (7.0 mm), *S. aureus* (8.0 mm) and *K. pneumoniae* (9.0 mm). Further studies on this plant are essential to identify and isolate bioactive compounds in the extracts and determine their potential against different biological actions.

Declaration of Interest
The authors declare that there is no conflict of interest.

Acknowledgement
The authors are grateful to the Direct Support Program for Research Groups (ADG in Spanish) of the Council for Scientific, Humanistic, Technological and Arts Development of the Universidad de Los Andes, Mérida, Bolivarian Republic of Venezuela. Research Group: Natural Products and Medicinal Chemistry; Research Institute, Natural Products Section, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Mérida, the Bolivarian Republic of Venezuela.

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