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Antibacterial and cytotoxicity activities and phytochemical analysis of three ornamental plants grown in Mexico

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Abstract*:* This study evaluates the antibacterial, cytotoxic activities, and phytochemical composition, of *Callistemon citrinu*s, *Hibiscus rosa-sinensis* and *Plumbago auriculata* leaves and flowers, three ornamental plants in Mexico. However, in other countries offers a range of other uses. Ethanol extracts of *C. citrinus* leaf and flower presented stronger antibacterial activity than the extracts obtained from the other two plants. *C. citrinus* leaf showed low cytotoxicity (LC_{50} <600 µg/mL) on the brine shrimp test, whereas the ethanol extracts of *H. rosa-sinensis* and *P. auriculata* leaves showed no cytotoxic activity. Flower extracts obtained from the three plants did no exhibit cytotoxicity. GC-MS analysis revealed that the ethanol extract of *P. auriculata* leaf contained lupeol triterpene and lupeol acetate, neither of them have been previously reported in this genus. Gamma sitosterol was present in the leaf and flower extracts of *P. auriculata*. Higher contents of linoleic and linolenic acids were found in extracts of *H. rosa-sinensis* leaves and flowers. The ability of the ethanol extracts of *C. citrinus* leaves and flowers to inhibit the growth of Gram-positive and Gram-negative bacteria indicates a potentially broad antimicrobial spectrum. Moreover, the absence of cytotoxicity suggests the potential use of this plant to treat microbial infections.

Key words: Antibacterial, *Artemia salina, Callistemon citrinus*, cytotoxic, lupeol.

INTRODUCTION

The genus *Callistemon* (commonly known as bottlebrush) belongs to the family Myrtaceae. Native of Australia and spread all over the world. *Callistemon* species have various applications. In several countries, *C. citrinus* is used for forestry, essential oil production and weed control, whereas in Mexico it is used as an ornamental plant. Additionally, the presence of biological properties in *Callistemon* species have been reported. For instance, *C. viminalis* exhibits anti-inflammatory and antifungal activities, while *C. rigidus* and *C.*

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citrinus show antibacterial and antioxidant activities (Goyal et al. 2012). Previously, we reported the presence of 74 and 64 terpenic compounds in leaves and flowers, respectively, of *C. citrinus* from Mexico (Petronilho et al. 2013).

The genus *Plumbago* (Plumbaginaceae) is distributed in tropical and subtropical regions. *P. auriculata* has potential therapeutic activities, including antiatherogenic, cardiotonic, hepatoprotective, neuroprotective and central nervous system stimulant properties (Tharmaraj and Antonysamy 2015). Paiva et al. (2005) reported plumbagin (2-methyl-5-hydroxy-1,4-naphthoquinone), epi-isoshinanolone, palmitic acid and sitosterol in the roots of *P. auriculata*. Ariyanathan et al. (2011) reported capensisone for the first time in the roots of *P. capensis.*

The genus *Hibiscus* (Malvaceae) is widely grown in tropical regions. In Mexico, only two species are well represented: *Hibiscus sabdariffa,* whose flower infusion is used to prepare beverages, and *Hibiscus rosa-sinensis* used only as an ornamental plant. In other countries, *H. rosa-sinensis* is used to treat bronchial catarrh, menorrhagia, hair loss and infertility. Additionally, antimicrobial, hypoglycemic, anticancer and antioxidant activities have been reported for this plant (Kumar et al. 2012). A phytochemical investigation reported the presence of alkaloids, flavonoids, phenols, tannins and terpenoids in leaf, stem and root extracts of *H. rosa-sinensis* (Divya et al. 2013). Ethanol extract of flower showed hexadecanoic acid, adipic acid and squalene as the major components (Bhaskar et al. 2011).

On the other hand, the indiscriminate usage of antibiotics has created the problem of antibiotic resistance; consequently, the search for new antimicrobial agents is a global concern. There is a particular revival of interest in herbal medicine assuming that medicine obtained from natural sources presents fewer side effects than synthetic pharmaceuticals. The aim of this study is to investigate and compare the antibacterial and cytotoxic activities and determine the chemical composition of the ethanol extracts of *C. citrinus, H. rosa-sinensis* and *P. auriculata* leaves and flowers, in an effort to add new uses of these plants in Mexico.

MATERIALS AND METHODS

PLANT MATERIAL

Leaves and flowers of *Callistemon citrinu*s, *Hibiscus rosa-sinensis* and *Plumbago auriculata* were collected in Morelia, Michoacán, Mexico: latitude 19°40'45" and longitude 101°22'0". Professor Patricia Silva, a plant taxonomist in the Department of Botany of the University of Michoacán, certified the taxonomic identity of the plant materials. Voucher specimens were deposited in the herbarium of the University of Michoacán (EBUM). The voucher numbers are as follows: *C. citrinus* is EBUM23538, *H. rosa-sinens*is is EBUM24073 and *P. auriculata* is EBUM24074.

PREPARATION OF PLANT EXTRACTS

Fresh leaves and flowers of *C. citrinu*s, *H. rosa-sinensis* and *P. auriculata* were collected during the morning in April. Then, they were crushed using a blender and macerated in 90% ethanol or water (1:10 w/v). The mixtures were left at room temperature and protected from light for a week. Afterwards, the ethanolic extracts were evaporated under reduced pressure at 45 °C using a rotary evaporator. Each concentrated crude extract, individually prepared, was weighed and stored at 4 ºC.

MICROORGANISMS

Bacterial strains were obtained from a local hospital in Morelia, Michoacán, Mexico. The microorganisms were Gram-negative: *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella pneumoniae*; and Gram-positive: *Staphylococcus*

aureus, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus.*

ANTIBACTERIAL ACTIVITY DETERMINATION

The antibacterial activity of *C. citrinu*s, *H. rosa-sinensis* and *P. auriculata* extracts was investigated by the agar diffusion method. Briefly, a suspension of the tested microorganism 10^8 cells/ml was spread on Petri plates with Luria agar. Dilutions of the stock solution containing 25, 50, 75 and 100 µg/mL were prepared in dimethyl sulfoxide (DMSO). Then, 5 µl of each dilution was used to test the antimicrobial activity. The plates were incubated for 24 hours at 37 ºC. The inhibition zone diameter was measured using a ruler and expressed in millimeters. The antibiotic cefotaxime (30 µg/mL) was used as a positive control and the solvent used for dissolving the extracts was a mixture 90% ethanol/water in DMSO served as negative control. Six replicates for each concentration and controls were done.

BRINE SHRIMP LETHALITY ASSAY

An *in vitro* lethality assay of brine shrimp nauplii (*Artemia salina*) was used to detect cell toxicity. Brine shrimp eggs were placed in artificial seawater (3.8% w/v sea salt in distilled water) and incubated at 24-28 ºC in front of a lamp. Eggs were hatched within 24-h period providing a large number of larvae (nauplii). Ten nauplii (97 µl) and 3 µl of the extracts dissolved in DMSO (1 – 1000 µg/mL) were placed in each well of an ELISA plate. The plate was incubated at room temperature for 24 h. The number of dead (non-motile) nauplii was determined by visual examination under a stereo microscope. Then, all nauplii were scarified by the addition of 100 µl of methanol to each well. After 20 minutes, the total number of nauplii/well were counted. Berberine sulfate was used as a positive control (IC_{50} 10 µg/mL), and DMSO was used as a negative control.

GC-EI-MS ANALYSIS

One microliter from the resultant extracts was injected into an Agilent Technologies 7890A gas chromatograph equipped with a 60 m x 250 µm (I.D.), 0.25 µm film thickness DB1MS fused silica capillary column (J&W Scientific Inc., Folsom, CA, USA) that was interfaced with an Agilent Technologies 5975C quadrupole spectrophotometer in MS detector with electron impact as the ionization source. A split injection ratio of 1:100 was used. The injection port temperature was set at 250 ºC. The oven temperature was programmed from 70 ºC to 300 ºC with an increase of 5 ºC/min and the transfer line was heated at 250 ºC. The ion source was set at 150 ºC and the quadrupole at 250 ºC. Helium was used as carrier gas with a flow of 1.0 ml/min and a column head pressure of 12 psi. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV, with a scan range of 50-500 *m/z* in a 2^{-s} cycle, in a full scan acquisition mode. When available, identification of volatile compounds was achieved by comparing the GC retention times and mass spectra with those of the standard compounds. In addition, the mass spectra of the samples were compared with the Wiley (G1035B; Rev D.02.00; Agilent Technologies, Santa Clara, CA, USA) and NIST Mass Spectral Search Program; version 2.0f (Nist Data Center, Gaithersburg, MD, USA) libraries reference spectral bank. All measurements were made with at least three replicates, each of them representing the analysis of one different aliquot of the extract. The GC peak area data obtained were used to estimate the relative content of each component. The samples of *H. rosa-sinensis* were pre-derivatized using trimethylsilylation.

RESULTS AND DISCUSSION

ANTIBACTERIAL ACTIVITY

Tables I and II display the antibacterial activity of the ethanol extracts of *C. citrinu*s, *H. rosa-sinensis* and *P. auriculata* leaves and flowers. *C.*

TABLE I
Antibacterial activity of ethanol extracts of *Callistemon citrinus, Plumbago auriculata* and *Hibiscus rosa-sinensis* leaves.

Bacterial strains	*C. citrinus*	*P. auriculata*	*H. rosa-sinensis*	Cefotaxime
	25 µg/mL	100 µg/mL	100 µg/mL	30 µg/mL
Escherichia coli	13.10 ± 0.37	8.00 ± 1.00	6.30 ± 1.15	16.00 ± 0.22
Salmonella typhimurium	14.10 ± 0.78	7.30 ± 0.57	6.30 ± 0.15	17.00 ± 0.49
Klebsiella pneumoniae	13.20 ± 0.98	10.60 ± 2.51	10.33 ± 0.57	22.00 ± 0.94
Staphylococcus aureus	14.10 ± 0.32	12.00 ± 1.73	6.60 ± 0.87	20.00 ± 0.26
Staphylococcus epidermidis	15.10 ± 0.67	7.33 ± 0.57	6.60 ± 0.59	22.00 ± 0.84
Staphylococcus saprophyticus	16.00 ± 1.00	11.00 ± 1.00	0.00	21.00 ± 0.59

Inhibition zone (mm); Values are given as mean ± SD (n=6). In the negative control, no inhibition was observed.

TABLE II
Antibacterial activity of ethanol extracts of *Callistemon citrinus, Plumbago auriculata* and *Hibiscus rosa-sinensis* flowers.

Bacterial strains	*C. citrinus*	*P. auriculata*	*H. rosa-sinensis*	Cefotaxime
	25 µg/mL	100 µg/mL	100 µg/mL	30 µg/mL
Escherichia coli	14.20 ± 0.83	9.60 ± 0.57	0.00	16.00 ± 0.22
Salmonella typhimurium	15.60 ± 0.43	9.60 ± 0.57	0.00	17.00 ± 0.49
Klebsiella pneumoniae	18.00 ± 0.52	11.00 ± 0.00	0.00	22.00 ± 0.94
Staphylococcus aureus	19.40 ± 0.49	17.60 ± 0.57	0.00	20.00 ± 0.26
Staphylococcus epidermidis	18.00 ± 1.30	9.60 ± 0.57	0.00	22.00 ± 0.84
Staphylococcus saprophyticus	17.20 ± 0.73	11.30 ± 2.30	0.00	21.00 ± 0.59

Inhibition zone (mm); Values are given as mean ± SD (n=6). In the negative control, no inhibition was observed.

citrinus flowers exhibited the strongest activity (as determined by the zone of inhibition) against Gram-negative and Gram-positive bacteria: *E. coli* (14.20 ± 0.83), *S. typhimurium* (15.60 ± 0.43), *K. pneumoniae* (18.00 ± 1.30 mm), *S. aureus* (19.40 ± 0.49 mm), *S. saprophyticus* (17.20 ± 0.73 mm) and *S. epidermidis* (18.00 ± 1.30 mm). Additionally, *C. citrinus* leaves exhibited good activity against *E. coli* (13.10 ± 0.37 mm), *Salmonella typhimurium* (14.10 ± 0.78 mm), *K. pneumoniae* (13.20 ± 0.98 mm), *S. aureus* (14.10 ± 0.32 mm), *S. saprophyticus* (16.00 ± 1.00 mm) and *S. epidermidis* (15.10 ± 0.67 mm).

It is well known that Gram-positive bacteria are more susceptible to antibiotics than Gram-negative bacteria. This is attributed to structural variations observed in the cell envelope between Gram-positive and Gram-negative bacteria (Palombo and Semple 2001). Fayemi et al. (2017) reported that 2% of methanolic extract of *C. citrinus* leaves inhibits the growth of *Listeria monocytogenes* in beef burger. Cock (2012) reported that *C. citrinus* leaf methanol extract was unable to inhibit the growth of *E. coli* and *K. pneumonia*, while the *C. citrinus* flower had only an effect against *K. pneumoniae.* Conversely, we found that the ethanol extracts of *C. citrinus* leaves and flowers exhibited strong antibacterial activities against Gram-negative, similar to previously reported results for the crude extracts of *C. linearis* (Haque et al. 2013). Scur et al. (2016) reported that aqueous extract and essential oil of *Psidium cattleianum* another Myrtaceae showed weak activity against *K. pneumoniae* and *S. epidermidis*. However, in the

present work, the aqueous extracts of *C. citrinus* did not show antibacterial activity. In addition, activity of solvents used as negative controls did not show inhibition.

As shown in Table I, *P. auriculata* leaf ethanol extract exhibited good activity against *S. aureus* (12.00 ± 1.73 mm), *S. saprophyticus* (11.00 ± 1.00 mm) and *K. pneumoniae* (10.60 ± 2.51 mm), while inhibition against *E. coli* (8.00 ± 1.00 mm), *Salmonella typhimurium* (7.3 ± 0.57 mm) and *S. epidermidis* (7.33 ± 0.57 mm) was moderate. These results are in agreement with the study of Tharmaraj and Antonysamy (2015). *P. auriculata* flower ethanol extract exhibited a stronger activity against *S. aureus* (17.60 ± 0.57 mm), good activity against *K. pneumoniae* (11.0 ± 0 mm) and *S. saprophyticus* (11.30 ± 2.30 mm) and moderate activity against *E. coli*, *S. typhimurium* and *S. epidermidis* (Table II). Aqueous extracts of *P. auriculata* leaves and flowers did not show antimicrobial activity.

Ahmad et al. (1998) reported that aqueous and alcoholic extracts from the roots of *P. zeylanica* showed antibacterial activity against *S. aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris* and against the yeast *C. albicans*. However, Jeyachandran et al. (2009) reported that methanol and chloroform extracts of *P. zeylanica* L. exhibited good activity against *E. coli*, *Salmonella typhy* and *S. aureus*, moderate activity against *K. pneumoniae*, *Serratia marcescens* and *Bacillus subtilis* and low activity against *Proteus vulgaris* and *Pseudomonas aeruginosas*. Paiva et al. (2003) found that plumbagin isolated from the roots of *P. scandens* completely inhibited the growth of *S. aureus* and *C. albicans*; however, it was ineffective against *E. coli* and *S. typhimurium*. Dhale and Markandeya (2011) showed that *P. zeylanica* ethanol and chloroform leaf extracts have antibacterial activity against two Gram-negative bacteria: *E. coli* and *Pseudomonas aeruginosa* and two Gram-positive bacteria: *S. aureus* and *Bacillus subtilis*.

In this study, the ethanol extract of *H. rosa-sinensis* leaf exhibited a good activity against *K. pneumoniae* (10.33 ± 0.57) and moderate activity against *E. coli* (6.30 ± 1.15), *S. typhimurium* (6.30 ± 0.15), *S. aureus* (6.60 ± 0.87) and *S. epidermidis* (6.60 ± 0.54). Nonetheless, it was unable to inhibit the growth of *S. saprophyticus* (Table I). Aqueous extracts of *H. rosa-sinensis* leaf and flower failed to inhibit bacterial growth. We found that the ethanol extract of *H. rosa-sinensis* red flower was unable to inhibit the growth of the bacteria tested (Table II). Meanwhile Mak et al. (2013) showed that aqueous and ethanol extracts of fresh *H. rosa-sinensis* flowers selectively inhibited the growth of *S. typhimurium* and *S. aureus*, respectively. Ruban and Gajalakshmi (2012) showed that methanol extracts of dried flowers presented a maximum zone of inhibition against *B. subtillis* and *E. coli*. The authors did not mention the flower color. However, Patel et al. (2012) studied the antimicrobial activity of five cultivars of *H. rosa-sinensis*, and they found differences in their effectiveness against *S. aureus*, *B. subtillis*, and *S. epidermidis* depending on the color of the flower red, yellow, orange, pink and white.

Based on the diameters of the inhibition zone observed for all microorganisms, *C. citrinus* leaves and flowers have stronger and broader spectrums of antimicrobial activities than those of *P. auriculata* and *H. rosa-sinensis*. Previously, we reported high levels of terpenoids in *C. citrinus*; thus, the antimicrobial activity may be attributed to these compounds (Petronilho et al. 2013). The ethanol extracts of *P. auriculata* and *H. rosa-sinensis* leaves and flowers not presented a minimal inhibitory concentration (MIC) as they were active only at 100 µg/mL. On the other hand, the ethanol extracts of *C. citrinus* leaves and flowers exhibited a MIC value of 25 µg/mL against all bacterial strains. This value is lower than the cefotaxime used as positive control, suggesting that the major compounds

present in the extracts possess antibacterial activity that could be used therapeutically.

CYTOTOXIC ACTIVITY

The cytotoxic activity of both flowers and leaves extracts of *C. citrinus, P. auriculata* and *H. rosa-sinensis* were tested *in vitro* against brine shrimp nauplii using the procedure described by Meyer et al. (1982). It was important to test the cytotoxic activity of the plants studied, in order to elucidate completely the biological response to their extracts. The brine shrimp lethality assay is a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity, which, in most cases, is correlated with cytotoxic and anti-tumor properties (Maridass 2008). Ali et al. (2011) reported cytotoxic activity of the methanol *C. citrinus* crude fruit extract with an LC_{50} of 65 µg/mL.

In the results presented in Table III, *C. citrinus* flower extract did not show cytotoxic activity against brine shrimp nauplii, while the leaf extract showed a LC_{50} <600 µg/mL. A plot of the relationship between shrimp nauplii killed and the concentrations of the extract allows us to determinate the LC_{50} value of the plant extract. A regression analysis then determines the best-fit line. In a toxicity evaluation of plant extracts using a brine shrimp lethality bioassay, Ayo et al. (2007) reported that LC_{50} values smaller than 1000 µg/mL are considered bioactive. Therefore, *C. citrinus* flower and leaf extracts can be considered non-toxic, justifying the continuation of biological experimentation to evaluate their pharmacological effects. Similarly, the extracts of *P. auriculata* and *H. rosa-sinensis* leaves and flowers did not show significant values in the mortality percentage among the different concentrations (Table III). In addition, DMSO used as negative control did not show cytotoxic activity.

CHEMICAL COMPOSITION

Jazet et al. (2009), Oyedeji et al. (2009) and Silva et al. (2010) studied the chemical composition of *C. citrinus* from Cameroon, South Africa and Brazil by GC-MS. They reported variances in the yields and chemical constituents that they attributed to a difference in geographical and environmental factors. Nevertheless, all studies showed the presence of three monoterpenenes: 1,8-cineole, α-pinene and 4-terpineol.

In a previous study, our group found that the major components of the leaf extract of *C. citrinus* from 4-year-old plants were 1,8-cineole (14.73%), limonene (9.12%), α-terpineol (5.41%) and

TABLE III
Mortality rate % of brine shrimp nauplii with different concentrations of *Callistemon citrinus, Plumbago auriculata* and *Hibiscus rosa-sinensis* extracts.

	C. citrinus		*P. auriculata*		*H. rosa-sinensis*	
Conc µg/mL	**Leaf**	**Flower**	**Leaf**	**Flower**	**Leaf**	**Flower**
1000	82	0	23	22	12	5
900	79	0	15	17	11	5
800	76	0	13	16	11	4
700	70	0	8	14	11	4
600	56	0	5	13	8	2
500	49	0	5	12	8	2
100	3	0	3	10	7	2
10	0	0	3	10	5	0
1	0	0	2	7	5	0

Berberine sulfate was used as a positive control (IC_{50} 10 µg/mL).

spathulenol (4.51%), whereas the major components in flower extracts of plants of the same age were *p*-cymene (9.45%), limonene (9.23%), 1,8-cineole (6.98%) and spathulenol (6.97%) (Petronilho et al. 2013). Concentrations of terpenoids are generally higher in reproductive structures and the foliage during and immediately after flowering.

The antibacterial activities observed here could be attributed to the presence of some of the reported compounds that have been associated with this activity. 1,8-cineole, displays antimicrobial (Hendry et al. 2009) and anti-inflammatory activities (Ganjewala and Luthra 2010). Limonene has antibacterial effects (Soković et al. 2010) and protective activity against many types of cancer (Gershenzon et al. 2000).

In this study, the ethanolic extracts of *C. citrinus* of leaf and flower exhibited strong antibacterial activity, as reported by Larayetan et al. (2017) for essential oil of *C. citrinus*. This antimicrobial activity could be associated with the high percent of 1,8-cineole, limonene and spathulenol that are the major constituents of the leaf and flower. Antibacterial activities of these compounds have been reported by other studies (Bevilacqua et al. 2010, Boligon et al. 2014); other components such 4-terpineol, linalool and α-terpinolene contributed to antibacterial activity as well (Park et al. 2012). It is also possible that some components will be involved in some type of synergism as Simsek and Duman (2017) for 1,8-cineole with chlorhexidine gluconate against *S. aureus*, *E. coli* and *K. pneumoniae*. Leaf and flower extracts of 20–year-old *C. citrinus* plants present high levels of these compounds (Petronilho et al. 2013).

The high levels of 1,8-cineole, limonene, α-terpinolene and spathulenol, in *C. citrinus* suggest that it may constitute an alternative commercial source of these compounds. Finally, evidence of antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria from the ethanol extracts obtained from the leaves and flowers of *C. citrinus*, along with the lack of cytotoxic effects against *A. salina*, shows the potential of this plant for medicinal uses in Mexico.

This is the first study of a phytochemical screening using GC-MS analysis in *Plumbago auriculata*. This analysis identified eight compounds in the leaf and flower extracts (Figure 1, Table IV). The major constituents found in the leaves were phytol (31.60%), lupeol acetate (20.50%), lupeol (7.35%), ethyl α-D-glucopyranoside (4.55%), gamma-sitosterol (3.17%) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one or DDMP (1.50%). The present study revealed the presence of lupeol triterpene and lupeol acetate, both of which have not been previously reported in this genus. Additionally, the steroid gamma sitosterol has only been reported in the roots of *Plumbago* species (Paiva et al. 2005). Na et al. (2009) reported that lupeol and lupenone inhibit protein tyrosine phostase 1B, which appears to be an attractive target of new drugs in development for type 2 diabetes and obesity. Gallo and Sarachine (2009) also reported anticancer, antiprotozoal, chemopreventive and anti-inflammatory properties.

Concerning *P. auriculata* flowers, their major constituents were gamma-sitosterol (23.30%), phytol (21.93%), caffeine (4.66%), DDMP (2.47%), theobromine (1.97%) and ethyl α-D-glucopyranoside (1.14%). Gamma-sitosterol has demonstrated strong cytotoxity on breast and lung cancer cell (Sundarraj et al. 2012). Phytol is a diterpenes that presents antimicrobial and anticancer activities (Saeb and Gholamrezaee 2012). In our study, we found that phytol is the major compound in the leaves and flowers of *P. auriculata* and both extracts have the highest antibacterial activity against *S. aureus*. Santos et al. (2013) reported that phytol inhibit the growth of *S. aureus,* and presents antinociceptive and antioxidant activities. Therefore, this compound is probably related to its antibacterial activity. The presence of caffeine

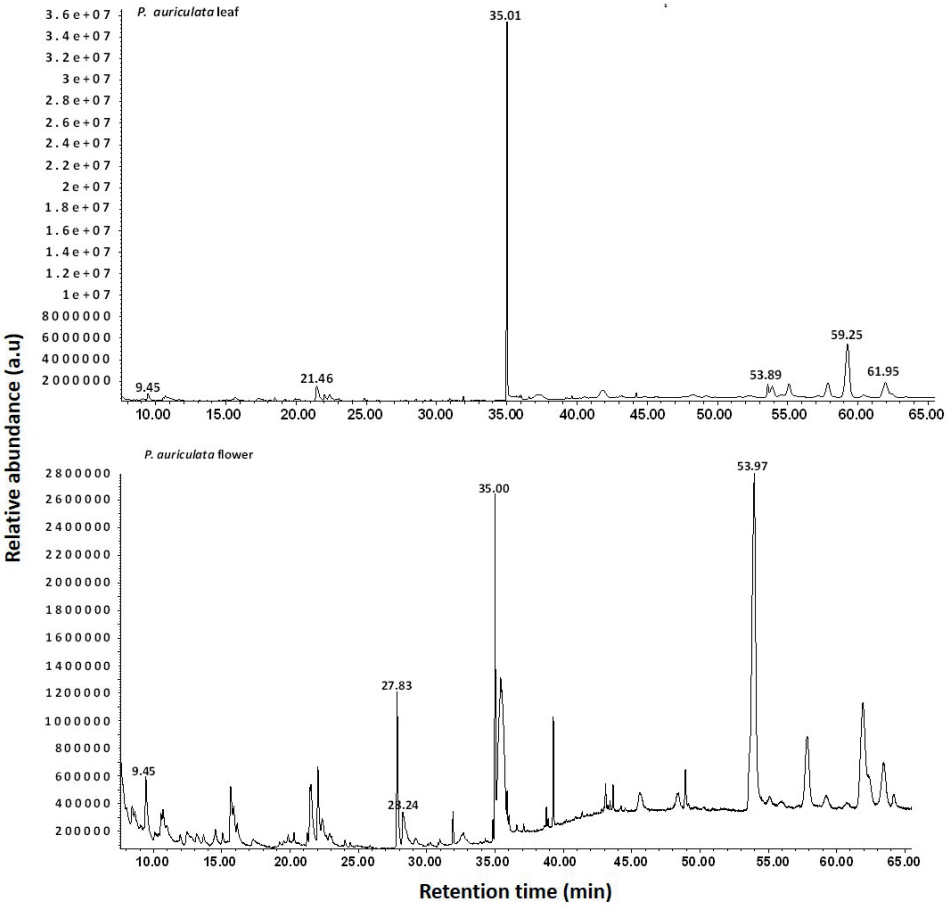

Figure 1 - Gas chromatogram of *Plumbago auriculata,* peak labels correspond to major compound identification given in Table IV.

and theobromine in the flower might be useful for making alternative tea beverages.

Pyo et al. (2004) showed that 5-hydroxymethyl-2-furancarboxaldehyde has anti-platelet activity. Several researchers found important biological activities of DDMP such anti-alpha-glucosidase activity in patients with diabetes mellitus, (Quan et al. 2003), reactive oxygen scavenging activity (Hwang et al. 2013) and anti-tumor activity (Ban et al. 2007).

GC-MS analysis of the ethanol extracts of *H. rosa-sinensis* leaves and flowers led to the identification of 16 compounds in the leaves and seven in the flowers. Figure 2 and Table V display the identified constituents with their respective percentages and retention time. The major constituents of the leaves were fructose (18.60%), 9,12-octadecadienoic acid (10.36%), 9,12,15-octadecatrienoic acid (7.67%) and glycerol (6.18%), while the main constituents of the flowers were 9,12-octadecadienoic acid (14.65%), 9,12,15-octadecatrienoic acid (11.67%), fructose (10.04%) and *n*-hexadecanoic acid (4.13%). There are some studies on the fatty-acid composition of the seeds of several *Hibiscus* species (Mohamed et al. 2007).

There are numerous varieties, cultivars and hybrids of *Hibiscus rosa-sinensis*. Their flowers colors can range from white, yellow, orange, scarlet and shades of pink, and can contain both single and double sets of petals. Patel et al. (2012) showed that the levels of some compounds were different in the

TABLE IV
Screening of the compounds identified in ethanol extracts of *Plumbago auriculata* L. leaves and flowers, using GC-MS.

tr (min)[a]	*m/z* fragmentation pattern	Compounds	Percentage in leaf (%)[b*]	Percentage in flower (%)[b*]
9.44	144 (37), 101 (31), 73 (14), 72 (20), 55 (25), 45 (25), 44 (76), 43 (100), 42 (9), 41 (5)	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran4-one	1.50 (8)	2.47 (4)
10.56	126 (78), 125 (15), 109 (14), 97 (100), 69 (33), 53 (15), 51 (12), 41 (73), 39 (36), 29 (17)	5-Hydroxymethyl-2-furancarboxaldehyde	0.29 (4)	0.54 (5)
21.46	75 (21), 74 (16), 73 (34), 71 (15), 60 (100), 57 (25), 47 (25), 45 (21), 43 (40), 42 (43)	Ethyl α-D-glucopyranoside	4.55 (9)	1.14 (8)
27.83	195 (10), 194 (100), 193 (14), 110 (9), 109 (74), 82 (30), 81 (8), 67 (46), 55 (43), 42 (15)	Caffeine	n.d	4.66 (2)
28.24	181 (10), 180 (100), 137 (13), 109 (24), 82 (23), 67 (35), 55 (29), 40 (7), 28 (10), 15 (10)	Theobromine	n.d	1.97 (5)
31.91	101 (70), 88 (100), 73 (23), 71 (19), 70 (15), 69 (23), 57 (40), 55 (50), 43 (57), 41 (48)	*n*-Hexadecanoic acid	0.40 (2)	0.67 (2)
35.01	123 (18), 81 (22), 71 (100), 69 (24), 68 (20), 57 (33), 56 (17), 55 (26), 43 (38), 41 (26)	Phytol	31.60 (5)	21.93 (6)
53.97	414 (35), 145 (32), 107 (44), 95 (45), 81 (48), 69 (36), 57 (56), 55 (67), 43 (100), 41 (50)	γ-Sitosterol	3.17 (3)	23.32 (6)
59.25	424 (63), 205 (100), 123 (43), 121 (47), 109 (76), 108 (39), 107 (46), 95 (67), 93 (40), 81 (49)	Lupenone	20.50 (9)	n.d
62.95	109 (61), 95 (72), 93 (68), 81 (76), 69 (73), 68 (98), 67 (78), 55 (87), 43 (100), 41 (67)	Lupeol	7.35 (3)	n.d

[a] tr (min): retention times (in minutes) of each compound identified.
[b] Percentage of each component is calculate as peak area of analyte divide by total peak area
n.d. (not detected).
*Relative Standard Deviation (RSD, % in parentheses).

leaves and stems from five cultivars with different flower colors. Prasad (2014) showed the presence of different phytochemical constituents in nine different varieties of *Hibiscus* species. These huge varieties in *H. rosa-sinensis* make it difficult to compare with the compounds previously reported in these plants.

CONCLUSION

This study found strong antibacterial activities against to Gram-negative and Gram-positive bacteria and no cytotoxity activities of *C. citrinus* extracts that supports the potential use of phytomedicine in microbial infections. The antibacterial property may be attributed to terpenes present in the ethanolic extracts of leaves and flower. Further studies are required to isolate and identify active compounds. The phytochemical screening using GC-MS analysis in *Plumbago auriculata* revealed for the first time high level of lupeol, lupeol acetate and DDMP, compounds reported to have pharmaceutical properties. Moreover, *H. rosa-sinensis* showed potential application as a nutritional source of essential fatty acids. Finally, the extracts of the three plants tested seemed to be innocuous on short-term use.

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TABLE V
Screening of the compounds identified in ethanol extracts of *Hibiscus rosa- sinensis* leaves and flowers using GC-MS.

tr[a]	***m/z* fragmentation pattern**	**Compounds**	**Leaf (%)[b*]**	**Flower (%)[b*]**
7.40	147 (20),144 (16), 133 (15), 132 (92), 116 (100), 103 (19), 75 (30), 73 (92), 57 (17), 45 (15)	Serine	0.87 (4)	n.d
7.76	218 (17), 206 (13), 205 (63), 147 (57), 133 (16), 117 (28), 103 (30), 75 (11), 73 (100), 45 (10)	Glycerol	6.18 (5)	n.d
8.72	247 (10), 246 (20), 245 (100), 147 (35), 143 (12), 133 (7), 83 (8), 75 (21), 73 (45), 45 (12)	Fumaric acid	0.89 (7)	n.d
12.28	258 (7), 230 (8), 158 (5), 157 (15), 156 (100), 147 (19), 75 (10), 74 (7), 73 (78), 45 (13)	Proline	0.42 (2)	n.d
20.06	465 (21), 375 (38), 347 (40), 363 (43), 275 (14), 274 (32), 273 (100), 147 (69), 75 (15), 73 (90)	Isocitric acid	1.52 (3)	n.d
20.35	437 (23), 219 (12), 218 (22), 217 (100), 205 (15), 204 (25), 147 (27), 103 (11), 75 (18), 73 (94)	Fructose	18.60 (5)	10.04 (8)
21.18	319 (13), 219 (9), 218 (21), 217 (100), 205 (7), 191 (21), 147 (20), 75 (7), 74 (7), 73 (82)	Galactose	1.13 (2)	2.04 (4)
23.46	101 (56), 89 (14), 88 (100), 73 (16), 69 (13), 57 (22), 55 (23), 43 (36), 41 (27), 29 (23)	*n*-Hexadecanoic acid	1.31 (3)	4.13 (7)
23.75	321 (13), 320 (27), 319 (94), 307 (27), 217 (37), 206 (14), 205 (72), 147 (34), 103 (31), 73 (100)	Glucitol	0.18 (7)	n.d
24.40	218 (7), 217 (27), 206 (8), 205 (20), 204 (100), 192 (7), 191(39), 147 (17), 129 (6), 73 (55)	Talose	2.64 (3)	2.04 (2)
25.70	339 (28), 338 (100), 323 (43), 308 (25), 293 (13), 249 (43), 219 (15), 75 (27), 73 (86), 45 (18)	Cinnamic acid	0.44 (6)	n.d
25.91	319 (13), 318 (30), 305 (22), 217 (26), 204 (13), 191 (18), 147 (32), 129 (7), 74 (9), 73 (100)	Inositol	2.98 (7)	n.d
27.17	96 (48), 95 (68), 82 (57), 81 (92), 69 (45), 68 (61), 67 (100), 55 (77), 54 (53), 41 (71)	9,12-Octadecadienoic acid	10.36 (8)	14.65 (4)
27.28	108 (43), 95 (52), 93 (52), 91 (31), 81 (44), 80 (41), 79 (100), 67 (63), 55 (46), 41 (40)	9,12,15-Octadecatrienoic acid	7.67 (5)	11.67 (7)
29.22	341 (90), 342 (27), 145 (42), 132 (58), 129 (38), 117 (95), 75 (78), 73 (100), 55 (26), 43 (35)	*n*-Octadecanoic acid	2.56 (3)	1.97 (6)

[a] tr (min): retention times (in minutes) of each compound identified.
[b] Percentage of each component is calculated as peak area of analyte divided by total peak area
n.d. (not detected).
*Relative Standard Deviation (RSD, % in parentheses).

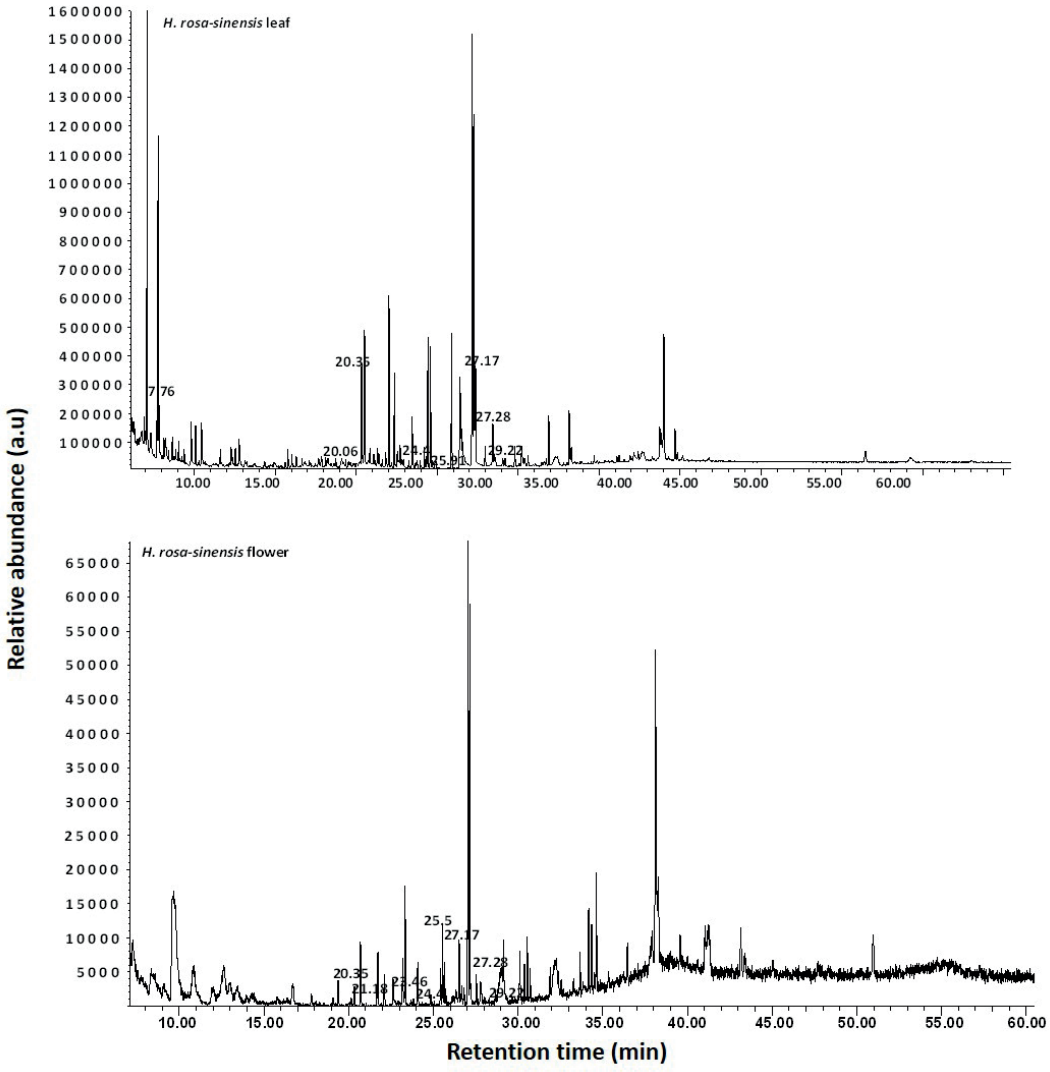

Figure 2 - Gas chromatogram of *Hibiscus rosa-sinensis*, peak labels correspond to major compound identification given in Table V.

AUTHOR CONTRIBUTIONS

Dra. Patricia Ríos-Chávez was involved in the manuscript preparation, designed and supervised this research. Student Jordy Perez-Gonzalez performed the laboratory tests; Dr. Rafael Salgado-Garciglia supervised cytotoxity experimentation; MSc. Enrique Ramirez-Chavez and Dr. Jorge Molina-Torres supervised phytochemical analysis; Miguel Martinez-Trujillo and Dra. Yazmin Carreon-Abud supervised antibacterial experimentation. All authors approved the manuscript.

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