Original Article

Ethanol extract of *Schinus molle L.* (*Molle*) and *Erythroxylum coca Lam* (*Coca*): Antibacterial Properties at Different Concentrations against *Streptococcus mutans*: An *In Vitro* Study

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Received : 28-04-20
Revised : 11-06-20
Accepted : 14-06-20
Published : 28-09-20

ABSTRACT

Objective: The aim of this study was to compare *in vitro* the antibacterial activity of an ethanol extract of *Erythroxylum coca Lam* (EEE) and *Schinus molle L.* (EES) at 50% and 75% versus *Streptococcus mutans* ATCC 25175. **Materials and Methods:** This was a prospective, comparative, longitudinal experimental study. The ethanol extract of *coca* and *molle* leaves was obtained by the vacuum filtration method at concentrations of 50% and 75% and was compared with a positive control (0.12% chlorhexidine). *Streptococcus mutans* strains were isolated in a culture medium (Mitis Salivarius Agar) ideal for the growth of bacterial colonies. The antibacterial activity of the ethanol extract was carried out following the Kirby–Bauer disk-diffusion method in Mueller–Hinton agar to measure bacterial sensitivity. A value of $P < 0.05$ was considered statistically significant. **Results:** Evaluation of the antibacterial effect of EEE and EES at 24 and 48 h showed that a concentration of 75% for both groups had the highest antimicrobial activity against *S. mutans* (11.2 ± 0.7 mm; 11.6 ± 0.5 mm and 11.3 ± 0.7 mm; 11.8 ± 0.5 mm, respectively). So, the results have shown that the concentration of EEE and EES of 75% has a greater efficacy than the concentration of 50%, but both concentrations are not as effective as chlorhexidine. **Conclusion:** EEE and EES at concentrations of 50% and 75% present antibacterial activity against *S. mutans* ATCC 25175.

KEYWORDS: *Antibacterial activity, Erythroxylum coca Lam, ethanol extract, Schinus molle L.*

INTRODUCTION

The study of the main microorganisms producing disease in humans is important to develop preventive strategies and treatments. The pharmaceutical industry has shown great interest in developing pharmaceutical products with antibacterial characteristics.[1-4] On the contrary, some medicinal herbal remedies have a great advantage over chemical products due to their biological properties which do not usually accumulate in the body and produce limited adverse effects.[1,5] It is important to know that treatment options against seek to eliminate the oral microbiota, even if several species in dental plaque show protective characteristics.[2]

A clear example of an herbal remedy is *Erythroxylum coca Lam*. which is a shrub ranging from 90 cm to 2 m height and has a strong root. The stem is firm and with branches with lenticels. The leaves are ovoid in

Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_237_20

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How to cite this article: Loyola D, Mendoza R, Chiong L, Rueda M, Alvítez-Temoche D, Gallo W, *et al.* Ethanol extract of *Schinus molle L.* (molle) and *Erythroxylum coca Lam* (coca): Antibacterial properties at different concentrations against *Streptococcus mutans*: An *in vitro* study. J Int Soc Prevent Communit Dent 2020;10:579-84.

shape with a yellowish or brownish-green color and are aromatic and bitter in taste. The creamy white-colored flowers are in groups of cymosan inflorescences, the chalice is gamosépalo and the corolla gamopétala, and its fruit is reddish in color.[6-9] Another example is *Schinus molle L.* This shrub is of up to 10 m in height, with a stem that has hanging curtain-shaped branches. A white-colored resin is produced in the case of damage to the shrub. Its leaves are alternately distributed, and the closer the leaves are to the apex the smaller they are in size. Its flowers are small, uni- or bisexual, and are very numerous. The fruits of *S. molle L.* are spherical in shape with a diameter of 5 mm and have a dark pink color and very smooth dark seeds.[10-13]

The alimentary as well as the medicinal properties of plants have long been described by our ancestors and studied in depth in the literature. Scientific evidence related to their utility for medicinal purposes has led to the development of many drugs.[1-5] *Erythroxylum coca Lam*. and *S. molle L*. are medicinal plants found in Peru with which many studies have been performed in relation to the field of stomatology and the cause–effect relationship they have on various microorganisms of the oral cavity.[4-9] Indeed, the main active element of *E. coca Lam* is cocaine, which has negative effects on the nervous system (increased excitement, tremor, nervousness, and convulsions) and the circulatory system (arrhythmia, cardiac arrest). On the contrary, similar to mate, cocaine has also been described as having beneficial effects in alleviating stomach and intestinal cramps and in relieving pain due to inflammation of the mouth.[8]

Taking all of the above into account, it has been hypothesized that ethanol extracts of *E. coca Lam* (EEE) and *S. molle L.* (EES) contain some metabolites with antibacterial properties which could inhibit the *S. mutans* strain ATCC 25175 production. Therefore, the aim of this study was to evaluate *in vitro* the antibacterial activity of EEE and EES at different concentrations versus *S. mutans* ATCC 25175.

Materials and Methods

Study design

A longitudinal, comparative, experimental *in vitro* study was carried out in the Microbiology Laboratory of the *Universidad Nacional Federico Villarreal* (UNFV) and the Faculty of Pharmacy and Biochemistry of the *Universidad Nacional Mayor de San Marcos* (UNMSM).

Sampling method and selection criteria

The sample size (n = 75) was obtained using data previously recorded in a previous study.[3,4] Whatman paper discs embedded with the ethanol extracts were divided into five groups. It should be noted that the outcome has been evaluated as a reduction in the extent of bacterial growth in mm. The selection criteria were the following:

Inclusion criteria

- Petri dishes inoculated with strains of *S. mutans*
- EEE at 50% and 75%
- EES at 50% and 75%

Exclusion criteria

- Petri dishes inoculated with strains of *S. mutans* presenting contamination and/or alterations due to bad incubation or poor operator maneuvers.
- EEE not belonging to the species of *E. coca Lam.*
- EES not belonging to the species of *S. molle L.*
- EEE and EES at concentrations other than those used in the analyses.

Allocation

Group No. 1: 50% EEE inoculated with *S. mutans*

Group No 2: 75% EEE inoculated with *S. mutans*

Group No. 3: 50% EES inoculated with *S. mutans*

Group No. 4: 75% EES inoculated with *S. mutans*

Group No. 5: Chlorhexidine 0.12% inoculated with *S. mutans*

Botanical classification

Taxonomic classification of the plant samples (stem with leaves and flower buds) [Figures 1 and 2] was carried out at the *Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos* (Boucher of botanical specimen No. 343-USM-2018 and N ° 328-USM-2018), obtaining the following information:

• Schinus molle L.

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Sapindales

Family: Anacardiaceae

Genre: *Schinus*

Species: *Schinus molle L.*

• Erythroxylum coca Lam

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Linales

Family: Erythroxylaceae

Genre: *Erythroxylum*

Species: *Erythroxylum coca Lam.*

Preparation of ethanol extracts

The specimens of *E. coca Lam.* (*coca*) and *S. molle L.* (*molle*) were transferred and prepared at the facilities of the Faculty of Pharmacy and Biochemistry of the UNMSM, where analytical control of the ethanol extraction was also performed (Analysis Protocol No. 00420-CPF -2018 and No. 00421-CPF-2018). Subsequently, the coca and molle leaves were separated from the stem, washed, and dried at room temperature. The leaves were placed in an oven at 40°C until they were completely dry for later grinding. Then 250 g of each species were weighed; 1500 mL of C_2H_5OH absolute ethanol (Merck EMPARTA ACS) was added to each amber bottle and macerated for 10 days. The extract was then filtered by vacuum filtration. The filtrate was poured into a drying dish and placed in an oven at 40°C for 3 days for concentration. The drying dish was removed from the stove on the last day, and the total content of 30 g that was poured into an amber bottle was scraped off. Concentrations of 50% and 75% of the mass of coca leaf and molle extract were prepared and stored in individual jars and kept refrigerated [Figure 3].

Figure 1: *Erythroxylum coca Lam* (*Coca*) leaves

Figure 2: *Schinus molle L* (*Molle*) leaves

Minimum inhibitory concentrations

For the evaluation of the minimum inhibitory concentration, the microdilution method was used. The EES (*molle*) and EEE (*coca*) were diluted between 0 and 75 µg/mL. Then all wells were supplemented with 100 µL of MHB (Mueller Hinton Broth) and 100 µL of bacterial suspension (McFarland Turbidity Standard 0.5) was added. Finally, the samples were incubated at 37 ° C for 24 h.

Microbiological seeding and measurement

Reactivation of the *S. mutans* ATCC 25175 strain from the laboratory (Gen Lab del Peru SAC.) was done by seeding in Mitis Salivarius agar and incubation at 37°C for 24 h. After this time, the growth of bacterial colonies was achieved and sensitivity tests were developed. *Streptococcus mutans* was seeded in each petri dish prepared with Mueller–Hinton agar. Subsequently, five discs were prepared for each petri dish; the concentrations of the ethanolic extract of *coca* and *molle* leaves were placed in four petri dishes, with chlorhexidine being placed in the last disc as a positive control group. At 24 and 48 h after seeding, the inhibition halos were measured using the Kirby–Bauer technique with a 12 mm × 300 mm Mitutoyo calibrator [Figure 4].

Statistical analysis

A comparison test of means was performed using Stata 12.0 software. Data were collected in an Excel database and were analyzed using the same software. First, normality was determined with the Shapiro–Wilk test, and then the Levene variance homogeneity test was carried out to decide the type of test for multiple comparisons. The Student's *t* test and analysis of

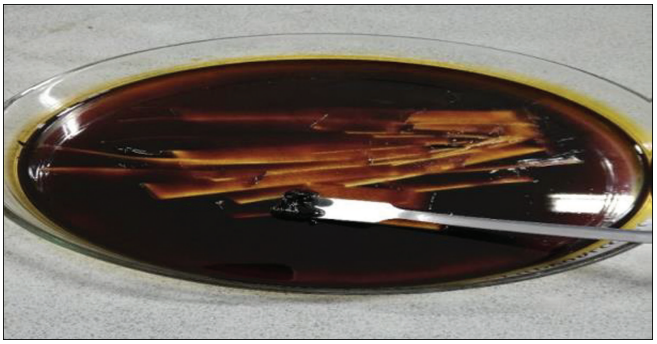

Figure 3: Crude ethanol extract

variance (ANOVA) were used for multiple comparisons. The level of significance was set at $P < 0.05$.

Results

Evaluation of the antibacterial effect of EEE and EES at 24 h showed that the concentration of 75% of both ethanol extracts had the highest antimicrobial activity against *S. mutans*, being 11.2 ± 0.7 mm and 11.6 ± 0.5 mm, respectively [Table 1]. There were statistically significant differences between the concentrations of 50% and 70% of EEE and EES, with a $P < 0.001$.

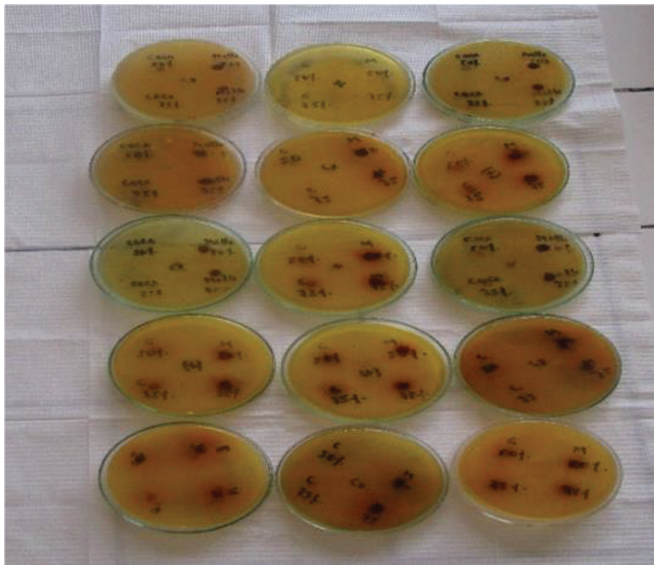

Figure 4: Petri dishes seeded at different concentrations

Similarly, the antibacterial effect of EEE and EES at 48 h at a concentration of 75% presented the highest antibacterial activity (11.3 ± 0.7 mm and 11.8 ± 0.5 mm, respectively) [Table 2], with statistically significant differences between the two groups ($p<0.05$).

Finally, there were statistically significant differences on comparing the antibacterial activities of EEE and EES with the control group (chlorhexidine) ($P < 0.001$).

Discussion

The plant of *E. coca Lam* has the following healing properties: the leaves have antirheumatic properties and can heal wounds and facilitate digestion and dental hygiene. The fruit prevents urinary retention, favors menstruation, is a bronchial expectorant, fights parasites, and, similar to the leaves, has antirheumatic properties. The bark and resin have antirheumatic and healing capacity. Other uses of this plant include: staining, fermentation of drinks with the fruit, and the bark can be used as flavoring.[1-4]

The present experimental investigation determined the antibacterial effect of EEE and EES at different concentrations in strains of *S. mutans* and found that

Table 1: *In vitro* comparison of the antibacterial activity of ethanol extracts of *Erythroxylum coca Lam* and *Schinus molle L.* at 24 h after inoculation

Plant	Concentration (%)	Mean	SD	Min	Max	*P**	*P***
Erythroxylum coca	50	10.1	0.7	9	11.5	0.001	<0.001
	75	11.2	0.7	10	12		
Schinus molle L.	50	10.7	0.7	11	12	0.005	
	75	11.6	0.5	11	13		
Clorhexidine	0.12	14.1	0.7	13	15		

All groups were measured in mm

Shapiro–Wilk test $P > 0.05$: all groups presented normality

* Student's *t* test $P < 0.05$ statistically significant

**ANOVA test $P < 0.05$ statistically significant

Table 2: *In vitro* comparison of the antibacterial activity of ethanol extracts of *Erythroxylum coca Lam* and *Schinus molle L.* at 48 h after inoculation

Plant	Concentration (%)	Mean	SD	Min	Max	*P**	*P***
Erythroxylum coca	50	10.5	0.6	10.0	12.0	0.012	<0.001
	75	11.3	0.7	10.0	12.0		
Schinus molle L.	50	10.9	0.7	10.0	12.0	0.008	
	75	11.8	0.5	11.0	13.0		
Clorhexidine	0.12	14.2	0.7	13.0	15.0		

All groups were measured in mm

Shapiro–Wilk test $P > 0.05$: all groups presented normality

* Student's *t* test $P < 0.05$ statistically significant

** ANOVA test $P < 0.05$ statistically significant

EEE and EES concentrations at 75% are more effective against *S. mutans* than concentrations of both extracts at 50%, whereas 0.12% chlorhexidine presented a higher inhibitory halo on EEE and EES against *S. mutans*. In addition, a greater antibacterial effect was observed at 48 h than at 24 h for both extract and chlorhexidine.

Gehrke *et al.*[14] described that *S. lentiscifolius Marchand* (without *Schinusweinmannifolius Engl*) is an oriental plant found in southeastern Brazil that is used as an antimicrobial agent in multiple diseases. However, despite being commonly used, there is scarce literature supporting its effectiveness. These authors concluded that the antibacterial activity of some components of *S. lentiscifolius* provides a solid scientific basis for use in different diseases. Similarly, De Wet[15] described some of the therapeutic uses of the leaf and bark extracts of four of the five South African species of *Erythroxylaceae* (*E. delagoense*, *E. emarginatum*, *E. pictum* and *Nectaropetalumcapense*), showing good antibacterial activity against *Bacillus subtilis, Klebsiella pneumoniae* and *Staphylococcus aureus*. In addition, a study by Restrepo *et al.*[16] found that the species *E. coca* and *E. novogranatense* have a high tropane alkaloid content, providing food, phytotherapeutic products, and other metabolites derived from this natural resource.[17-20]

On the contrary, in relation to *S. molle L*. the study of Martins *et al.*[11] showed S. *molle* to be effective against the *P. brasiliensis* fungus, which is a very prevalent fungal disease in Latin America. In addition, do Prado *et al.*[10] also showed that essential oils from the leaves and fruits of *S. molle* have antimicrobial activity, thereby demonstrating their usefulness in the pharmaceutical industry. These results are consistent with those of this study and those described by Gundidza[13] who described that the essential oil of this plant has antibacterial activity against some bacteria such as *K. pneumoniae, Alcaligenes faecalis, Pseudomonas aeruginosa*, *Enterobacter aerogenes*, among others.

The main limitation of this research was the scarce literature available on these plants in Peru making comparison with other studies difficult. The second drawback was where to prepare the ethanol extracts, as the reagents needed are regulated by the Peruvian state. Finally, contamination of the petri dishes had to be carefully controlled when seeding. Nonetheless, despite these limitations, the study was carried out successfully. The results of this study provide knowledge of the properties of plants found in Peru which may be useful against *S. mutans* strain ATCC 25175, providing benefits to the dental profession and the public alike like other studies.[21-24] Indeed, in contrast to antibacterial drugs produced by the pharmaceutical industry which may produce adverse effects in the long term, EEE and EES do not seem to have secondary effects. Oral microbial diseases are the main source of loss of tooth structure, and the positive effects and low cost of EEE and EES make their future use in the form of a toothpaste or mouthwash promising. In recent years, various technical techniques have been carried out to investigate the oral microbiota. However, the reduction in economic resources has impacted timely medical access. Research is needed on the role of oral microorganisms and on how to effectively control them.[25-28] Another limitation was that only the antibacterial effect against a *S. mutans* strain was evaluated, since this bacterium is the main microorganism involved in the dental caries development process; however, it is not the only bacterial factor that has a great impact on oral health. For reasons of availability and laboratory logistics, it was only evaluated against *S. mutans*, thus leaving a line of research that can be continued to expand the results of the present investigation.

Finally, research should be carried out on the EEE and EES in animal biomodels to verify whether they coincide with the results obtained in this *in vitro* study. Further studies using higher concentrations of these plant extracts are needed to establish the antibacterial effects of *Coca* and *Molle* leaves on chemical products. In addition, studies involving a longer exposure time of the ethanol extracts should be undertaken to verify possibly greater antibacterial effects over time and the possible cytotoxicity in humans.

Conclusion

According to the results of the study, concentrations of EEE and EES at 50% and 75% present antibacterial activity against *S. mutans* ATCC 25175. This *in vitro* activity was significantly greater for both extracts at 48 h.

Acknowledgement

We wish to thank the Faculty of Pharmacy and Biochemistry of the Universidad Nacional Mayor de San Marcos for providing the facilities for the development of this research. We also acknowledge the Universidad Cientifica del Sur (UCSUR) for constantly supporting us in the elaboration of the manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

None to declare.

Authors contributions

Study conception (DL, RM, LCH, MR), data collection (RM, DL, LCH, MR), data acquisition and analysis (DAT, WG, FMT), data interpretation (DAT, FMT), manuscript writing (FMT, WG, DAT).

Ethical policy and institutional review board statement

This project is exempted from ethical approval due to it was an experimental *in vitro* study.

Patient declaration of consent

Not applicable.

Data availability statement

The data that support the study results are available from the author (Dr. Frank Mayta-Tovalino, e-mail: fmaytat@ucientifica.edu.pe) on request.

References

1. Mummed B, Abraha A, Feyera T, Nigusse A, Assefa S. In vitro antibacterial activity of selected medicinal plants in the traditional treatment of skin and wound infections in eastern Ethiopia. Biomed Res Int 2018;2018:1862401.
2. Patini R, Cattani P, Marchetti S, Isola G, Quaranta G, Gallenzi P. Evaluation of Predation Capability of Periodontopathogens Bacteria by Bdellovibrio Bacteriovorus HD100. An in Vitro Study. Materials (Basel) 2019;12:2008.
3. Mayta-Tovalino F, Gamboa E, Sánchez R, Rios J, Medina R, García M, *et al*. Development and formulation of the experimental dentifrice based on *Passiflora mollissima* (tumbo) with and without fluoride anion: Antibacterial activity on seven antimicrobial strains. Int J Dent 2019;2019:9056590.
4. Calderon A, Salas J, Dapello G, Gamboa E, Rosas J, Chávez J, *et al*. Assessment of antibacterial and antifungal properties and *in vivo* cytotoxicity of peruvian *Passiflora mollisima*. J Contemp Dent Pract 2019;20:145-51.
5. Bussmann RW, Paniagua Zambrana NY, Moya Huanca LA, Hart R. Changing markets—Medicinal plants in the markets of La Paz and El Alto, Bolivia. J Ethnopharmacol 2016;193:76-95.
6. Biondich AS, Joslin JD. Coca: The history and medical significance of an ancient Andean tradition. Emerg Med Int 2016;2016:4048764.
7. Biondich AS, Joslin JD. Coca: High altitude remedy of the ancient Incas. Wilderness Environ Med 2015;26:567-71.
8. Novák M, Salemink CA, Khan I. Biological activity of the alkaloids of *Erythroxylum coca* and *Erythroxylum novogranatense*. J Ethnopharmacol 1984;10:261-74.
9. Casale JF, Mallette JR, Jones LM. Chemosystematic identification of fifteen new cocaine-bearing *Erythroxylum* cultigens grown in Colombia for illicit cocaine production. Forensic Sci Int 2014;237:30-9.
10. do Prado AC, Garces HG, Bagagli E, Rall VLM, Furlanetto A, Fernandes Junior A, *et al*. *Schinus molle* essential oil as a potential source of bioactive compounds: Antifungal and antibacterial properties. J Appl Microbiol 2019;126: 516-22.
11. Martins Mdo R, Arantes S, Candeias F, Tinoco MT, Cruz-Morais J. Antioxidant, antimicrobial and toxicological properties of *Schinus molle* L. Essential oils. J Ethnopharmacol 2014;151:485-92.
12. Hayouni el A, Chraief I, Abedrabba M, Bouix M, Leveau JY, Mohammed H, *et al*. Tunisian salvia officinalis L. And *Schinus molle L.* Essential oils: Their chemical compositions and their preservative effects against salmonella inoculated in minced beef meat. Int J Food Microbiol 2008;125:242-51.
13. Gundidza M. Antimicrobial activity of essential oil from *Schinus molle linn*. Cent Afr J Med 1993;39:231-4.
14. Gehrke IT, Neto AT, Pedroso M, Mostardeiro CP, Da Cruz IB, Silva UF, *et al*. Antimicrobial activity of *Schinus lentiscifolius* (anacardiaceae). J Ethnopharmacol 2013;148:486-91.
15. De Wet H. Antibacterial activity of the five South African Erythroxylaceae species. African J Biotechnol 2011;10: 11511-4.
16. Restrepo DA, Saenz E, Jara-Muñoz OA, Calixto-Botía IF, Rodríguez-Suárez S, Zuleta P, *et al*. *Erythroxylum* in focus: An interdisciplinary review of an overlooked genus. Molecules 2019;24:2-27.
17. White DM, Islam MB, Mason-Gamer RJ. Phylogenetic inference in section archerythroxylum informs taxonomy, biogeography, and the domestication of coca (*Erythroxylum* species). Am J Bot 2019;106:154-65.
18. Cruz RA, Almeida H, Fernandes CP, Joseph-Nathan P, Rocha L, Leitão GG. A new tropane alkaloid from the leaves of *Erythroxylum* subsessile isolated by ph-zone-refining counter-current chromatography. J Sep Sci 2016;39:1273-7.
19. Sauvain M, Rerat C, Moretti C, Saravia E, Arrazola S, Gutierrez E, *et al*. A study of the chemical composition of *Erythroxylum coca* var. Coca leaves collected in two ecological regions of Bolivia. J Ethnopharmacol 1997;56: 179-91.
20. Lucas-Filho MD, Silva GC, Cortes SF, Mares-Guia TR, Perpétua Ferraz V, Serra CP, *et al*. ACE inhibition by astilbin isolated from *Erythroxylum gonocladum* (mart.) O.E. Schulz. Phytomedicine 2010;17:383-7.
21. Mayta-Tovalino F, Sedano-Balbin G, Romero-Tapia P, Alvítez-Temoche D, Álvarez-Paucar M, Gálvez-Calla L, *et al*. Development of new experimental dentifrice of peruvian *Solanum tuberosum* (tocosh) fermented by water stress: Antibacterial and cytotoxic activity. J Contemp Dent Pract 2019;20:1206-11.
22. Poma-Castillo L, Espinoza-Poma M, Mauricio F, Mauricio-Vilchez C, Alvítez-Temoche D, Mayta-Tovalino F. Antifungal activity of ethanol-extracted *Bixa orellana* (L) (achiote) on *Candida albicans*, at six different concentrations. J Contemp Dent Pract 2019;20:1159-63.
23. Medina-Flores D, Ulloa-Urizar G, Camere-Colarossi R, Caballero-García S, Mayta-Tovalino F, del Valle-Mendoza J. Antibacterial activity of Bixa orellana L. (achiote) against *Streptococcus mutans* and *Streptococcus sanguinis*. Asian Pacific J Trop Biomed 2016;6:400-3.
24. Camere-Colarossi R, Ulloa-Urizar G, Medina-Flores D, Caballero-García S, Mayta-Tovalino F, del Valle-Mendoza J. Antibacterial activity of Myrciaria dubia (Camu camu) against Streptococcus mutans and Streptococcus sanguinis. Asian Pacific J Trop Biomed 2016;6:740-4.
25. Martellacci L, Quaranta G, Fancello G, *et al*. Characterizing Peri-implant and sub-gingival microbiota through culturomics. First isolation of some species in the oral cavity. A pilot study. Pathogens 2020;9:E365.
26. Patini R. Oral microbiota: Discovering and facing the new associations with systemic diseases. Pathogens 2020; 9:313.
27. Martellacci L, Quaranta G, Patini R, Isola G, Gallenzi P, Masucci L. A literature review of metagenomics and culturomics of the peri-implant microbiome: Current evidence and future perspectives. Materials (Basel) 2019; 12:3010.
28. Patini R, Staderini E, Lajolo C, Lopetuso L, Mohammed H, Rimondini L, *et al*. Relationship between oral microbiota and periodontal disease: A systematic review. Eur Rev Med Pharmacol Sci 2018;22:5775-88.