Antimicrobial activity of three plant species from the Cañada region in Oaxaca state against bacteria responsible for foodborne diseases

Lucio González Montiel[1]*, Norma Güemes Vera[2], Sergio Soto Simental[2], Jesús M Campos Pastelin[1]

[1]Universidad de la Cañada. Cuerpo Académico Aprovechamiento Integral de Productos Agroindustriales. Carr. Teotitlán-San Antonio Nanahuatipán Km. 1.7 s/n. Paraje Titlacuatitla. Teotitlán de Flores Magón, Oaxaca, México. C.P. 68540. [2]Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Agropecuarias, Rancho Universitario, Tulancingo, Hidalgo, México. C.P. 43600.

Abstract

Consumers nowadays expect food to be nutritious, organoleptically attractive, quick and easy to prepare, as well as safe to eat. However, many foods and drinking water represent risks to public health, mainly due to the presence of pathogenic microorganisms, toxins, pesticides or dangerous substances responsible for foodborne diseases (FD). In the present study, the antibacterial activity of hexane, dichloromethane and methanolic extracts of three plant species collected in the Cañada region, in the state of Oaxaca, Mexico was evaluated: *Philodendron* sp., *Sideroxylon palmeri* and *Verbesina crocata*, which are traditionally used in the treatment of gastrointestinal diseases as well as digestive and inflammatory disorders. The antibacterial activity was evaluated against *Escherichia coli*, *E. coli* ATCC 8739, *Salmonella typhimurium*, *Staphylococcus aureus* ATCC 29213, *Vibrio cholerae* INDRE 206, *Vibrio cholerae* CDC V12 and *Yersinia enterocolitica*. The hexane and dichloromethane extracts of *Sideroxylon palmeri* and the hexane extract of *Verbesina crocata* showed greater activity against all of the strains evaluated, while none of the *Philodendron* sp. extracts presented antibacterial activity. It is concluded that these extracts could be used during food preparation or manufacturing as an important source of natural compounds that can prevent or inhibit the growth of bacteria responsible for foodborne diseases.

Keywords: Antibacterial activity, *Sideroxylon palmeri*, *Verbesina crocata*, *Philodendron* sp

Introduction

Foodborne diseases (FD) are defined as a set of signs and symptoms that are caused by physical, chemical or biological agents that enter the body through the intake of water and contaminated food in sufficient quantities to affect the health of the consumer (Zambecchi, 2010). The main pathogenic groups with regard to food infections are bacteria, protozoa and viruses, while for food poisoning, they are bacteria and mold (Oliva, 2008). In general, only small amounts of microorganisms contaminate food, so they must find the right conditions to survive and multiply until reaching the required levels to infect or produce enough toxins to cause disease symptoms (Parrilla *et al*., 1993). FD symptoms can last from a few hours to a few days and in most cases include vomiting, abdominal pain, diarrhoea and even occasionally fever (Zambecchi, 2010).

More than 250 types of FD have been reported worldwide. In 2010, some 33 million cases were reported which resulted in medical care costs of USD 23 million, and this figure does not take into account the economic losses of time off work due to illness, as well as other factors. In Latin America, FDs represent about 70% of cases of acute diarrheal disease according to the World Health Organization (Cisan, 2011). In 2010, 5.681 million cases of these diseases were

reported in Mexico according to the National Epidemiological Surveillance System (SINAVE for its Spanish acronym), however, it is thought that there are many more cases than those recorded (Osuna, 2012). Statistics relating to acute gastrointestinal diseases reported by SINAVE include illnesses transmitted by food (potential FDs) such as intestinal amebiasis, amebic liver abscess, giardiasis, teniasis, cysticercosis, cholera, typhoid fever, paratyphoid fever, salmonellosis, shigellosis, brucellosis and viral hepatitis (Flores, 2006).

A great diversity of foods has been involved in disease outbreaks, such as eggs, meat, chicken, dairy products, canned products, among others, and these vary according to the characteristics of each country, as well as production patterns and consumption habits among the local population (Parrilla *et al*., 1993). Therefore, the presence of these microorganisms in food leads us to look for alternatives to inhibit and eliminate them with a high possibility of application, one of which is the use of natural products. There is scientific evidence of several plant species that have antimicrobial, antifungal and other phytotherapeutic applications, which can be used in pharmaceutical activities, pest control in the agricultural sector and food preservation (Rodríguez-Sauceda, 2011).

The Cañada region of the State of Oaxaca is part of the Tehuacán-Cuicatlán Valley (Briones, 2000). The flora of this region is composed of about 3,000 species of plants and 30% of this flora is used in different ways by the local population, which is mainly as a food source, for construction, feed for livestock and medicinal purposes (Téllez *et al*., 2008). In terms of medicinal purposes, the following flora can be used in different ways as described in the following section:

***Philodendron* sp.** Commonly known as rabbit sweet potato root and belongs to the Araceae family. According to the inhabitants of San Mateo Yoloxochitlán in the Cañada region of Oaxaca State, the root of this plant is used to treat stomach pain and diarrhoea. The presence of terpenes in the methanolic extract has been reported (Rosas *et al*., 2012). Bezerra *et al*. (2002), identified various chemical components such as β-pinene, limonene, spatulenol, α-pinene, caryophylene oxide, α-humulene, β-elemene and β-cariophylene, which are responsible for its antimicrobial activity.

***Sideroxylon palmeri* (Rose) Pennington**. The unripened fruit of this plant is commonly known as *tempesquistle* and is consumed in various municipalities of the Cañada region of Oaxaca State. Research on *tempesquistle* is scarce and there are no previous reports of phytochemical studies having been carried out. However, there is evidence about other species of the *Sideroxylon* genus, such as the root of *S. cubense*, which presents saponins (sideroxyloside A and 3-O-β-D-glucopyranosyl-protobasic acid) (Jiang *et al*., 1994), as well as sideroxyposide B and sideroxycoside C in *S. foetidissimum* Jacq. subsp. foetidissimum (Nicola *et al*., 1995). In addition, the presence of various phenolic compounds, triterpenes, phytosterols and flavonoids has been reported from saponins.

***Verbesina crocata* (Cav.) Less. ex DC**. Commonly known as *Arnica*, the presence of various alkaloids has been reported (Ordaz *et al*., 2012). Xu *et al*. (2009) reported the presence of sesquiterpenoids, diterpenoids and triterpenoids in the genus *Verbesina*, presenting various biological activities, such as antimicrobial, antiviral, antifungal and antibacterial activity, mainly against Gram-positive species (Chaturvedi, 2011).

Taking the aforementioned into account, this work aims to evaluate the antibacterial activity of the organic extracts (hexane, dichloromethane and methanolic) from the root of *Philodendron*

sp., the *Sideroxylon palmeri* fruit and the *Verbesina crocata* leaf against seven pathogenic bacterial strains responsible for foodborne diseases.

Materials and Methods

Location of experiments. The experiments relating to this work were carried out in the chemistry and biology laboratories of the University of La Cañada.

Plant material. Three plants were selected (Table 1) based on their popularity and empirical use, according to the inhabitants of the Cañada region, for treating gastrointestinal discomforts (stomach pain, diarrhoea, dysentery, gastritis, gastroenteritis, among others) associated with food consumption.

Table 1. Plants studied.

Common name	Scientific name	Part used	Area collected
Arnica	*Verbesina crocata*	Leaves	Teotitlán de Flores Magón
Rabbit sweet potato root	*Philodendron sp.*	Root	San Mateo Yoloxochitlán
Tempesquistle	*Sideroxylon palmeri*	Fruit	Teotitlán de Flores Magón

Obtaining plant extracts. The plant material was dehydrated in a free convection dryer at room temperature and kept under shade for ten days. The dried material was fragmented in a KRUPS electric grinder. The extraction was carried out by thorough maceration at room temperature with a proportion of 1:40 p/v (25 g of plant material in a liter of solvent) for 72 hours, using three different solvents of increasing polarity: hexane, dichloromethane and methanol (Aburto, 2006). The extraction solvent was then decanted and filtered (Whatman filter paper No. 1). In order to obtain the crude extracts, a digital rotary evaporator (BÜCHI model R-215) was used at 40°C. Five concentrations were prepared from the crude extracts: 40, 20, 10, 5 and 2.5 mg / mL using distilled water as a diluent for methanolic extracts and DMSO for hexane and dichloromethane extracts. In order to dissolve the hexane extracts of *S. palmeri* and dichloromethane of *V. crocata*, they were placed in a water bath at 60°C, and a sonicator was then used (BRANSON ultrasonic cleaner).

Activation of bacterial strains. Seven bacterial strains responsible for FD were used, six of which were Gram-negative bacteria and one of which was Gram-positive bacteria; *Escherichia coli*, *E. coli* ATCC 8739, *Salmonella typhimurium*, *Vibrio cholerae* INDRE 206, *Vibrio cholerae* CDC V12, and *Yersinia enterocolitica* and a Gram positive; *Staphylococcus aureus* ATCC 29213 (Strains donated by Dr. Margarita Canales Martínez, Faculty of Higher Education-Iztacala, UNAM). A colony was seeded in tubes with 10 mL of sterile Müeller Hinton broth, and incubated at 37°C for 24 hours. In another tube with sterile broth, a sample from the previous culture was added and incubated at 37°C for 24 hours. This operation was repeated two more times (a total of 4 procedures). For the active growing cultures, tubes were prepared by adjusting turbidity to 0.5 on the Mc Farland nephelometer ($1X10^8$ CFU / mL).

Controls. An antifungal-antibiotic solution (100 ×), consisting of 10,000 units of penicillin, 10 mg of streptomycin and 25 µg of amphotericin B per mL (Sigma-Aldrich Química, S. de R.L. de C.V. A5955) was used as a positive control. Regarding negative control, distilled water was

used for the methanolic extract, while 100% dimethylsulfoxide (DMSO) was used for the dichloromethane and hexane extracts.

Evaluation of antibacterial activity. The evaluation of antimicrobial activity was carried out by means of the agar diffusion method (Cruz-Carillo *et al*., 2010; González, 2012; Jaime, 2008). Seeding was performed in sterile 100 x 15 mm Petri dishes, which involved adding 1 mL of the bacterial suspension as well as 20 mL of Müeller Hinton agar with a sterile test tube at 45 °C. These were homogenized with orbital movements and allowed to stand until solidification. Seven wells measuring 0.55 cm in diameter were then made. In each well, 25 µL of the extracts' corresponding dilutions (40, 20, 10, 5 and 2.5 mg / mL) and positive and negative controls were added, while the boxes were incubated at 37 °C for 24 hours (Incubator-digital JEIO-TECH brand IB-I5G). The extracts were evaluated in triplicate.

Reading and interpretation of results. The zone of inhibition was measured using a Vernier caliper (Pie de Rey, manufactured by SCALA), with the average diameter of the zone of inhibition for each of the extracts recorded. The minimum inhibitory concentration (MIC) was determined taking into account the lowest concentration of the extract against each of the bacteria studied.

Results and Discussion

None of the *Philodendron* sp. (rabbit sweet potato) root extracts showed antibacterial activity against the strains evaluated. The hexane extract of *Verbesina crocata* (Arnica) only exhibited inhibitory activity in the 40 and 20 mg/mL concentrations, while the extracts with methanol and dichloromethane did not show any inhibitory activity. On the other hand, the hexane and dichloromethane extracts of *Sideroxylon palmeri* (tempesquistle) showed zones of inhibition against all of the strains evaluated and at all concentrations.

Table 1 shows the results of the antibacterial activity of the hexane and dichloromethane extracts of the *Sideroxylon palmeri* fruit. It is worth mentioning that the methanolic extract did not show antibacterial activity against any evaluated strain. The antibacterial activity is a function of the compounds present in the extract, as well as the concentration used. All concentrations of the hexane extract showed greater inhibitory activity against *V. cholerae* CDC V12, as well as *S. typhimurium* which recorded no inhibition for the 2.5 mg/mL concentration. We therefore consider the 5 mg/mL concentration as the minimum inhibitory concentration (MIC). For the rest of the strains, the zones of inhibition were similar, with the strain with the highest sensitivity being *V. cholerae* CDC V12.

Canales *et al*. (2011), Hernández *et al*. (2009), Hernández *et al*. (2005), Hernández *et al*. (2003), mention that the inhibition of the hexane extract of various plants against S. aureus, *Y. enterocolitica*, *V. cholerae* INDRE 206, and *V. cholerae* CDC V12 may be due to the presence of compounds such as carvacrol, α-terpenyl acetate, thymol, terpineol, cimeno, cineole, linalool, β-pinene and the methyl ester of unsaturated fatty acids.

Table 1. Antibacterial activity of the hexane and dichloromethane extracts of *Sideroxylon palmeri* and hexane extract of *Verbesina crocata* against bacteria responsible for foodborne diseases.

Strain	Diameter of Zone of Inhibition (mm)			
	Concentration (mg/mL)	*Sideroxylon palmeri*		*Verbesina crocata*
		Hexane Extract	Dichloromethane Extract	Hexane Extract
Escherichia coli	40	14.17 ± 0.67	11.63 ± 0.23	8.20 ± 0.26
	20	11.10 ± 0.17	9.83 ± 0.38	7.13 ± 0.15
	10	9.80 ± 0.53	7.93 ± 0.29	0 ± 0
	5	7.67 ± 0.91	7.23 ± 0.21	0 ± 0
	2.5	7.73 ± 0.06	6.77 ± 0.06	0 ± 0
	Control +	17.55 ± 0.49	16.10 ± 0.78	17.75 + 0.07
	Control -	0 ± 0	0 ± 0	0 + 0
***Escherichia coli* ATCC 8739**	40	13.80 ± 0.20	12.10 ± 0.35	9.17 ± 0.21
	20	11.83 ± 0.25	9.57 ± 0.38	7.83 ± 0.46
	10	10.30 ± 0.26	8.47 ± 0.45	0 ± 0
	5	8.33 ± 0.23	7.40 ± 0.17	0 ± 0
	2.5	7.90 ± 0.26	6.97 ± 0.46	0 ± 0
	Control +	19.10 ± 1.13	17.80 ± 0	19.75 ± 0.35
	Control -	0 ± 0	0 ± 0	0 ± 0
Salmonella typhimurium	40	13.77 ± 0.25	12.20 ± 0.50	8.23 ± 0.49
	20	11.50 ± 0.40	9.93 ± 0.12	0 ± 0
	10	9.63 ± 0.45	8.03 ± 0.75	0 ± 0
	5	**7.50 ± 0.46**	**7.60 ± 0.00**	0 ± 0
	2.5	0 ± 0	0 ± 0	0 ± 0
	Control +	17.80 ± 0.14	18.80 ± 0.28	19.90 ± 0.28
	Control -	0 ± 0	0 ± 0	0 ± 0
***Staphylococcus aureus* ATCC 29213**	40	14.03 ± 0.15	12.00 ± 0.20	9.00 ± 0.44
	20	12.03 ± 0.21	10.27 ± 0.40	7.87 ± 0.15
	10	10.40 ± 0.26	8.77 ± 0.06	0 ± 0
	5	8.03 ± 0.31	7.87 ± 0.23	0 ± 0
	2.5	7.73 ± 0.46	7.17 ± 0.47	0 ± 0
	Control +	14.95 ± 0.35	15.80 ± 0.00	15.65 ± 0.65
	Control -	0 ± 0	0 ± 0	0 ± 0
***Vibrio cholerae* INDRE 206**	40	13.70 ± 0.20	11.80 ± 0.20	8.07 ± 0.49
	20	11.80 ± 0.20	9.77 ± 0.15	7.45 ± 0.07
	10	10.17 ± 0.06	8.20 ± 0.35	0 ± 0
	5	8.40 ± 0.44	7.73 ± 0.59	0 ± 0
	2.5	7.63 ± 0.60	7.03 ± 0.53	0 ± 0
	Control +	18.90 ± 1.27	18.70 + 0.00	20.60 ± 0.57
	Control -	0 ± 0	0 + 0	0 ± 0
***Vibrio cholerae* CDC V12**	40	14.53 ± 0.15	12.40 ± 0.56	9.27 ± 0.42
	20	12.20 ± 0.26	10.70 ± 0.53	8.20 ± 0.35
	10	10.60 ± 0.20	8.87 ± 0.76	0 ± 0
	5	7.67 ± 0.25	7.67 ± 0.31	0 ± 0
	2.5	7.97 ± 0.65	7.13 ± 0.06	0 ± 0
	Control +	24.00 ± 0	19.13 ± 0.67	22.35 ± 0.35

	Control -	0 ± 0	0 ± 0	0 ± 0
Yersinia enterocolítica	40	13.83 ± 0.21	12.40 ± 0.50	8.53 ± 0.25
	20	11.83 ± 0.15	10.00 ± 0.70	0 ± 0
	10	10.43 ± 0.06	8.30 ± 0.46	0 ± 0
	5	7.57 ± 0.15	7.33 ± 0.65	0 ± 0
	2.5	7.93 ± 0.40	7.05 ± 0.21	0 ± 0
	Control +	18.87 ± 0.85	18.95 ± 0.92	20.40 ± 0.71
	Control -	0 ± 0	0 ± 0	0 ± 0

Control + = antifungal-antibiotic solution. Control - = distilled water for the methanolic extract and dimethylsulfoxide (DMSO) for dichloromethane and hexane extracts.
The values represent the average of 3 experiments performed independently + the standard deviation.

The dichloromethane extract of *Sideroxylon palmeri* showed greater inhibition against *V. cholerae* CDC V12 and *Y. enterocolitica* with the 40 mg/mL concentrations as the most sensitive strains. For the 2.5 mg/mL concentration, no zones of inhibition were observed for *S. typhimurium*, with the minimum inhibitory concentration (MIC) 5 mg/mL. For the rest of the strains, the zones of inhibition were very similar. This effect may be due to the presence of carvacrol, thymol (Qaralleh *et al*., 2009) and phenols which have the ability to break the barrier that provides permeability to the cell membrane, causing cell death (Namian *et al*., 2013). Nantachit and Tuchinda (2009), mention that the antibacterial activity of diclomethane extracts is due to triterpenoids. *Syed et al*. (2013) report the presence of tannins, flavonoids, alkaloids, saponins, terpenes and glycosides, in dichloromethane extracts have an inhibitory effect on Gram negative bacteria.

The hexane extract of the *Verbesina crocata* leaves at a concentration of 40 mg/mL shows the greatest inhibition of bacterial growth against the *V. cholerae* CDC V12 strain with a zone of inhibition of 9.27 ± 0.42 mm, followed by the *E. coli* ATTC 8739 strain with 9.17 ± 0.21 mm. On the other hand, the lowest inhibition at this concentration was presented against *V. cholerae* INDRE 206, with diameters of 8.07 ± 0.49 mm. At a concentration of 20 mg/mL, this extract exhibited zones of inhibition ranging from 7.13 to 7.87 mm in strains of *E. coli*, *E. coli* ATCC 8739, *S. aureus* ATCC 29213 and *V. cholerae* INDRE 206. Furthermore, the greatest inhibition at this concentration was observed for *V. cholerae* CDC V12 with diameters of 8.2 mm. It is important to mention that *Salmonella typhimurium* and *Yersinia enterocolitica* did not show inhibitory activity at this concentration, meaning their minimum inhibitory concentration (MIC) was 40 mg/mL. Niño *et al*. (2006) evaluated the antibacterial activity of eight hexane extracts from plants belonging to the Asteraceae family, including *Verbesina nudipes* at concentrations of 5, 2.50, 1.25, 0.62 and 0.31 mg / mL against *E. coli* and *S. aureus*. No antimicrobial activity was recorded, and the presence of secondary metabolites of a phytochemical nature was not detected.

Conclusion

This study showed that of the three plant species studied from the Cañada region, only *Sideroxylon palmeri* (*tempesquistle* fruit) and *Verbesina crocata* (*Arnica* leaves) demonstrated antibacterial activity against *E. coli*, *E. coli* ATCC 8739, *Salmonella typhimurium*, *S aureus* ATCC 29213, *Y. enterocolitica*, *V. cholerae* INDRE 206 and *V. cholerae* CDC V12. This, therefore, confirms its effectiveness against bacteria responsible for foodborne diseases, while *Philodendron* sp. (rabbit sweet potato) does not exhibit such activity. It can be concluded that

Sideroxylon palmeri (*tempesquistle* fruit) and *Verbesina crocata* (*Arnica* leaves) could be used as an important source of naturally occurring antimicrobials.

References

Aburto R. (2006). Estudio químico y biológico preliminar de las especies *Penstemon barbatus, P. campanulatus, Castilleja tenuiflora y Vitex mollis.* Tesis de Maestría. Universidad Autónoma del Estado de Morelos.

Bezerra B. M. Z., Andrejo-Neto M., De Freitas M. R., Feitosa M. C. and De Andrade M. I. (2002). The root of *Philodendron imbe* Schott (Araceae). *Journal of Essential Oil Research*. 14(1):56-57.

Briones M. (2000). Lista anotada de los mamíferos de la región de la Cañada, en el Valle de Tehuacán-Cuicatlán, Oaxaca, México. *Acta Zoológica Mexicana* (Nuevas Serie). 81:83-103.

Canales M., Hernández T., Rodríguez-Monroy M. A., Flores C. M., Jiménez-Estrada M., Hernández L. B., Durán A., Hernández-Moreno M., Trejo N. A., Hernández A. B., Ramírez J. J., Orozco J., Eleno M. G. and Martínez K. E. (2011). Evaluation of the antimicrobial activity of *Acalypha monostachya* Cav. (Euphorbiales: Euphorbiaceae). *African Journal of Pharmacy and Pharmacology*. 5(5):640-647.

Chaturvedi D. (2011). Sesquiterpene lactones: Structural diversity and their biological activites. En: Vinod K. T. and Bhuwan B. M., editors. Opportunity, Challenge and Scope of Natural Productos in Medicinal Chemistry. *Publishing Research Signpost. India*. pp. 313-334.

[Cisan-2011]. Consejo para la Información sobre la Seguridad de los Alimentos y Nutrición. Brotes de enfermedades transmitidas por alimentos en 2011. (En línea). Disponible en www.cisan.org.ar

Cruz-Carillo A., Rodríguez N. N. y Rodríguez C. E. (2010). Evaluación in vitro del efecto antibacteriano de los extractos de *Bidens pilosa, Lantana camara, Schinus molle y Silybum marianum. Revista U. D. C. A. Actualidad & Divulgación Científica.* 13(2):117-124.

Flores J. L. (2006). El subregistro de enfermedades diarreicas. *Red sanitaria.* 1(4):8.

González L. M., Mosquera O. M. y Niño J. (2012). Ciclótidos de la especie *Guettarda crispiflora* (Rubiaceae). *Scientia Et Technica*. XVII (51):203-210.

Hernández T., Canales M., Avila J. G., Duran A., Caballero J., Romo de Vivar A. and Lira R. (2003). Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlán de las Salinas, Puebla (México). *Journal of Ethnopharmacology*. 88(2-3):181–188.

Hernández T., Canales M., Avila J. G., García A. M., Martínez A., Caballero J., Romo de Vivar A. and Lira R. (2005). Composition and antibacterial activity of essential oil of *Lantana achyranthifolia* Desf. (Verbenaceae). *Journal of Ethnopharmacology*. 96(3):551–554.

Hernández T., Canales M., Avila J. G., García A. M., Meraz S., Caballero J. and Lira R. (2009). Composition and antibacterial activity of essential oil of *Lippia graveolens* H. B. K. (Verbenaceae). *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*. 8(4):295-300.

Jaime C. (2008). Elucidación estructural por GC-MS de metabolitos del extracto en diclorometano de la Melastomatácea *Clidemia rubra*. Tesis de Licenciatura. Universidad Tecnológica de Pereira.

Jiang Y., Oulad-Ali A., Guillaume D., Bernard W. and Anton R. (1994). Triterpenoid saponins from the root of *Sideroxylon cubense*. *Phytochemistry.* 35(4):1013-1015.

Namian P., Talebi T., Germi K. G. and Shabani F. (2013). Screening of biological activities (Antioxidant, Antibacterial and Antitumor) of *Nerium oleander* leaf and flower extracts. *American Journal of Phytomedicine and Clinical Therapeutics*. 1(4):378-384.

Nantachit K. and Tuchinda P. (2009). Antimicrobial activity of hexane and dichloromethane extracts from *Momordica cochinchinensis* (Lour.) Spreng leaves. *Thai Pharmaceutical and Health Science Journal*. 4(1):15-20.

Nicola G., Oulad-Ali A., Guillaume D., Lobstein A., Weniger B. and Anton R. (1995). Triterpenoid saponins from the root of *Sideroxylon foetidissimum*. *Phytochemistry.* 38(1**)**:225-228.

Niño J., Narváez D. M., Mosquera O. M. and Correa Y. M. (2006). Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and two Rubiaceae plants from Colombian biodiversity. *Brazilian Journal of Microbiology*. 37(4):566-570.

Oliva M. M. (2008). Enfermedades infecciosas transmitidas por alimentos. *Revista de Ciencias Médicas de la Habana*. 14(3):102-115.

Ordaz E. A., Gómez T. S., Saucedo M. M., Prado E. N., Rosas R. y Aburto R. (2012). Evaluación fitoquímica y biológica de algunas plantas medicinales de la Cañada Oaxaqueña. 8va. Reunión Internacional de Investigación en Productos Naturales. Huatulco, Oaxaca. p. 299.

Osuna J. (2012). Aumentan enfermedades transmitidas por alimentos. *Noticias nuevas. Estatales, Sinaloa.* Disponible en: www.inforural.com.mx/spip.php?article89594 (Último acceso el 7 de diciembre del 2017).

Parrilla M. C., Vázquez J. L., Saldate E. O. y Nava L. M. (1993). Brotes de toxiinfecciones alimentarias de origen microbiano y parasitario. *Salud Pública de México*. 35(5):456-463.

Qaralleh H. N., Abboud M. M., Khleifat K. M., Tarawneh K. A. and Althunibat O. Y. (2009). Antibacterial activity *in vitro* of *Thymus capitatus* from Jordan. *Pakistán Journal of Pharmaceutical Sciences*. 22(3):247-251.

Rodríguez-Sauceda E. N. (2011). Uso de agentes antimicrobianos naturales en la conservación de frutas y hortalizas. *Revista Científica Ra Ximhai*. 7(1):153-170.

Rosas R., Gómez T., Ordaz E. A., Saucedo M., Criollo A. y Aburto R. (2012). Estudio fitoquímico y evaluación de la toxicidad contra *Artemia salina* de 5 plantas medicinales. 8va. Reunión Internacional de Investigación en Productos Naturales. Huatulco, Oaxaca. p. 303.

Syed F., Jahan R., Ahmed A. and Khan S. (2013). *In vitro* antimicrobial activities of *Glycyrrhiza glabra* and *Fagonia arabica*. *Journal of Medicinal Plant Research*. 7(30): 2265-2270.

Téllez O., Reyes M., Dávila P., Gutiérrez K., Téllez O., Álvarez R., González A., Rosas I., Ayala M., Hernández M., Murgía M. y Guzmán U. (2008). Las plantas del Valle de Tehuacán-Cuicatlan. Guía ecoturística. CONABIO. p. 12.

Xu W.-H., Jacob M. R., Agarwal A. K., Clark A. M., Liang Z.-S. and Li X.-G. (2009). Verbesinosides A−F,15,27-cyclooleanane saponins from the American native plant *Verbesina virginica*. *Journal of Natural products*. 72(6):1022–1027.

Zambecchi M. (2010). Evaluación de conocimientos en el manejo higiénico de los alimentos en consumidores. Tesis de Licenciatura. Universidad de Belgrano.