# Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2022.321.327

# Research Article
# Antimicrobial and Antioxidant Properties of *Tropaeolum tuberosum* Extracts from Ecuador

[1]Freddy Bayas-Chacha, [1]Maribel Bermeo-Sanchez, [2]Byron Herrera-Chavez and [1]Favian Bayas-Morejon

[1]Agroindustrial Engineering Career, Biotechnological Research and Development Center, State University of Bolivar, Guaranda EC020150, Ecuador
[2]Animal Production and Industrialization Research Unit, Engineering Faculty, National University of Chimborazo, Riobamba EC060150, Ecuador

## Abstract

**Background and Objective:** Nowadays, there is a growing interest in the use of natural antioxidants and antimicrobials for the treatment of diseases and this is where Andean tubers are playing an important role. Whereby, the objective of this work was to study the antimicrobial and antioxidant properties of *Tropaeolum tuberosum* extracts. **Materials and Methods:** In the present investigation, the extracts of 2 varieties of mashua were studied, white and yellow (*Tropaeolum tuberosum*), the extracts of the tuber were obtained by soxhlet and Supercritical Fluids (SCF) techniques, to these extracts were analyzed for their antioxidant activity by ABTS method and antimicrobial by disc-plate diffusion on 3 bacterial strains: *Salmonella* spp., *Escherichia coli* and *Arcobacter* spp. The extracts were characterized by mass coupled gas chromatography (GC-MS). **Results:** After the analysis of the antioxidant activity, the highest concentration presented the white mashua extract by supercritical fluids with a value of 6596.10 µmol trolox/g. In antimicrobial activity, the best inhibitory effect was evidenced on *Salmonella* strains with a halo diameter $\geq$8 mm, especially in the extract obtained from FSC, highlighting the white mashua (with an inhibition diameter of up to 20 mm). Through GC-MS, between 21 and 11 compounds were identified, where the compounds with the largest area were: Campesterol, β-Sitosterol, γ-Sitosterol, 4-Methoxybenzyl isothiocyanate and α-Tocopherol, which have been described as having antioxidant and antimicrobials effects. **Conclusion:** Consequently, it is concluded that the extracts can be used as a natural food additive.

Key words: Mashua, antioxidant activity, antimicrobial effect, SCF, GC-MS, campesterol, disco-plate diffusion

Citation: Chacha, F.B., M.B. Sanchez, B.H. Chavez and F.B. Morejon, 2022. Antimicrobial and antioxidant properties of *Tropaeolum tuberosum* extracts from Ecuador. Asian J. Plant Sci., 21: 321-327.

Corresponding Author: Favian Bayas-Morejon, Agroindustrial Engineering Career, Biotechnological Research and Development Center, State University of Bolivar, Guaranda EC020150, Ecuador



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

In recent years, there is a growing interest in the use of antioxidants and antimicrobials for the treatment of diseases, as well as their important role in preventing the development of some pathologies. Antimicrobial resistance has generated new interest in the search for drugs with antimicrobial effects, linking products of plant origin that have sources of bioactive molecules with antimicrobial properties. Globally, around 250 causative agents of foodborne diseases (ETAS) have been described and many of them are related to pathogenic bacteria with high resistance to antibiotics for clinical use, some of these show a high incidence, such as *E. coli*, *Salmonella* spp., *Staphylococcus aureus*, *Clostridium* spp., *Listeria monocytogenes*, in fact, *Salmonella* spp. and *Escherichia coli* have been detected with a high effect of resistance to antibiotics[1,2]. In the case of the *Arcobacter* genus, they are not part of the intestinal flora and humans can become infected due to the consumption of food of animal origin or in water contaminated with the pathogen[3].

Therefore, one of the guidelines recommended by the who has been to improve initiatives to favour research for the development of new drugs and the improvement of existing ones, especially the generation of natural drugs[4].

The antioxidant and antimicrobial capacity of foods depends on their nature and concentration of antioxidant compounds, which varies within the same family of the plant kingdom. This explains that different foods differ in their ability to prevent chronic diseases not transmissible to oxidative stress[4,5].

In our research, we have focused on mashua (*Tropaeolum tuberosum*), which is a tuber of the Tropaeolaceae family, native to the central Andes, which, compared to potatoes, melloco and goose, has a higher antioxidant capacity, with a high content of anthocyanins, carotenoids, proteins, carbohydrates, fibre, ascorbic acid (vitamin C) and calories. This tuber also has a high concentration of aromatic glucosinolates that when hydrolyzed are transformed into isothiocyanates, these components are known for their antibiotic, insecticidal, nematicidal and anticancer properties[6,7].

Consequently, several researchers are recommending the use of natural agents such as extracts and vegetable oils as an alternative to deal with problems of cellular oxidation and bacterial resistance[4].

In the present investigation, the antioxidant and antimicrobial activity of the extracts of 2 varieties of mashua, obtained using soxhlet and supercritical fluids, was evaluated.

## MATERIALS AND METHODS

**Study area:** This study was carried out from January-August, of 2021. Where, the raw material for obtaining the extracts of "Yellow Mashua" was obtained from the Guaranda, Salinas parish and "White Mashua" from the Ambato, Quisapincha parish. Later these were transferred to the facilities of the Research laboratories of the State University of Bolivar.

**Study factors:** The present investigation will consider 2 factors, factor A, varieties of tubers, factor B, Extraction methods (extraction by soxhlet, extraction by supercritical fluids), according to the following description Table 1. For which, a block design with factorial arrangement A×B was applied, each experimental unit is formed from 2000 g. It should be noted that the mashua samples were previously lyophilized.

**Obtaining extracts using soxhlet:** Ten grams of each sample were weighed into the thimbles, 2 balloons of 1000 mL were placed on the warming blankets, 400 mL of methanol was added to each 1000 mL balloon, thimbles were placed in each extractor body, the solvent was added in the corresponding balloon, then the soxhlet equipment was turned on considering the water reflux, the most suitable temperature (60°C), It was expected to perform 7 refluxes, in which we obtained an extract from each matrix. For the preparation of samples of soxhlet extracts, a 1:72 dilution was carried out (25 µL of sample+1800 µL of methanol in a 2 mL volume Eppendorf tube in the amount 1225 µL ABTS+75 µL Extract.

**Obtaining extracts using supercritical fluids:** The extraction is carried out in a Helix SFE system, which consists of a solvent tank ($CO_2$, Linde SA with 99.95% purity), a $CO_2$ pump module/control module and a vessel or base module. In each extraction, the container is loaded with 15 g of ground sample, it was worked at constant temperature and pressure considering a static time of 30 min and a dynamic time of 2 hrs. The extractions were carried out at a temperature of 50°C and a pressure of 200 Bar. With a volumetric flow rate of $CO_2$ of 1L ±0.5 L $min^{-1}$. The extract was collected in a 50 mL vial and stored at -10°C. Each of the tests was performed 7 times. For the preparation of the extracts by supercritical fluids of the 2 varieties of mashua, a 1:80 dilution (25 µL of sample+2000 µL Dimethyl Sulfoxide) was made in an Eppendorf tube with a volume of 2 mL, in an amount of

Table 1: Study factors

| Factors | Code | Levels |
|---|---|---|
| Varieties of tubers | A | $a_1$ : Yellow mashua |
| | | $a_2$ : White mashua |
| Methods | B | $b_1$ : Soxhlet extraction |
| | | $b_2$ : Supercritical fluid extraction |

1425 µL of ABTS+75 µL of extract. A NanoDrop mini photometer was used to perform the readings, which was previously configured for reading at 734 nm.

**Method for the determination of antioxidant capacity (ABTS test):** Total antioxidant activity test. Total antioxidant activity was measured according to the method described by Hsu *et al.*[8]. For which, dilutions of ABTS and buffer were prepared.

**Determination of volatile compounds by EM gas chromatography:** The detection of compounds was carried out by Gas Chromatography Coupled to a Mass Spectrometer (CG-MS) in Agilent Technologies brand equipment (7890A GC and 5975C inert XL MSD system with triple-axis detector). An HP-5MS capillary column (30 m×250 µm 0.25 µm) was used with phenyl methyl polysiloxane (0.25 µm film thickness) as stationary phase and helium as stripping gas (0.8 mL $min^{-1}$). One µL of the derivatized (previously filtered) sample 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 ramp of 5°C $min^{-1}$ then increased to 92°C with a ramp of 3°C $min^{-1}$ for 5 min, then increased to 165°C at a rate of 4°C $min^{-1}$ and finally at 29°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 UMA or 40-550 Dalton.

**Antimicrobial activity by diffusion disk plate**

**Bacterial reactivation:** In the present investigation, we worked with isolates of *Arcobacter*, *Salmonella* and *Escherichia coli* conserved in the Bank of Microorganisms of the Laboratory of Molecular Biology of the State University of Bolivar.

**Reactivation of *Arcobacter* spp. isolates:** Ten *Arcobacter* strains were selected on blood agar (*Arcobacter* broth+bacto agar+5% lamb's blood) and it was incubated at 37°C for 24-48 hrs under microaerophilic conditions (10% $CO_2$, 5% $O_2$ and 85% $N_2$).

**Reactivation of *Escherichia* spp. isolates:** Six *Escherichia* isolates were selected, for which, nutrient agar boxes (NutriAgar) were prepared and left to incubate at 37°C for 24 hrs under aerobic conditions.

**Reactivation of *Salmonella* spp. isolates:** Four *Salmonella* isolates were selected, for this, XLD agar boxes (Xylose Lysine Deoxycholate) will be prepared and then these were incubated in aerobiosis at 37°C for 24 hrs.

**Inoculum preparation and antimicrobial activity using the Kirby Bauer method (disk-plate diffusion):** From the cultures in an exponential growth phase, a bacterial suspension of each strain was prepared in 10% saline water up to the 0.5 McFarland scale. Subsequently, with the use of a sterile swab, the seeding was carried out in Müller Hinton Agar (MH) plates (Pronadisa, 1058.00, USA) in a homogeneous way. After a few minutes at rest and with the help of sterile forceps, the discs were placed on the surface of the agar. Previously, the blank discs were immersed in mashua extracts obtained by the soxhlet method[9]. On the other hand, for supercritical fluid extracts the blank discs were immersed in diluted extracts of 5, 10 and 20 mg of extract diluted with 400 µL of Dimethyl Sulfoxide. Moreover, Ciprofloxacin, Streptomycin and Penicillin discs were tested as a control. Finally, the plates were incubated under conditions under controlled atmospheres at 37ºC for 24 and 48 hrs as appropriate. After the incubation time, the diameters of the zones of inhibition of the discs were measured. The results were interpreted according to the criteria established by Clinical Laboratory and Standards Institute (CLSI 2010, M45-A2).

## RESULTS AND DISCUSSION

**Antioxidant activity:** The ABTS method is based on the oxidation of ABTS with potassium persulfate to become a free radical ABTS+ in which it gives us a greenish gum colour, that is, while there is the presence of antioxidant activity in the samples, they discolour or the radical is eliminated free ABTS+.

**Results of ABTS antioxidant activity:** The variety that shows the lowest concentration of antioxidant capacity was the yellow mashua extract by the soxhlet method with a value of 76.30 µmol $g^{-1}$, on the other hand, the white mashua extract by the soxhlet method presented a value of 101.66 µmol $g^{-1}$ in Table 2. The antioxidant capacity of the yellow mashua extract by supercritical fluids showed a value of 5732 µmol $g^{-1}$

Table 2: Absorbance and antioxidant activity values

| Varieties | Number of soxhlet reads 1 | 2 | 3 | Mean | S | Unit µmol Trolox/g |
|---|---|---|---|---|---|---|
| Yellow mashua | 0.30 | 0.13 | 0.13 | 0.13 | 0.00 | 76.43 |
| White mashua | 0.27 | 0.25 | 0.27 | 0.26 | 0.012 | 101.66 |
| | Number of supercritical fluid reads | | | | | |
| | 1 | 2 | 3 | | | |
| Yellow mashua | 0.51 | 0.50 | 0.52 | 0.51 | 0.010 | 5732.10 |
| White mashua | 0.56 | 0.56 | 0.57 | 0.56 | 0.006 | 6596.10 |

Table 3: Antimicrobial activity of the extracts against *Arcobacter*

| | Halos of inhibition antimicrobial activity mm diameter | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Supercritical fluids | | | | | | | | |
| | | | Concentrations (mg µL$^{-1}$) | | | | | | | | |
| | Soxhlet | | Yellow mashua | | | White mashua | | | Control antibiotics | | |
| Extracts | Yellow mashua | White mashua | 5 | 10 | 20 | 5 | 10 | 20 | Ciprofloxacin | Penicillin | Streptomycin |
| *Arcobacter* Q 14 C1 | 2 | 2 | 18 | 9 | 9 | 4 | 20 | 10 | 27 | 17 | 31 |
| *Arcobacter* Q3 C1 | 0 | 0 | 7 | 7 | 6 | 7 | 11 | 16 | 29 | 14 | 30 |

and the extract of the white mashua extracted by supercritical fluids gave us a value of 6596.10 µmol g$^{-1}$, these being the ones that showed the highest value. The work developed by Guevara-Freire *et al.*[10], on the antioxidant capacity in different varieties of mashua presented values 16.20-45.70 µmol equivalent of Trolox/g ABTS, when comparing with those obtained in our experiment, it can be said that ours exceed the values reported by the authors, while in work of Inostroza *et al.*[11], the antioxidant capacity equivalent to Trolox obtained was 15.80±0.2 µmol g$^{-1}$ PF, applying the DPPH test and 17.0±0.2 µmol g$^{-1}$ PF with ABTS. In addition, Peñafiel[12] reported the different mashua accessions evaluated, the FRAP activity ranged from 376.89-2327.18 mM $Fe_{2+}$ 100 g$^{-1}$ FW, while the DPPH radical scavenging activity varied from 376.89-68.25±1.80 µM TEAC 100 g$^{-1}$ FW. With the previously mentioned, the differences are due especially to the methodology used. When performing the statistical comparison, it can be considered that there is a statistical difference between the treatments, where the $A_2B_2$ treatment (extract of white mashua by SCF) presented a better antioxidant effect, followed by the $A_1B_2$ treatment (extract of yellow mashua by SCF), where it is evidenced that the FSP method is the best.

**Results of antimicrobial activity:** To comply with the 4th objective of our research, the extracts obtained from the 2 varieties of mashua previously described were evaluated for antimicrobial activity using the plate disk diffusion method against the bacterial strains, *Arcobacter* (*Arcobacter* Q 14 C1 and *Arcobacter* Q3 C1), *Escherichia*: (*Escherichia coli* Pt 46 and *Escherichia coli* 127 C1) y *Salmonella*: (*Salmonella* Pt 42 and *Salmonella* C36).

**Antimicrobial activity in *Arcobacter* spp.:** Table 3 shows the results of the inhibition halos of the *Arcobacter* strain, where the mashua extracts by the soxhlet method showed values of 2 mm for the first strain and 0 mm for the second. On the other hand, in the extracts of supercritical fluids of yellow mashua with a concentration of 5 mg µL$^{-1}$ presented a halo of 18 mm in diameter, in terms of the concentration of 10 mg µL$^{-1}$, the inhibitory effect continues to be favourable for the first strain, being the white mashua the one with the best value with 20 mm of halo. Whereas in the concentration of 20 mg µL$^{-1}$, the effect decreased, except for the 2nd strain in white mashua extract, which was 16 mm. When comparing our results with the control antibiotics, the white mashua extract was the best with data close to the effect generated by penicillin, although far from ciprofloxacin and streptomycin. It should be noted that it is the 1st time that the antibiotic effect of mashua extracts has been studied against the pathogen *Arcobacter*, in the same way, our results are encouraging, since as established by Ponce *et al.*[13], which corresponds to a degree of susceptibility of a microorganism to agents of plant origin because the inhibition range is

Table 4: Antimicrobial activity of the extracts against *Escherichia coli*

| Halos of inhibition antimicrobial activity mm diameter | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Supercritical fluids | | | | | | | | |
| | | | Concentrations (mg μL$^{-1}$) | | | | | | | | |
| | Soxhlet | | Yellow mashua | | | White mashua | | | Control antibiotics | | |
| Extracts | Yellow mashua | White mashua | 5 | 10 | 20 | 5 | 10 | 20 | Ciprofloxacin | Penicillin | Streptomycin |
| *Coli* Pt 46 | 0 | 2 | 2 | 3 | 4 | 2 | 2 | 6 | 32 | 16 | 24 |
| *Escherichia coli* 127 C1 | 0 | 0 | 2 | 4 | 4 | 2 | 4 | 6 | 31 | 28 | 34 |

Table 5: Antimicrobial activity of the extracts against *Salmonella* spp.

| Halos of inhibition antimicrobial activity (mm ) diameter | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Supercritical fluids | | | | | | | | |
| | | | Concentrations (mg μL$^{-1}$) | | | | | | | | |
| | Soxhlet | | Yellow mashua | | | White mashua | | | Control antibiotics | | |
| Extracts | Yellow mashua | White mashua | 5 | 10 | 20 | 5 | 10 | 20 | Ciprofloxacin | Penicillin | Streptomycin |
| *Salmonella* Pt 42 | 8 | 4 | 8 | 10 | 10 | 8 | 8 | 20 | 31 | 0 | 25 |
| *Salmonella* C36 | 6 | 6 | 8 | 8 | 10 | 8 | 8 | 8 | 31 | 0 | 26 |

>8 mm, in our case even halo size results were evidenced, even close to or equal to 20 mm. Therefore, our results are comparable and equal to penicillin for clinical use.

**Antimicrobial activity in Escherichia coli:** In table 4, the results of the inhibition halos of the *Escherichia* strain are reported, where the extracts by soxhlet did not present antimicrobial activity, while the extracts of supercritical fluids the best values were obtained at a concentration of 20 mg μL$^{-1}$ in white mashua extract. However, these results are lower than those recommended to be considered with an antimicrobial agent the values obtained are not at all close to the control antibiotics. It is important to note that there are studies in which the authors also reported a low inhibitory effect of mashua extracts against *S. aureus* and *E. coli* strains[14,15]. Nonetheless, Mejía Lotero *et al.*[16], obtained a satisfactory inhibitory effect of yellow mashua extracts against strains of *Escherichia coli* with similar values to the clinical antibiotics controls.

**Antimicrobial activity in *Salmonella* spp.:** After the analysis of the antimicrobial activity in *Salmonella* isolates, it was evidenced that with the extract obtained by soxhlet only that of yellow mashua presented a better effect in 1 of the 2 strains. On the other hand, the extracts obtained by supercritical fluids, all presented a favourable anti-salmonella effect, being the white mashua extract at a concentration of 20 mg μL$^{-1}$ that presented a larger halo size, very close to the effect produced by streptomycin in Table 5. Likewise, it is important to consider that these 2 strains were resistant to penicillin, this could be because this antibiotic is considered effective against gram-positive bacteria[17]. It is important to emphasize that for these strains, the soxhlet method presented a better effect of the yellow mashua extract, also for supercritical fluids a positive antimicrobial effect is evidenced with halos greater than 8 mm in both strains, these were similar to the established by Ponce *et al.*[13], which corresponds to a degree of susceptibility of a microorganism to agents of plant origin.

The antimicrobial action presented by Chirinos *et al.*[18], reported activity in the mashua extract against *Salmonella*. Therefore, our work is the 1st to have analyzed the inhibitory effect of mashua extracts against strains of the *Salmonella* genus.

After the chromatographic analysis of the extracts obtained, it is important to note that some compounds beneficial to health were identified in the different extracts of the 2 varieties of mashua studied, among these we have: Stigmasterol, which includes campesterol, β-Sitosterol are compounds containing provitamin $D_2$, very useful for the prevention of diseases such as ovarian, prostate, breast and colon cancer[19]. Also, γ-Sitosterol is a potent $C_1$ complex inhibitor that has demonstrated its potential as a treatment for diabetes in this study tested by Rangachari *et al.*[20] and

Gutiérrez *et al.*[19]. Similarly, 4-Methoxybenzyl isothiocyanate and α-Tocopherol have been considered important agents due to their antifungal and antibacterial activity[21,22].

This implies that in the not too distant future Andean tubers could play an important role in the pharmacological industry, especially in obtaining antioxidant and antibacterial components, in the same way, in their use as food additives. Thus, it is highly recommended the development of new works that allow determining the antioxidant effect using biological models, also, it is recommended to carry out more studies of antibiotic activity of the extracts with a greater number of bacterial strains. In the present study, the economic factor limited the extension of the investigation.

## CONCLUSION

Consequently, it is concluded that the extracts of mashua black obtained by supercritical fluids can be used as a natural food additive, especially due to its antioxidant and anti-salmonella effect, demonstrated especially by the presence of Campesterol, β-sitosterol, γ-sitosterol, 4-methoxybenzyl isothiocyanate and α-tocopherol.

## SIGNIFICANCE STATEMENT

This study discovers the possible parallel antioxidant and antimicrobial effect of white and yellow mashua extracts, which can be a natural alternative to deal with certain diseases. This study will allow the researcher to consider the use of natural extracts as alternatives for their use in obtaining biopharmaceuticals. Thus, in new research, it will be possible to determine the optimal conditions for the use of natural extracts to avoid cellular oxidation and bacterial resistance.

## REFERENCES


1. Bintsis, T., 2017. Foodborne pathogens. AIMS Microbiol., 3: 529-563.
2. Caffer, M.I., C. Lucero and M. Pichel, 223. *Salmonella*, *Edwardsiella*, *Citrobacter*. In: Manual of Clinical Microbiology of the Argentine Association of Microbiology, Lopardo, H.A., S.C. Predari and C. Vay, Archivo Digital, Argentina, ISBN-13 ISBN: 978-987-26716-8-6, pp: 181.
3. Gonzalez, A. and M.A. Ferrus, 2011. Study of *Arcobacter* spp. contamination in fresh lettuces detected by different cultural and molecular methods. Int. J. Food Microbiol., 145: 311-314.
4. Bayas-Morejón, F., R. Ramón, M. García-Pazmiño and G. Mite-Cárdenas, 2020. Antibacterial and antioxidant effect of natural extracts from *Baccharis latifolia* (chilca). Caspian J. Environ. Sci., 18: 489-493.
5. Pérez, D.Q., 2017. Antimicrobial resistance: Evolution and current perspectives in the context of the "one health" approach. Rev. Cubana Med. Trop., Vol. 69.
6. Gonzales-Torre, H., I. Aliaga-Barrera and F.F. Velásquez-Barreto, 2020. Effect of chlorpropham on the sprouting and behavior of bioactive compounds in purple mashua (*Tropaeolum tuberosum* Ruíz & Pavón) during postharvest storage. Bioagro, 32: 49-58.
7. Amaral, A.B., M.V. da Silva and S.C. da Silva Lannes, 2018. Lipid oxidation in meat: Mechanisms and protective factors-a review. Food Sci. Technol., 38: 1-15.
8. Hsu, H.K., Y.C. Yang, J.H. Hwang and S.J. Hong, 2003. Effects of *Toona sinensis* leaf extract on lipolysis in differentiated 3T3-L1 adipocytes. Kaohsiung J. Med. Sci., 19: 385-389.
9. Okonkwo, C.O. and O.C. Ohaeri, 2020. Comparative study of steam distillation and soxhlet for the extraction of botanical oils. Asian J. Biol. Sci., 13: 62-69.
10. Guevara-Freire, D.A., L. Valle-Velástegui, M. Barros-Rodríguez, C. Vásquez, H. Zurita-Vásquez, J. Dobronski-Arcos and P. Pomboza-Tamaquiza, 2018. Nutritional composition and bioactive components of mashua (*Tropaeolum tuberosum* Ruiz and Pavón). Trop. Subtropical Agroecosyst., 21: 53-68.
11. Inostroza, L.A., A.J. Castro, E.M. Hernández, M. Carhuapoma, R.A. Yuli, A. Collado and J.S. Córdova, 2015. Antioxidant activity *Tropaeolum tuberosum* Ruiz & Pavón (mashua) and its application as colorant for yogurt. Sci. Res., 18: 83-89.
12. Peñafiel, L. and A. Castro, 2017. Phenolic compounds, *in vitro* antioxidant and anticolagenase activities of the lyophilized extract of *Tropaeolum tuberosum* Ruiz & Pavón, "black mashua". Científica, 14: 28-38.
13. Ponce, A.G., S.I. Roura, C.E. del Valle and M.R. Moreira, 2008. Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: *In vitro* and *in vivo* studies. Postharvest Biol. Technol., 49: 294-300.
14. Jane, T.C., L.Q. Jorge and T.C. Gloria, 2017. Antibacterial and antifungal activity from leaf extracts of *Luma chequen* (molina) a. gray myrtle against pathogens of clinical origin. J. Venezuelan Soc. Microbiol., 37: 10-16.
15. Bonilla, C.J.E., C. Iván, F. Mancheno and T.E.S. Herrera, 2021. Bromatological and microbiological characterization of andean tubers mashwa (*Tropaeolum tuberosum*) and oca (*Oxalis tuberosa*). ESPOCH Congresses: Ecuadorian J. S.T.E.A.M., 1: 1301-1310.
16. Lotero, F.M.M., J.E.S. Gil, S.V. Londoño, J.A.S. Jiménez and L.S.T. Valenzuela, 2018. Antimicrobial and antioxidant capacity of andean tubers (*Tropaeolum tuberosum* and *Ullucus tuberosus*). Rev. U.D.C.A Actualidad Divulgación Científica, 21: 449-456.

17. Kapoor, G., S. Saigal and A. Elongavan, 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. J. Anaesthesiology Clin. Pharmacol., 33: 300-305.
18. Chirinos, R., D. Campos, M. Warnier, R. Pedreschi, J.F. Rees and Y. Larondelle, 2008. Antioxidant properties of mashua (*Tropaeolum tuberosum*) phenolic extracts against oxidative damage using biological *in vitro* assays . Food Chem., 111: 98-105.
19. Gaitén, Y.I.G., M.M. Martínez, A.B. Alarcón, S.V. Hernández and R.M. de Oca Porto, 2011. Chemical characterization using gas chromatography/mass spectrometry of two extracts from *Phyllanthus orbicularis* HBK. Rev. Cubana Farmacia, 45: 405-413.
20. Balamurugan, R., V. Duraipandiyan and S. Ignacimuthu, 2011. Antidiabetic activity of γ-sitosterol isolated from *Lippia nodiflora* L. in streptozotocin induced diabetic rats. Eur. J. Pharmacol., 667: 410-418.
21. Saladino, F., K. Bordin, F.B. Luciano, M.F. Franzón, J. Mañes and G. Meca, 2017. Antimicrobial Activity of the Glucosinolates. In: Glucosinolates, Mérillon, J.M. and K.G. Ramawat (Eds.), Springer, ISBN: 978-3-319-25461-6, Swizerland, pp: 249-274..
22. Bastidas, J.P., P.U. Valencia and M.R. Oliva, 2017. Antimicrobial effect of vitamin C, vitamin E and essential oil of rosemary (*Rosmarinus oficinalis*) in frankfurt chicken sausages. Ind. Data, 20: 27-36.