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PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL CHARACTERIZATION OF *HEDERA HELIX* L. EXTRACT

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Abstract: The present research describes the components of the ivy (*Hedera helix* L.) plant extract. It is known that ivy presents a lot of health benefits, like antibacterial, antimicrobial, anticancer properties or skin care, due to their components. So, the aim of our study was to characterize quantitative (polyphenols, tannins, flavonoids, terpenoids) and qualitative (saponins, proteins, steroids) screening for phytochemical compounds and antioxidant activity of the hydroalcoholic extract obtained by ivy leaves. The sample was analyzed by UV-VIS, FTIR, TLC techniques. The antioxidant activity was evaluated using DPPH method. The antimicrobial activity was demonstrated on bacteria, yeast and mold species.

Keywords: analytical techniques, antioxidant activity, *Hedera helix* L., phytocompounds, phytosynthesis.

Introduction

Ivy (*Hedera helix* L.), is a genus containing approximately 15 species of climbing evergreen plants. On suitable surfaces, trees and rock faces, ivy has the ability to climb 25-30 m above the ground and hold fast to vertical surfaces, like liana [XIA & al. 2011; LUTSENKO & al. 2010].

Hedera helix contains saponins, flavonoids, polyacetylenes and phenolic compounds (flavonoids, anthocyanins, coumarins and phenolic acids), aminoacids, steroids, vitamins, volatile and fixed oils, which are reported for medicinal benefits: antifungal, antispasmodic, antimicrobial, antimutagenic or cytotoxic activities [BUSECK & al. 2011; MEDEIROS & al. 2002; MIAO & al. 2015].

As we said above, the main constituents in the crude extract ivy plant are triterpen saponins, with the predominant substance Hederacozide C, presented in Figure 1.

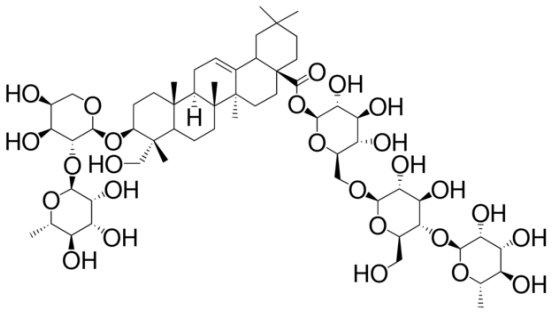

Figure 1. Structure formula of hederacozide C.

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Material and methods

Ivy plant (*Hedera helix* L.) was collected from ICECHIM institute garden, Bucharest city. All solvents used for extraction were of analytical grade. Methanol was purchased from Merck. Distilled water was internal laboratory obtained, using Liston equipment. For determinations of phytochemical methods, it was used $AlCl_3$ (from Sigma-Aldrich), $NaNO_2$, NaOH, H_2SO_4, Na_2CO_3, HCl and Folin–Ciocalteu reagent (from Merck) substances. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate stable free radical, Merk) was utilized for antioxidant determination. For qualitative determinations, Benedict's and Millon's reagents were purchased from Sigma – Aldrich.

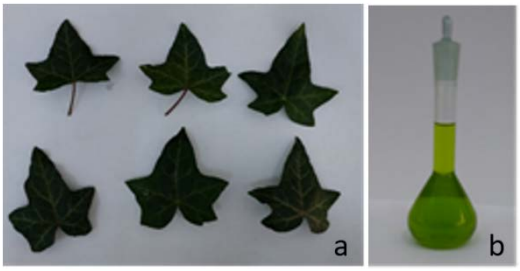


Figure 2. a. Ivy leavesand b. hydroalcoholic ivy extract.

The ivy leaves (Figure 2a) were washed and dried at room temperature, 5 days. These where extracted in a mixed solution (ethanol: distilled water) using an ultrasonicator bath (50 ºC, 90 min, 100 power). After that, the extract was kept 6 days at maceration, at room temperature. Then it was filtered and the final solution (Figure 2b) was kept in the fridge, in order to avoid carotenoid degradation.

UV-VIS Spectroscopy. The absorption spectra of the samples were recorded on a double beam M400 Carl Zeiss Jena UV-VIS spectrophotometer from 250 to 750 nm, at the resolution of 1 nm, with 1 nm slit width and 0.3 nm/s scan rate.

FTIR Spectroscopy. For Fourier transformed IR spectroscopy, the spectra were collected using a Perkin Elmer Spectrum GX instrument. Spectra were registered using ATR technique, in the range of 4000-600 cm^{-1} at a spectral resolution of 4 cm^{-1}.

Antioxidant activity (*AA%*). The principle of AA% method consists in reducing the presence of an antioxidant molecule, giving rise to colored methanol solutions. The utilization of DPPH method gives an easy and rapid result to antioxidant activity against free radicals [SUICA-BUNGHEZ & al. 2020].

Phytochemical analyses. The phytochemical analyses were used for the determination of total tannins, total flavonoids, total polyphenols, total terpenoids and carotenoids existent in the hydroalcoholic ivy extract.

Qualitative screening for phytochemical compounds. The qualitative screening refers mainly to the change in color of the aqueous ivy (*Hedera helix* L.) extract when known reagents are added, indicating the presence or absence of different phytochemicals such as carbohydrates, tannins, saponins, proteins, alkaloids and glycosides.

TLC method. It was used to observe the presence of β-carotene, chlorophylls A and B pigments, in the ivy extract sample. To achieve thin layer chromatography, some specific steps for this technique, was followed. The ivy extract sample was evaporated to concentrate

the pigments. The chromatographic tank was prepared, using a solvent mixture: hexane 70%: acetone 30%. The samples were spotted on the silica gel plate, and this was introduced into the chromatographic tank. The compound elution was stopped when it reached 2 cm from top of the plate. The spots formatted on the chromatograms, were visualized with the use of a UV lamp. The final step was to calculate R_f (retention factor) values, utilizing the equation:

$$R_f = \text{distance spot moved/distance solvent moved}$$

Antimicrobial activity. The in vitro antimicrobial activity of the extract was evaluated by the disk-diffusion and agar dilution methods. For the disk-diffusion technique the agar plates were inoculated with standardized inoculums of the test microorganism equivalent to the 0.5 McFarland standards by streaking, and after that the paper discs (about 6 mm in diameter) were placed on the agar and inoculated with 20 µl of the extract. The Petri plates were placed in incubator for 24 hours, at 35 °C for bacteria and 28 °C for the fungi. Following the incubation time, the inhibition zone sizes were measure using a ruler. In the agar dilution test, 20 µl of the extract was used by spotted on the agar: Mueller Hinton Agar for bacteria and Sabouraud for the fungal strains. The ivy extract sample was tested on *Escherichia coli* and *Staphylococcus aureus* bacteria, *Candida albicans* yeast and *Aspergillus niger* mold.

Results and discussions

The components and phytosynthesis of extracts were confirmed by modern analytical techniques (UV-VIS and FTIR spectroscopy) and by TLC (thin layer chromatography) method

UV-VIS results. UV-visible spectroscopy was used to characterize the hydroalcoholic ivy extract (Figure 3). The wavelength spectrum was registered between 250-750 nm. It was identified the wavelengths specific to flavonoids and phenolic acids at 300-350 nm. Another peak appears between 400-420 nm specific to carotenoids, the peaks between 420-460 nm and 600-650 nm are specific to chlorophyll B and the peak at 660 nm it is characteristic for chlorophyll A [LICHTENTHALER, 1987; BRITTON, 1995].

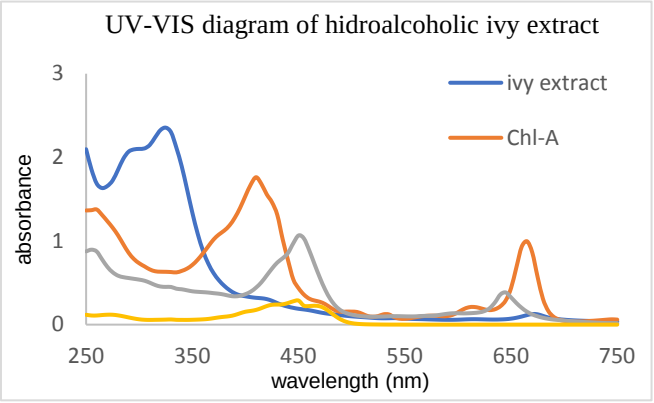


Figure 3. UV-VIS spectra of ivy leaves extract.

FTIR results. The infrared spectral analysis was carried out to characterize the type of functional group existent in ivy leaves extract. FTIR spectra (Figure 4) of the different phytocompounds in ivy extract were recorded to observe the functional groups contained in this type of plant. The spectrum (Figure 3) was recorded between 4000-600 cm^{-1}. The bands C–N, C=C and C=O are found at 1600 cm^{-1} region. The CO groups, from esters, hydroxy flavones, catechins and type II amides and the C–H bending aliphatic amine functional groups are predominant in the range of 1268-1206 cm^{-1}. The peak at 1045 cm^{-1} was associated with vibration of the CO–C bond typically found in carbohydrates.

The ivy extract exhibit weak IR bands between 814-643 cm^{-1} specific for C-N stretching vibrations of aliphatic amines or C-O stretching vibrations of alcohols or phenols, which are found due to different types of phytocomponents present in the fruit extract (polyphenols, polysaccharides and proteins) [SUICA-BUNGHEZ & ION, 2017].

At 1693 cm^{-1} are found the COO- functional groups. The peaks around 1600 cm^{-1} and 1449 cm^{-1} represents the amide II bands and these are standard protein peaks, indicating the presence of N–H in-plane bending, and the stretching vibrations of C–N and C–C. The broad band present at 3330 cm^{-1} in the ivy sample indicates that both O–H bonds and N–H vibration were present [HEMMALAKSHMI & al. 2017].

Alkanes are characterized by stretching and bending vibrations of C-H groups at 2927 cm^{-1}. The aromatic group of the amide of type I and II are observed in the region between 1387 and 1365 cm^{-1}. A narrow adsorption band at 1660–1693 cm^{-1} could be assigned to carbonyl group. Moreover, stretching vibrations of C-O groups can be detected at 1030 cm^{-1}. The presence of stretching vibrations of C=O groups suggest the structure of oleanane-type triterpenoid saponins, most likely bidesmosides [ZDARTA & al. 2019; SCHULZ & al. 2005].

At 2927 cm^{-1} are identified asymmetric stretching of –CH (CH_2) vibration. The peak around 2275 cm^{-1} is characteristic to C-C triple bond. Between 1990-1938 cm^{-1} appears carbonyl compound frequency [AROCKIA SAHAYARAJ & al. 2015].

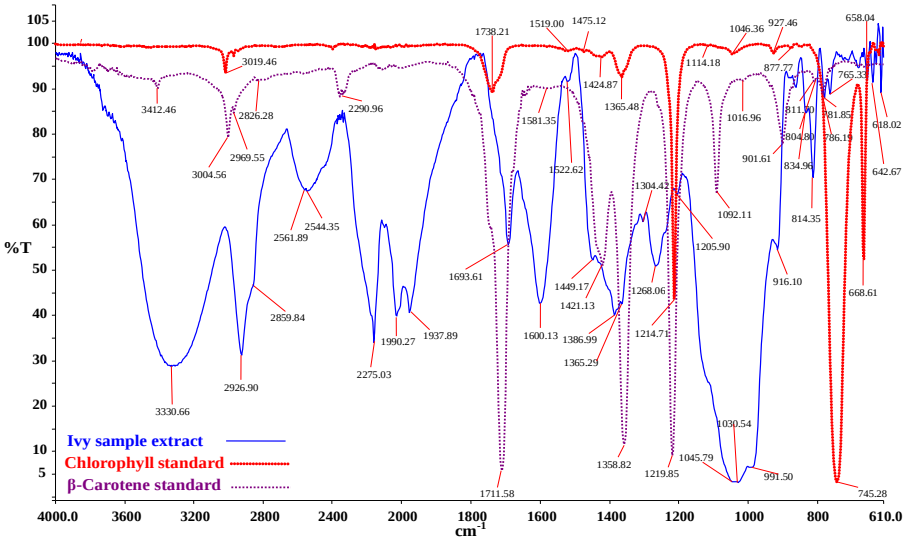


Figure 4. FTIR spectrum of ivy extract sample, chlorophyll and beta-carotene standards.

Phytochemical results are presented in Table 1.

Table 1. Phytochemical results of ivy extract sample.

Assays	Ivy extract results
Total tannins	13.5 mg/L
Total flavonoids	212.5 mg/L
Total terpenoids	107.77 mg/L
Total polyphenols	779.66 mg/L
Total carotenoids	2.586 mg/mL
Antioxidant activity	75.88%

Qualitative phytochemical results (Table 2), related the presence of saponins, tanins and terpenoids in the ivy extract sample.

Table 2. Qualitative phytochemical results of ivy extract sample.

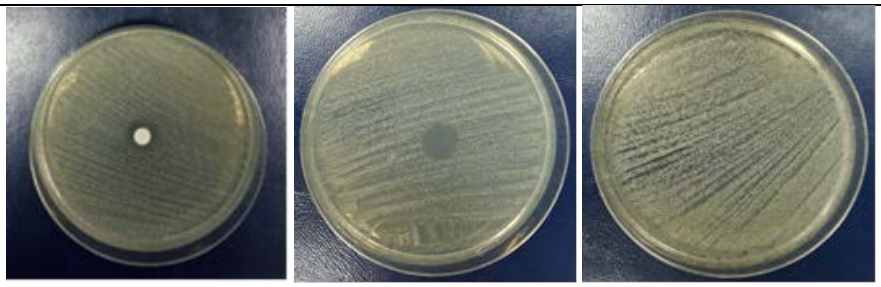
Phytochemical test	Ivy extract results (+) presence; (-) absence
Saponins	+
Tannins	+
Proteins and aminoacids (Millon's test)	-
Protein and aminoacids (copper sulphate test)	-
Steroids	-
Terpenoids	+

Thin layer chromatography (*TLC*) *analysis of the ivy extract.* The solvent mixture introduced in chromatographic tank was C_6H_{14}:C_3H_6O (70%:30%). Using a UV lamp, the spots migrated on the chromatogram were observed at 366 nm wavelength. The TLC results are represented in Table 3.

Table 3. R_f results of ivy extract sample and standards (β-carotene, chlorophyll A and B).

Sample	R_f value
Chlorophyll A	0.905
Chlorophyll B	0.908
β-carotene	0.930
Ivy extract	0.918

Antimicrobial activity. The antimicrobial activity of plant extracts is due to chemical constituents, such as alkaloids, polyphenols, saponins and essential oils [GAZDARU & ION 1994]. One of the aim of this research was to highlight the in vitro anti-bacterial and anti-fungal potential of phenolic extracts and compounds from the ivy extract. A qualitative method it was used, using blank, disc diffusion and spot. The ivy extract sample was tested on bacteria, yeast and mold. No antimicrobial activity on *Escherichia coli*, *Staphylococcus aureus* bacteria was observed. On *Aspergillus niger* mold, it was saw a slight reduction in growth, which led to the conclusion that a more concentrated sample could have a better effect. But for *Candida albicans* yeast, it was observed a good antimicrobial activity.



a. disc diffusion b. spot c. blank

Figure 5. The antimicrobial activity of the *Hedera helix* leaf extract on *Candida albicans*, a. disc diffusion, b. spot and c. blank.

Conclusions

In the present study were determinated total flavonoids, polyphenols, terpenoids, tannins, carotenoids and antioxidant activity of ivy (*Hedera helix* L.) plant. Also, the sample extract compounds were characterized using different types of analytical methods (FTIR, UV-VIS). The existence of phenolic compounds in the ivy leaves was confirmed by the Folin-Ciocalteu method. ATR-FTIR results demonstrated the major amount of carbohydrates, aminoacids, proteins, phytoingredients, hydroxyl functional groups (polyphenols) and chlorophyll and carotene pigments. TLC, also demonstrated the presence of photosynthetic pigments in ivy extract. The antioxidant capacity was measured by the free radical scavenging methods DPPH. The methanolic solutions of the ivy extract showed high antioxidant capacity (AA = 75.88%). All results of phytochemical analyses were made in triplicate and calculated using calibration curves results, with very good regression indices. The *Hedera helix* L. extract sample was tested on *Escherichia coli* and *Staphylococcus aureus* bacteria, *Candida albicans* yeast and *Aspergillus niger* mold. It was observed a law activity on *A. niger* and a better activity on *C. albicans.*

Notes on contributors

Ioana-Raluca SUICA-BUNGHEZ is a chemist, PhD, with a special interest in the phytochemical components and properties of Romanian extracts plant (ornamental and medicinal) and noble nanoparticles. Her work focuses in the UV-VIS determination and systematic characterization of the indigenous plants.

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