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Research Article
Antioxidant and Antimicrobial Properties Determination of two Varieties of Malanga: White Malanga (*Xanthosoma sagittifolium*) and Purple Malanga (*Xanthosoma violaceum*) Cultivated in Ecuador

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Abstract

Background and Objective: Malanga is a tuber introduced in Ecuador, rich in thiamin, riboflavin, flavonoids, protein, fibre and vitamins, ideal for a balanced diet, which today contributes enormously to the socio-economic development of some areas of the country. In this sense, the objective of this work was to know the antioxidant and antimicrobial properties of two varieties of malanga (White and purple) grown in Ecuador. **Materials and Methods:** For which, the extract of the two malanga varieties was obtained using two extraction methods (soxhlet and supercritical fluids "SCF"). The extracts obtained were analyzed for their antioxidant activity by the DPPH method and their antimicrobial activity by disc-plate diffusion, against strains of *Escherichia coli*, *Salmonella*, *Arcobacter* and *Staphylococcus aureus.* Gas chromatography analysis coupled to a Mass Spectrometer (to know the bioactive compounds that act) was performed. **Results:** After these analyses, the SCF extracts showed higher antioxidant activity. With respect to antimicrobial activity, no values greater than 8 mm in halo size were found in any of the extracts, however, it was in the *S. aureus* strain that there was the greater activity of SCF extracts against the pathogen. After chromatography, the most representative compounds were phthalic acid, bis (2-ethylhexyl), linoleic acid, palmitic acid, linolein, 2-mono, furfural, pyranone, all of these together act as antioxidants, anti-inflammatory and antibacterial. **Conclusion:** Finally, it was concluded that malanga extracts by SCF could act as inhibitors only against Gram-positive bacteria.

Key words: Malanga, *Xanthosoma sagittifolium*, *Xanthosoma violaceum*, antioxidant, antimicrobial

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The tubers are generally underground, accumulating starch in the roots as their main reserve substance, in which they provide nutrients, minerals and are also rich in vitamin E, ideal for a balanced diet, acting as antioxidants that protect us against free radicals, tubers are part of the diet, especially in rural communities, contributing to their socioeconomic development[1]. Malanga is a little-known tuber that was introduced in Ecuador in 1995, as it is a tropical species, it adapted perfectly to the Amazon region, with the province of Sucumbíos having the largest plantation and being exported to other countries, especially to United States[2].

According to Milián-Jiménez[3], malanga is an annual herbaceous plant that behaves perennially if not harvested, this species belongs to the monocotyledons within the family of edible herbaceous *Araceae* and it is produced in the tropical areas of the world. Currently, in Ecuador, two varieties of malanga are produced or also known as taros (malanga) which, due to their physiological, adaptable and nutritious characteristics, the first is white malanga (*Xanthosoma sagittifolium*), from the Antilles and the second is lilac or purple malanga (*Xanthosoma violaceum*) of Asian origin[4].

The malanga tuber has a high content of thiamine, riboflavin and flavonoids, constituting the daily intake of phenolic antioxidants, it also has proteins, fibre, vitamins A, C, Calcium and a high percentage of Phosphorus, the phytosterols present in the malanga inhibit the absorption of bad cholesterol (LDL) in the body[5]. Malanga contains high concentrations of polyphenols, flavonoids, condensed tannins and phytic acid that may provide antioxidant activity[6].

In addition, the tubers have the inhibitory capacity, elimination of fungi, microorganisms, pathogenic bacteria that cause foodborne illnesses, because it has an antibiotic effect against bacteria, such as *Escherichia coli*, *Staphylococcus*, *Salmonella*, among other bacteria, as well as the fungus *Candida albicans* and other microorganisms that cause foodborne disease[7,8]. In the case of the *Arcobacter* genus, these are not part of the intestinal flora and humans can become infected through the consumption of food of animal origin or contaminated water[9]. In such a way that one of the guidelines recommended by the WHO has been to improve initiatives to promote research for the development of new drugs and the improvement of existing ones, especially the generation of natural drugs, many of these are based on vegetable extracts and oils obtained using techniques such as Soxhlet, distillation and supercritical fluids[10,11].

Considering everything described, the object of the study was to determine the antioxidant and antimicrobial properties of two varieties of malanga (*Xanthosoma sagittifolium* L. Schott), white malanga (*Xanthosoma sagittifolium*), purple malanga (*Xanthosoma violaceum*).

MATERIALS AND METHODS

Research location: The present investigation was carried out in the Research Laboratory of the State University of Bolivarduring the months of January to December, 2021.

Collection of raw material: The white malanga and the purple malanga were collected in the community of San Luis de Ininkis, of the Ethnocultural Agricultural Association (ANENT) belonging to the parish Sevilla Don Bosco, canton Macas, province of Morona Santiago.

Study factors: The present investigation considered two factors of study: Factor A, varieties of tubers and factor B, Extraction Methods (Extraction by Soxhlet and Supercritical Fluids) in Table 1.

Description of obtaining the flour malanga reception, heavy, selection (The malanga corms of uniform size and without bruises were selected), Washing (to separate the remains of earth and roots), Bare, Sliced (2 mm thick), Deep freeze (-80°C), Lyophilization (initial drying lasting 10 hrs at a pressure of 0.030 mbar and a temperature of -57°C and the final drying lasted three days at the same conditions, finally grinding.

Obtaining extracts extraction by the Soxhlet method: To obtain the extracts of the white malanga and the purple malanga, Soxhlet equipment was used, which consists of a heating blanket, a 1000 mL balloon, an extractor body, a cooling body with its inlet and water outlet connected with silicone hoses and universal support that is holding all the equipment.

Extraction by the method of supercritical fluids (SCF): Obtaining the extracts of the two varieties of the tuber under study was carried out in a Helix SFE system, which consists of

Table 1: Study factors

Factors	Code	Levels
Varieties of tubers	A	a_1: White malanga (*Xanthosoma sagittifolium*)
		a_2: Purple malanga (*Xanthosoma violaceum*)
Extraction methods	B	b_1: Extraction by Soxhlet
		b_2: Extraction by supercritical fluid (SCF)

a CO_2 solvent tank, Linde SA with 99.95% purity, a CO_2 pump module/control module and a module that contains the vessel, finally, the pump that helps to inject the solvent.

Determination of antioxidant capacity: The antioxidant activity was analyzed by the free radical method 2.2-diphenyl-1-picrylhydrazyl (DPPH), this radical is susceptible to react with antioxidant compounds through a process characterized by the session of a hydrogen atom provided by the antioxidant agent. To determine the antioxidant capacity, a calibration curve was first made with a solution of the reference antioxidant Trolox in a concentration range of 0-800 µmol L^{-1}. The 1450 µL of DPPH plus 50 µL of the sample of the extract diluted in DMCO were taken and carefully homogenized, leaving it to stand for 30 min in the dark, then the samples were read at a wavelength of 517 nm in the UV spectrophotometer (NanoDrop). The results are expressed in µmoL trolox g^{-1} dry sample, the calculation was made using the following expression recommended by Singh *et al.*[12]:

$$Absreal = Bl_M\text{-}Abs_M$$

Correction of the actual absorbance of the sample for DPPH

$$DPPH = \frac{Abs - b}{a} \times \frac{V \times DF \times 100}{WS \times 1000}$$

Where:

Abs = Absorbance
a and b = Slope of the Trolox calibration curve and the cut-off point
V = Total volume
WS = Weight of the sample in gram
DF = Dilution factor

Determination of antimicrobial activity: For the antimicrobial activity, the diluted extracts in DMCO of the flours of the tubers understudy extracted by the Supercritical Fluids method were used and it was evaluated with a gram-positive battery (*Staphylococcus aureus*) and three gram-negative bacteria (*Arcobacter* sp., *Escherichia coli* sp. and *Salmonella* sp.), strains previously characterized and conserved in the Bank of Microorganisms of the Laboratory of Molecular Biology of the State University of Bolívar.

Bacterial resuscitation: To resuscitate the *Arcobacter* sp., strain, three isolates were selected and the blood agar medium was introduced, the *Escherichia coli* sp., strain in the nutrient agar medium, the *Salmonella* sp., strain in the XLD agar medium (Xylose, Lysine, Deoxycholate) and the *staphylococcus aureus* strain were revived in trypticase soy broth. Finally, they were incubated at 37°C for 24 hrs under aerobic conditions, except for *Arcobacter* sp., which, being a microaerophilic bacterium, requires controlled conditions (10% CO_2, 5% O_2 and 85% N_2) and was incubated for 48 hrs.

Preparation of the inoculum and antimicrobial activity by disk-plate diffusion method: For this analysis, bacterial cultures in the growth phase were used to prepare a bacterial suspension of each strain until a 0.5 McFarland scale was achieved. Subsequently, using a sterile swab, seeding was carried out on Müeller Hinton (MH) Agar plates (Pronadisa, 1058.00, USA), it was kept at rest for 15 min and then the blank discs were immersed in the extracts of malanga obtained. Finally, with the help of a sterile cloth, the discs were placed on the surface of the MH agar.

For supercritical fluid extracts, blank discs were immersed in diluted extracts of 5, 10 and 20 mg of extract diluted with 400 µL of Dimethyl Sulfoxide. In parallel, Levofloxacin disks were tested as a control. Finally, the plates were incubated under controlled conditions at 37°C for 24 hrs. After incubation, the diameters of the inhibition zones of the discs were measured. The results obtained were interpreted according to the criteria of the Clinical Laboratory and Standards Institute.

Determination of the chemical composition by Gas Chromatography The detection of compounds was carried out by Gas Chromatography Coupled to a Mass Spectrometer (GC-MS) in Agilent Technologies equipment (7890A GC system and 5975C inert XL MSD with triple-axis detector). An HP-5MS capillary column (30 m × 250 µm, 0.25 µm) with phenylmethyl polysiloxane (0.25 µm film thickness) as stationary phase and helium as carrier gas (0.8 mL min^{-1}) was used. The 1 µL of the derivatized sample (previously filtered) was injected in Split mode using the 1:20 ratio. The injection chamber temperature was 250°C. The oven temperature was maintained from 60-80°C with a 5°C min^{-1} ramp, then increased to 92°C with a 3°C min^{-1} ramp for 5 min, then increased to 165°C at a rate of 4°C min^{-1} and finally at 290°C at a rate of 2°C min^{-1} for 2 min, 70°C for 2 min and increased to 300°C at 5°C min^{-1} with a waiting time of 6 min. The compounds were identified by comparison with the mass spectra of the NIST 2011 library. The mass range used was between 40-550 Daltons.

Statistical analysis: The completely random design (DCA) was applied in a factorial arrangement A × B (2 × 2) with three repetitions. Also, the test of least significant difference (LSD), to determine if the corresponding treatments are significantly different.

RESULTS AND DISCUSSION

Volume and mass of extracts obtained: Soxhlet and supercritical fluid (FSC) methods were analyzed from malanga samples, whereby Soxhlet a larger volume was obtained in purple malanga with 10.7 mL and only 3.3 mL in white malanga in Table 2. This difference is due to its state of maturation and the numerous volatile compounds. Díaz *et al.*[13], in their research, obtained 1.1 mL of hexanic extract from the species *Xanthosoma maximiliani* Schott. While Guadalupe and Magali[14], in his study of Andean tubers obtained 55 mL of ethanolic extract in melloco and 21 mL of ethanolic extract in mashua. These comparisons were made because no bibliographical references to malanga extracts were found. On the other hand, concerning the extracts obtained utilizing FCS, greater mass was given by purple malanga with 1.24 g and white with 1.08 g, as can be seen in Table 2, this difference can be attributed to the starch, the variety of the tuber, to the state of maturation of the same. Regarding the extraction of the extracts by SCF of the malanga, no bibliographical references were found, therefore, the current study was the first to carry out extractions of said tubers by the mentioned method.

Table 2: Volume and mass of extracts obtained

Type of sample	Volume extracted from the rota-evaporator (mL)
Soxhlet extraction of the two tuber varieties	
White malanga	3.3
Purple malanga	10.7
Sample	Extract quantity (g)
Supercritical fluid extraction	
White malanga	1.0760
Purple malanga	1.2372

Table 3: Results of the antioxidant activity content of the DPPH method

	Tuber variety	
	White malanga	Purple malanga
Extraction methods	Sample (μmoL of trolox g^{-1})	
Soxhlet	4.61	11.32
SCF	207.87	167.64

Analysis of antioxidant activity, radical capture (DPPH): This method is used to determine the antioxidant capacity of foods and synthetic compounds, the DPPH radical, this free radical is capable of reacting with antioxidant compounds through a process characterized by the transfer of a hydrogen atom provided by the antioxidant agent.

After the analysis of antioxidant activity, a greater DPPH radical scavenging effect was evidenced by the SCF method with values of 207.8 μmoL of trolox g^{-1} sample in white malanga and 167.6 in purple malanga, being much higher than those obtained by the Soxhlet method in Table 3.

Moncayo *et al.*[15], analyzed the antioxidant capacity of ethanolic extract from leaves of 18 species of plants native to western Ecuador, in which Xanthosoma sagittifolium presents a percentage of 44.99 I% of antioxidant activity by the DPPH method. On the other hand, Kim *et al.*[16], carried out a study on the antioxidant capacity with the DPPH method of extract of *Colocasia esculenta* and identified a value of 4.52 μmoL of trolox g^{-1} sample, a value that was considerably lower than that obtained in this work, where the higher the value the higher the antioxidant capacity. The results obtained in this research differ significantly for each variety of tuber, this can be attributed to the extraction method, the analysis performed on starch, the type of tuber and the climatic conditions. As no previous work on the antioxidant activity of malanga was found, it was contrasted with work carried out on tubers of the same order and family.

Analysis of antimicrobial activity

Antimicrobial activity against *Escherichia coli*: The results obtained from the antimicrobial activity of the extracts obtained were expressed by measuring the diameter of the inhibition halo in mm against *E. coli* strains. Where in Table 4 inhibition halos of malanga extracts by supercritical fluids can be seen ranging from 2-4.3 mm in halo size, these values are much lower than those recommended by Ponce *et al.*[17], since this author considers that there is an antimicrobial effect of extracts and vegetable oils, this must be greater than or equal to 8 mm, however it is important to consider that the extract of the purple malanga at a dilution of 1:2 presented a greater inhibitory effect against the Cer3C1 strain with a value of 4.3 mm, concerning the control antibiotic (levofloxacin), the inhibition halo being considerably greater compared to the extracts. On the other hand, Sánchez-Bautista *et al.*[18], in the interpretation of sensitivity, considered the percentage of levofloxacin antibiotic sensitivity of 67.8% in *E. coli* bacteria. Pérez-Delgado *et al.*[19], in its antibiogram manual, discloses the inhibitory halos of *E. coli* against levofloxacin from 27-33 mm in diameter. It is important to emphasize that this is the first work on the antimicrobial activity of malanga extracts.

Antimicrobial activity against *Salmonella* sp.: The following table details the antimicrobial action against the extracts obtained by SCF, at different concentrations. After this analysis, Table 5 shows a poor antimicrobial effect of the extracts obtained against *Salmonella* strains, with halo size

Table 4: Inhibition halos of extracts of the two malanga varieties at different dilutions against *Escherichia coli* strains

	Extraction by SCF				
	White malanga		Purple malanga		
	Dilutions (mg mL^{-1})				Control antibiotic
Escherichia coli strains	1:2	1:4	1:2	1:4	Levofloxacin
Cer3C1	3.3	4	4.3	4	28
Res2C1	2.7	3.3	3.7	3	28
Cer2C1	2	3.3	2.3	3	28

Table 5: Inhibition halos of extracts of the two malanga varieties at different dilutions against *Salmonella* strains

	Extraction by SCF				
	White malanga		Purple malanga		
	Dilutions (mg mL^{-1})				Control antibiotic
Salmonella strains	1:2	1:4	1:2	1:4	Levofloxacin
S7	0.7	0.7	0.7	0.7	11.3
5M3	0	0	0	0	11.3
S8	2	1	1	1	28

Table 6: Halos of inhibition of extracts of the two varieties of malanga at different dilutions against *Arcobacter* strains

	Extraction by SCF				
	White malanga		Purple malanga		
	Dilutions (mg mL^{-1})				Control antibiotic
Arcobacter strains	1:2	1:4	1:2	1:4	Levofloxacin
Q1BC1	3.3	2.7	1.3	1.3	30
Q3NC2	1.3	2.7	1	1	16
Q1NC1	1.7	1.7	2.7	1.3	13

values of 0-1 mm. However, the Minimum Inhibitory Concentration (MIC), according to the Clinical Laboratory Standard Institute (CLSI) mentioned by Humphries *et al.*[20] considers that the Enterobacteriaceae family was resistant to the antibiotic levofloxacin when the inhibition halo is $\leq$16 mm in diameter. Similarly, Sfeir[21] considers levofloxacin resistant if the inhibition halo is $\leq$13 mm in diameter against the Enterobacteriaceae family, in such a way that it can be defined that the S7 and 5M3 strains were resistant to the drug.

Antimicrobial activity against *Arcobacter* sp.: In Table 6, the results of the inhibition halos were presented and low antimicrobial activity against *Arcobacter* can be observed, where halo size values of 1-3.3 mm are evidenced, being the 1:2 dilution the one that showed the greatest effect against the Q1BC1 strain, however, these values are very low compared to what is recommended by Ponce *et al.*[17]. On the other hand, works such as the one developed by Goyes[22], in his research on the susceptibility of the different *Arcobacter* strains, reveal the resistance of the bacteria to levofloxacin with a halo $\leq$14 mm in diameter, Humphries *et al.*[20], in the table of cut-off points for the interpretation of MICs and zone diameters, it mentions that the genus *Campylobacter* is susceptible with a halo $\geq$26 mm to the group of fluoroquinolone antibiotics in which levofloxacin is immersed. The interpretation of the results was carried out using the recommendations of the European Committee for Susceptibility Testing according to EUCAST and the CLSI[20]. In this sense, the Q1NC1 strain showed resistance to the reference antibiotic.

Antimicrobial activity against *Staphyloccocus aureus*: The results obtained from the antimicrobial action of the extracts of the two varieties of the tuber against the gram+*S. aureus* bacteria were expressed by measuring the diameter of the inhibition halo. In this analysis, the 1:2 dilution of the purple malanga extract presented the best effect with a halo size of 7 mm, followed by the white malanga extract in the 1:2 dilution with a 6.7 mm halo in Table 7, these results were quite close to the 8 mm recommended by Ponce *et al.*[17].

Table 7: Halos of inhibition of extracts of the two varieties of malanga at different dilutions against *Staphyloccocus aureus*

	Extraction by SCF				
	White malanga		Purple malanga		
	Dilutions (mg mL^{-1})				Control antibiotic
Staphylococcus aureus strain	1:2	1:4	1:2	1:4	Levofloxacin
Staphylococcus aureus	6.7	5.3	7	6	26

The extract of the white malanga in the different dilutions presents values greater than 5 mm in halo size. EUCAST for *S. aureus* reports that the halo $\leq$22 mm in diameter is resistant to the antibiotic levofloxacin and is susceptible when the inhibition halo is $\geq$50 mm, while CLSI, reported that inhibitory halos of *S. aureus* are 25-30 mm in diameter compared to levofloxacin. Concerning the antimicrobial activity of malanga, no reports were found in the literature, so it is the first investigation of the antimicrobial action of these tubers against the *S. aureus* bacteria. In short, the extracts of the purple and white malanga have low antimicrobial action compared to the control antibiotic used.

Identification of compounds present by gas chromatography (GC-MSD) in purple malanga extract obtained by SCF: After the chromatographic analysis of the obtained extracts, it is important to highlight that some beneficial compounds for health were identified in this work, among these we have: it is, Phthalic acids, bis (2-Ethylhexyl) ester with an area of 36.96% being an acid Phthalic, continuously with the Linoleic acid 13.31% being an essential fatty acid type compound, the Palmitic acid with an area 9.75% forming part of the essential fatty acid, the Linolein, 2-mono with an area 6.65% forming part of the fatty acids and finally Furfural with an area of 0.375 forming an aldehyde-type compound.

The Pyranone compound, which was identified in the extract of the white malanga, was also identified by Zakaria *et al.*[23], in the ethanolic extract of the fresh leaves of *Pandanus amaryllifolius*, a tropical plant from Southeast Asia, the aforementioned compound has a high capacity to act as an antioxidant in volatile substances and has been much discussed in recent years. The Linoleic acid compound was identified by Jiménez *et al.*[24], in the mashua *Tropaeolum tuberosum*, whose function is to be anti-inflammatory, in addition, it helps prevent coronary diseases, inhibiting angiogenesis, developing a cytotoxic activity in tumour cells. The Furfural compound was also identified by van der Maas *et al.*[25], this allows to inhibit microbial growth.

CONCLUSION

After this investigation, conclude that the extracts of purple malanga obtained by supercritical fluids can be used as a natural food additive, especially for its antioxidant and antibacterial effect, especially in the Gram-positive group, demonstrated especially by the presence of Furfural, Linoleic acid and Pyranone.

SIGNIFICANCE STATEMENT

This study determines the parallel antioxidant and antimicrobial effect of white and purple malanga extracts, they can be a natural alternative to treat bacterial infections, as well as their broad antioxidant benefit, in such a way that it will be possible in the future to obtain biopharmaceuticals.

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