GC-MS analysis of phytochemical compounds of *Opuntia megarrhiza* (Cactaceae), an endangered plant of Mexico

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ABSTRACT

*Opuntia megarrhiza* is an endemic plant used in Mexican traditional medicine for the treatment of bones fractures in humans and domestic animals. One of the most used technique for the detection and characterization of the structure of phytochemical compounds is the Gas Chromatography Coupled to Mass Spectrometry. The goals of the present study were to identify and characterize the phytochemical compounds present in wild individuals of *O. megarrhiza* using this analysis. We used chloroform and methanol extracts from cladodes, and they were analyzed by gas chromatography-electron impact-mass spectrometry. We obtained 53 phytochemical compounds, 19 have been previously identified with some biological activity. Most of these compounds are alkanes, alkenes, aromatic hydrocarbons, fatty acids, and ketones. We detected some fragmentation patterns that are described for the first time for this species. The variety of metabolites presents in *O. megarrhiza* justifies the medicinal use of this plant in traditional medicine and highlight it as a source of phytochemical compounds with potential in medicine and biotechnology.

INTRODUCTION

The members of Cactaceae represent a diverse evolutionary lineage endemic to America, over 1,450 species belonging to ca. 127 genera (*Barthlott & Hunt, 1993; Hunt, Taylor & Charles, 2006; Hernández-Hernández et al., 2011*). They are successful plants adapted to arid and semiarid environments, where the conditions imply a constant stress, so they have developed different phytochemical compounds with an important biological activity such as alkaloids, amino acids, antioxidant phenol components (betalains and flavonoids), carotenoids, coumarins, esters, fibers, phytosterols, tannins, terpenes,
tocopherols, and vitamins C and E (Piattelli, Minale & Prota, 1965; Stintzing, Schieber & Carle, 2001; Strack, Vogt & Schliemann, 2003; Paiz et al., 2010; Sim et al., 2010; Osorio-Esquível et al., 2011; Harlev et al., 2012; Aruwa, Amoo & Kudanga, 2018; Araújo et al., 2021). Bioactive phytochemical compounds are of great interest since their possible applications in biotechnology and industry, and they are usually categorized into phenolic and non-phenolic compounds and pigments (Martins et al., 2011; Aruwa, Amoo & Kudanga, 2018; Araújo et al., 2021). Some of them have nutritional benefits (Kris-Etherton et al., 2002; Kudanga, Nemadziva & Le Roes-Hill, 2017; Araújo et al., 2021; Yu et al., 2021), pharmaceutical applications (Aruwa, Amoo & Kudanga, 2018; Araújo et al., 2021; Patra et al., 2021; Yu et al., 2021), and are used in the production of nutraceuticals (Gil-Chávez et al., 2013; Aruwa, Amoo & Kudanga, 2018; Yu et al., 2021), in novel food formulations (Gurrieri et al., 2000; Pawar, Killedar & Dhuri, 2017; Aruwa, Amoo & Kudanga, 2018; Araújo et al., 2021), and for animal feed supplementation (Ennouri et al., 2006; Aruwa, Amoo & Kudanga, 2018; Araújo et al., 2021).

The species of genus *Opuntia* (Cactaceae) are native of Mexico, where they originated and diversified (Barthlott & Hunt, 1993; Reyes-Agüero, Aguirre & Carlín, 2004). Different cultures, ancient and modern, have used them as fuel, forage, fences, food, and particularly in traditional medicine (González-Durán, Riojas & Arreola, 2001; Reyes-Agüero, Aguirre-Rivera & Hernández, 2005; Andrade-Cetto & Wiedenfeld, 2011). Several *Opuntia* species have the ability to synthesize molecules with unique and complex structures with therapeutical potential (Shedbalkar et al., 2010; Bargougui, Le Pape & Triki, 2013; Weli et al., 2019). For example, *Opuntia dillenii* (Ker Gawl.) Haw. has beneficial effects for the human health as anti-inflammatory, analgesic, hypoglycemiant, hypocholesterolemiamt, and antioxidant (Perfumi & Tacconi, 1996; Park et al., 2001; Ghasemzadeh & Ghasemzadeh, 2011). Other edible species like *Opuntia ficus-indica* (L.) Mill. present antioxidant and antiproliferative activities useful in colon cancer (Serra et al., 2013; Yeddes et al., 2013a; Yeddes et al., 2013b), and have nutraceutical, anticarcinogen, and antiviral properties useful in the digestive processes, reducing the risks of obesity, gastrointestinal suffering and high levels of cholesterol (Feugang, 2006; Bensadón et al., 2010). Usually, the used parts are the fruit, stem, cladode, and root (Estrada-Castillón et al., 2012). It has been estimated that plants have ca. 200,000 different metabolites, primary and secondary (Pichersky & Gang, 2000; Fiehn, 2001; Fiehn, 2002), like amino acids, fatty acids, carbohydrates, lipids, and more (Velumurugan & Anand, 2017; Banakar & Jayaraj, 2018). Several studies in *Opuntia* have focused on the analysis of phytochemical compounds as alkaloids, carotenoids, flavonoids, phenols, and vitamins C and E in different plant species in order to discover and produce new drugs for several illnesses (Yahia & Mondragon-Jacobo, 2011; Weli et al., 2019), as well as nutritional supplements since they provide metabolites, mineral, and vitamins essential for the human organism (Caballero-Gutiérrez & Gonzáles, 2016). In the agriculture, some of these compounds are used for the control of phytopathogen microorganisms (De Corato et al., 2010). Some other, are used industrially to produce detergents, cosmetics and dermatological products, solvents, lubricants, textiles, and others (Kim et al., 2019).
The main chemical analysis for the detection and characterization of the structure of phytochemical compounds are the Thin-Layer Chromatography, the UV-Vis spectrophotometry, the Nuclear Magnetic Resonance, the liquid chromatography-mass spectrometry (LC-MS), and the Gas Chromatography Coupled to Mass Spectrometric (GC-MS) ([Robertson, 2005]; [Marquet, 2012]). The last one is the most used in metabolomics research since it is a very selective technique for the detection and characterization of metabolites ([Fiehn, 2016]). The LC-MS is a robust technique for general unknown screening, however its major drawback is the lack of universal reference libraries obtained with different instrument types ([Marquet, 2012]), as in GC-MS. The GC-MS together with the metabolomic analysis are a key for the profiling of metabolites in plants since they perform the qualitative and quantitative characterization of all the molecules (metabolites) present in their cells ([Harrigan & Goodacre, 2012]).

*Opuntia megarrhiza* Rose is a species endemic to Mexico, locally known as “nopal camote” (Fig. 1). It is restricted to some regions of the Chihuahuan Desert, particularly in the State of San Luis Potosi ([Hernández, Gómez-Hinostrosa & Bárcenas, 2001]), and it is listed as endangered in the IUCN Red List ([Hernández et al., 2013]). It grows in different habitats as xerophytic scrubs, oak forest, and other mountain forests ([Hernández, Gómez-Hinostrosa & Bárcenas, 2001]; [Segura-Venegas & Rendón-Aguilar, 2016]). This species is characterized by its massive roots, which are succulent, gross, and deeply buried in ground, 30 to 60 cm long and 5 to 10 cm diameter ([Bravo-Hollis & Sánchez-Mejorada, 1991]; [Figure 1 *Opuntia megarrhiza* from wild populations. (A) Herbarium specimen from the studied locality; (B) Flowering adult plant; (C) Adult plant showing part of its characteristic massive roots. Photos (A) and (B) by J.A. De Nova, (C) by P. Delgado. Full-size DOI: 10.7717/peerj-ochem.5/fig-1])

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The cladodes are relatively small contrasting with other *Opuntia* species. The flowers are yellowish-green to pink, 3 to 5.5 cm long and 2.5 to 6 cm diameter at anthesis. Fruits are ovoid, 2.5 cm long, and the seeds are ca. 4 mm diameter (*Bravo-Hollis & Scheinvar, 1999; Hernández, Gómez-Hinostrosa & Bárcenas, 2001*).

*Opuntia megarrhiza* is used by locals in the treatment of bones fractures, both animals and humans (*Hernández, Gómez-Hinostrosa & Bárcenas, 2001*). In Cerro El Borrego (Guadalcazar, San Luis Potosi), the root is applied directly in the fractures, but in other localities like Xoconoctle (Zaragoza, San Luis Potosí) people use cladodes or the whole plant, and sometimes is mixed with parts of *Cylindropuntia* spp., to make a paste that is applied with bandages in the injury (*Segura-Venegas & Rendón-Aguilar, 2016*). Given the ethnopharmacological value of *O. megarrhiza*, previously highlighted by the empirical traditional medicine, the goals of the present study were to identify and characterize the phytochemical compounds present in cladodes from wild individuals of the species, using GC-MS. There are not previous studies about the bioactive phytochemical compounds in this species, so its phytochemical characterization could contribute to increase the knowledge of the species and its potential biotechnological applications, but also improving bio-valorization and environment.

**MATERIALS AND METHODS**

**Sampling**

All the protocols involving plants were adhered to relevant ethical guidelines and permissions for plant sampling from herbaria JAAA and SLPM (SGPA/DGGFS/712/0501/18) were used. Non-lethal samples of cladodes were obtained from ten wild individuals, sampling randomly, of *Opuntia megarrhiza* located at Cerro San Pedro (San Luis Potosí). We do not collect whole plants and the identification was conducted in field by taxonomists Arturo De Nova and Eleazar Carranza form herbarium SLPM and registered with photographs then confirmed with Research Grade in iNaturalist (46236747, 46978331). The Fig. 1A show a reference herbarium voucher from the studied locality for identification (SLPM 22132).

**Extracts**

The extractions were conducted at Laboratorio de Biotecnología de la Facultad de Agronomía y Veterinaria, UASLP and Centro Regional de Biociencias, UASLP. Cladode fragments were cleaned using brushes and distilled water to eliminate possible associated microorganisms. We used 95 g of sample, which was macerated to make a semisolid paste, then either 25 ml of methanol (MeOH) for extraction 1 or 25 ml of chloroform (CHCl₃) for extraction 2. These solutions were vortex mixed one hour to prevent conglomeration and sedimentation of small particles. The extracts were filtered three times using Whatman paper, grade 1, 5 and 6 in order, in a vacuum chamber. The volumes were adjusted to 10 mg/ml for all extracts. Subsequently, we made a dilution for each solution using acetone as solvent (1:1): (1) acetone-chloroform ((CH₃)₂CO/CHCl₃), and (2) acetone-methanol ((CH₃)₂CO/MeOH). Before depositing in a vial, extracts were filtered through a polytetrafluoroethylene (PTFE) or polyvinylidene (PVDF) membranes.
(with different hydrophobic adsorption ranges and size exclusion pores), and transferred to a vial for analysis in the GC-MS.

**GC-MS analysis scan mode**

This process was conducted at Coordinacion para la Innovacion y la Aplicacion de la Ciencia y la Tecnologia, UASLP. We used the Hewlett Packard gas chromatograph HP 6890, coupled to mass spectrometry detector with electronic impact HP 5973 (Agilent Technologies, Palo Alto, CA, USA). The column exerted was an absorbed silica capillary column of 95% methyl-poly-siloxane and 5% phenyl (HP-5MS; length: 60 m, diameter: 0.25 mm and film 0.25 µm). Helium was used as carrier gas, and the flow rate was 1 ml/min. The GC oven temperature gradient was: 60 °C (hold for 3 min) initially, then increased 5 °C each minute until 300 °C, this final temperature was hold 5 minutes. The transfer line temperature was 280 °C. The ion source temperature was 230 °C and the MS was scanned at 50 to 550 mass range. The essays were processed in the ChemStations software (Houston, TX, USA) to generate the chromatograms for the interpretation of the spectra.

**Identification of phytochemical compounds**

The identification was performed by comparing the spectrum of unknown compounds with the spectrum of known compounds in the National Institute of Standards and Technology (NIST98) mass spectral library. Compound name, synonyms, molecular weight, and the mass spectrum for each compound were obtained from NIST Standard Reference 69 and PubChem databases to confirm the GC-MS results. Nomenclature for all compound names was standardized with IUPAC rules. Relative quantification of the compounds present in each sample was obtained from the relative area of the peaks in the chromatograms. Biological activity for each identified compound was obtained with an exhaustive search in scientific publications and from the Dr. Duke phytochemical and ethnobotanical databases. The identity of five compounds showing similarity above 90% (as recommended in Mangas-Marín et al., 2018) with phytochemical compounds previously reported with biological activity was verified by using the commercial pure standards: The used standards were 1,3-benzothiazole (Purity: minimum 97.0%), hentriacontane (Purity: minimum 98.0%), methyl hexadecanoate (Purity: minimum 98.5%), triacontane (Purity: minimum 98.0%), were purchased from Sigma (St. Louis, MO, USA). All standards were diluted in acetone as the final solvent at a concentration of 5 ppm and were analyzed in the GC-MS using the same parameters than in the samples.

**RESULTS**

A total of 53 phytochemical compounds were detected based on the analyses of the obtained chromatograms (Tables 1–4). The (CH₃)₂CO/MeOH extract showed 11 peaks with the PVDF membrane filter and 12 with the PTFE. The (CH₃)₂CO/CHCl₃ extract showed 23 peaks with PVDF and seven with PTFE (Fig. 2).
The analysis from (CH$_3$)$_2$CO/MeOH extract with PVDF membrane filter showed the presence of 11 phytochemical constituents. Five alkanes: 2,3,6,7-tetramethyloctane (1.17%), 2,3,5,8-tetramethyldecane (1.78%), heptadecane (1.03%), 7-methylhexadecane (1.0%), 2,3,6-trimethyldecane (1.80%). One aromatic hydrocarbon: 1,3-di-$t$-butylbenzene (4.19%). One ester: methyl (2$R$)-5-oxo-2-propan-2-ylhexanoate (2.78%). One ketone: 4-propan-2-ylcyclohexane-1,3-dione (2.19%). One Halogenated hydrocarbons: 1,54-dibromotetrapentacontane (1.41%). Two fatty acids: hexadecanoic acid (4.67%), octadecanoic acid (2.47%) (Fig. 2A, Table 1).

| Peak No. | RT (min) | Name of the compound | Molecular weight (g/mol) | Peak area (%) | Similarity (%) | Molecular formula | Compound nature |
|----------|----------|-----------------------|--------------------------|--------------|---------------|------------------|-----------------|
| 1        | 8.21     | 2,3,6,7-tetramethyloctane | 170.33                  | 1.17         | 64            | C$_{12}$H$_{26}$  | Alkane          |
| 2        | 8.33     | 2,3,5,8-tetramethyldecane | 198.38                  | 1.78         | 64            | C$_{14}$H$_{30}$  | Alkane          |
| 3        | 15.35    | 1,3-di-$t$-butylbenzene  | 190.32                  | 4.19         | 95            | C$_{14}$H$_{32}$  | Aromatic Hydrocarbon |
| 4        | 16.74    | methyl (2$R$)-5-oxo-2-propan-2-ylhexanoate | 186.25              | 2.78         | 53            | C$_{16}$H$_{16}$O | Ester            |
| 5        | 17.20    | 4-propan-2-ylcyclohexane-1,3-dione | 154.21              | 2.19         | 60            | C$_{10}$H$_{14}$O$_2$ | Ketone          |
| 6        | 17.28    | heptadecane            | 240.46                  | 1.03         | 59            | C$_{17}$H$_{36}$  | Alkane          |
| 7        | 21.25    | 7-methylhexadecane     | 240.46                  | 1.00         | 72            | C$_{17}$H$_{36}$  | Alkane          |
| 8        | 22.69    | 2,3,6-trimethyldecane  | 184.36                  | 1.80         | 64            | C$_{13}$H$_{28}$  | Alkane          |
| 9        | 31.75    | hexadecanoic acid      | 256.42                  | 4.67         | 97            | C$_{16}$H$_{32}$O$_2$ | Fatty acid      |
| 10       | 35.43    | octadecanoic acid      | 284.47                  | 2.47         | 93            | C$_{18}$H$_{36}$O$_2$ | Fatty acid      |
| 11       | 46.72    | 1,54-dibromotetrapentacontane | 917.2                | 1.41         | 76            | C$_{34}$H$_{108}$Br$_2$ | Halogenated hydrocarbon |

Notes:
1. Percentage of similarity to the reference spectrum of the NIST library.
2. RT, retention time.

| Peak No. | RT (min) | Name of the compound | Molecular weight (g/mol) | Peak area (%) | Similarity (%) | Molecular formula | Compound nature |
|----------|----------|-----------------------|--------------------------|--------------|---------------|------------------|-----------------|
| 1        | 8.33     | 4-methyldecane        | 156.30                   | 1.44         | 64            | C$_{11}$H$_{24}$  | Alkane          |
| 2        | 15.35    | 1,3-$d$-tert-butylbenzene | 190.32                | 3.53         | 95            | C$_{14}$H$_{32}$  | Aromatic Hydrocarbon |
| 3        | 17.20    | 4-propan-2-ylcyclohexane-1,3-dione | 154.21              | 1.84         | 53            | C$_{14}$H$_{22}$O$_2$ | Ketone          |
| 4        | 21.61    | heptacosane           | 380.73                   | 0.98         | 83            | C$_{27}$H$_{56}$  | Alkane          |
| 5        | 22.05    | 2,4-$d$-tert-butylphenol | 206.32                | 2.13         | 97            | C$_{14}$H$_{22}$O | Aromatic Hydrocarbon |
| 6        | 22.69    | pentacosane           | 352.68                   | 1.66         | 64            | C$_{25}$H$_{52}$  | Alkane          |
| 7        | 31.75    | 2,4-di-$t$-butylphenol | 308.07                | 0.98         | 90            | C$_{21}$H$_{44}$  | Alkane          |
| 8        | 48.04    | triacontane           | 422.81                   | 1.4          | 96            | C$_{30}$H$_{62}$  | Alkane          |
| 9        | 50.57    | hentriacontane        | 436.83                   | 1.63         | 93            | C$_{31}$H$_{64}$  | Alkane          |
| 10       | 54.33    | octacosane            | 394.76                   | 1.28         | 95            | C$_{28}$H$_{58}$  | Alkane          |
| 11       | 52.22    | (3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5S)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecacyclo[1H]-cyclopenta[a]phenanthren-3-ol | 414.70         | 1.76         | 90            | C$_{29}$H$_{50}$O | Lipids          |

Notes:
1. Percentage of similarity to the reference spectrum of the NIST library.
2. RT, retention time.

Cupido et al. (2022), PeerJ Organic Chemistry, DOI 10.7717/peerj-ochem.5
The analysis from the (CH$_3$)$_2$CO/MeOH extract with PTFE membrane filter detected 12 phytochemical constituents. Eight alkanes: 4-methyldecane (1.44%), heptacosane (0.98%), pentacosane (1.66%), henicosane (0.98%), nonacosane (2.28%), triacontane (1.4%), hentriacontane (1.63%), octacosane (1.28%). Two aromatic hydrocarbons: 1,3-ditetra-tert-butylbenzene (3.53%), 2,4-di-tert-butylphenol (2.13%). One ketone: 4-propan-2-ylcyclohexane-1,3-dione (1.84%). One lipid: (3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5S)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol (1.76%) (Fig. 2B, Table 2).

| Peak No. | RT (min) | Name of the compound | Molecular weight (g/mol) | Peak area (%) | Similarity* | Molecular formula | Compound nature |
|----------|----------|----------------------|--------------------------|--------------|-------------|------------------|----------------|
| 1        | 10.45    | 4-ethyl-1,2-dimethylbenzene | 134.21                   | 2.27         | 93          | C$_{10}$H$_{14}$  | Aromatic Hydrocarbon |
| 2        | 11.34    | 1,2,4,5-tetramethylbenzene | 134.21                   | 0.51         | 81          | C$_{10}$H$_{14}$  | Aromatic Hydrocarbon |
| 3        | 11.47    | 1,2,3,4-tetramethyl-5-methylenecyclopenta-1,3-diene | 134.21                   | 0.82         | 95          | C$_{10}$H$_{14}$  | Alkene |
| 4        | 14.53    | 1,3-benzothiazole | 135.18                   | 5.33         | 95          | C$_{7}$H$_{5}$NS  | Aromatic Hydrocarbon |
| 5        | 18.59    | (E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol | 296.53                   | 0.45         | 42          | C$_{20}$H$_{36}$O | Alcohol |
| 6        | 22.70    | 6-hexyloxan-2-one | 184.27                   | 0.58         | 59          | C$_{11}$H$_{20}$O | Ketone |
| 7        | 30.09    | (E)-octadec-5-ene | 252.47                   | 0.67         | 78          | C$_{12}$H$_{25}$  | Alkene |
| 8        | 30.95    | 7,9-ditetra-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione | 276.37                   | 1.61         | 50          | C$_{12}$H$_{25}$O | Ketone |
| 9        | 31.01    | methyl hexadecanoate | 270.45                   | 1.1          | 93          | C$_{12}$H$_{26}$O | Fatty acid |
| 10       | 33.98    | (E)-octadec-9-ene | 252.5                    | 1.65         | 89          | C$_{14}$H$_{30}$  | Alkene |
| 11       | 34.20    | methyl (9Z,12Z)-octadeca-9,12-dienoate | 294.47                   | 0.65         | 96          | C$_{14}$H$_{30}$O | Fatty acid |
| 12       | 34.31    | (3Z,13Z)-2-methyloctadeca-3,13-dien-1-ol | 280.5                    | 0.45         | 53          | C$_{14}$H$_{28}$O | Alcohol |
| 13       | 34.78    | methyl octadecanoate | 298.50                   | 0.97         | 93          | C$_{14}$H$_{28}$O | Fatty acid |
| 14       | 34.88    | tridecanedial | 212.33                   | 0.58         | 62          | C$_{13}$H$_{26}$O | Aldehyde |
| 15       | 34.97    | 1-(7-hydroxy-8,9-dimethoxy-17-oxa-5,15-diazahexacyclo[13.4.3.0$^{1,16}$,0$^{4,12}$,0$^{8,11}$,0$^{12,16}$]docosa-6,8,10-trien-5-yl)ethanone | 414.5                    | 0.84         | 52          | C$_{23}$H$_{30}$N$_2$O$_5$ | Ketone |
| 16       | 35.84    | butyl hexadecanoate | 312.53                   | 4.83         | 87          | C$_{19}$H$_{38}$O$_2$ | Fatty acid |
| 17       | 36.24    | dioctadecoxy(oxo)phosphonium | 585.9                    | 0.55         | 53          | C$_{19}$H$_{38}$O$_2$P | Fatty acid |
| 18       | 39.21    | 2-methylpropyl octadecanoate | 340.58                   | 3.10         | 87          | C$_{19}$H$_{38}$O$_2$ | Fatty acid |
| 19       | 39.39    | tetraatriocane | 478.91                   | 0.92         | 90          | C$_{25}$H$_{42}$  | Alkane |
| 20       | 40.96    | tetratetracontane | 759.45                   | 0.93         | 80          | C$_{25}$H$_{50}$  | Alkane |
| 21       | 42.48    | icosane | 282                      | 1.34         | 68          | C$_{25}$H$_{50}$  | Alkane |
| 22       | 45.81    | (6E,10E,14E,18E)-2,6,10,14,18-pentamethylcicosan-2,6,10,14,18-pentaene | 350.6                    | 4.80         | 74          | C$_{25}$H$_{50}$  | Alkene |
| 23       | 52.21    | 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,7,8,9,11,12,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthrene | 396.7                    | 13.80        | 60          | C$_{29}$H$_{48}$  | Alkene |

Notes: * Percentage of similarity to the reference spectrum of the NIST library.
RT, retention time.
The analysis from the (CH$_3$)$_2$CO/CHCl$_3$ extract with PVDF membrane filter showed the presence of 23 phytochemical constituents. Three alkanes: tetratriacontane (0.92%), tetrapentacontane (0.93%), icosane (1.34%). Three aromatic hydrocarbons: 4-ethyl-1,2-dimethylbenzene (2.27%), 1,2,4,5-tetramethylbenzene (0.51%), 1,3-benzothiazole (5.33%). Three ketones: 6-hexyloxan-2-one (0.58%), 7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione (1.61%), 1-(7-hydroxy-8,9-dimethoxy-17-oxa-5,15-diazahexacyclo[13.4.3.0$^1$.0$^2$.0$^3$.0$^4$.0$^5$.0$^6$.0$^7$.0$^8$.0$^9$.0$^{10}$]docosa-6,8,10-trien-5-yl)ethanone (0.84%). Two alcohols: (E,Z$^1$,7$^R$,11$^R$)-3,7,11,15-tetramethylhexadec-2-en-1-ol (0.45%), (3Z,13Z)-2-methyloctadeca-3,13-dien-1-ol (0.45%). One aldehyde: tridecanedial (0.58%). Five alkenes: 1,2,3,4-tetramethyl-5-methylidenecyclopenta-1,3-diene (0.82%), (E)$^1$-octadec-5-ene (0.67%), (E)$^1$-octadec-9-ene (1.65%), (6$^E$,10$^E$,14$^E$,18$^E$)-2,6,10,14,18-pentamethyllicos-2,6,10,14,18-pentaene (4.80%), 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,7,8,9,11,12,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthrene (13.80%). Six fatty acids: methyl hexadecanoate (1.1%), methyl (9Z,12Z)-octadeca-9,12-dienoate (0.65%), methyl octadecanoate (0.97%), butyl hexadecanoate (4.83%), dioctadecyloxy(oxo)phosphonium (0.55%), 2-methylpropyl octadecanoate (3.10%) (Fig. 2C, Table 3).

The analysis from (CH$_3$)$_2$CO/CHCl$_3$ extract with PTFE membrane filter seven compounds were observed. One alkane: 1-(6-methylheptan-2-yl)-4-(4-methylpentyl)cyclohexane (1.20%). Four aromatic hydrocarbons: 1-ethyl-2,4-dimethylbenzene (2.51%), 4-ethyl-1,2-dimethylbenzene (4.24%), 2-ethyl-1,3-dimethylbenzene (0.83%), 2,4-dietert-butylphenol (5.28%). One ester: 2-(2-ethylhexoxycarbonyl)benzoic acid (7.55%). One fatty acid: 2-methylpropyl octadecanoate (10.83%) (Fig. 2D, Table 4).

The analyses revealed different nature kinds for the identified compounds such as alkanes, aromatic hydrocarbons, esters, ketones halogenated hydrocarbons, alcohols, aldehydes, alkenes, lipids, and fatty acids, some of them with a biological activity previously reported (Tables 5–7). From the identified compounds, 19 shown similarities to

| Peak No. | RT (min) | Name of the compound | Molecular Weight (g/mol) | Peak area (%) | Similarity* (%) | Molecular formula | Compound nature |
|----------|----------|----------------------|--------------------------|--------------|----------------|------------------|-----------------|
| 1        | 10.25    | 1-ethyl-2,4-dimethylbenzene | 134.21                   | 2.51         | 64            | C$_{10}$H$_{14}$  | Aromatic Hydrocarbon |
| 2        | 10.45    | 4-ethyl-1,2-dimethylbenzene | 134.21                   | 4.24         | 80            | C$_{10}$H$_{14}$  | Aromatic Hydrocarbon |
| 3        | 11.48    | 2-ethyl-1,3-dimethylbenzene | 134.21                   | 0.83         | 74            | C$_{10}$H$_{14}$  | Aromatic Hydrocarbon |
| 4        | 22.07    | 2,4-dietert-butylphenol     | 206.32                   | 5.28         | 83            | C$_{14}$H$_{22}$O | Aromatic Hydrocarbon |
| 5        | 36.26    | 1-(6-methylheptan-2-yl)-4-(4-methylpentyl)cyclohexane | 280.53                   | 1.20         | 53            | C$_{20}$H$_{40}$  | Alkane |
| 6        | 39.22    | 2-methylpropyl octadecanoate | 340.58                   | 10.83        | 90            | C$_{22}$H$_{44}$O$_2$ | Fatty acid |
| 7        | 41.77    | 2-(2-ethylhexoxycarbonyl)benzoic acid | 278.34                   | 7.55         | 53            | C$_{16}$H$_{22}$O$_4$ | Ester |

Notes: * Percentage of similarity to the reference spectrum of the NIST library.
RT, retention time.
Figure 2. GC-MS chromatograms. (A) MeOH extract with PVDF membrane filter, (B) MeOH extract with PTFE membrane filter, (C) CHCl₃ extract with PVDF membrane filter, and (D) CHCl₃ extract with PTFE membrane filter.

Table 5. Number and type of phytochemical compounds in *Opuntia megarrhiza* by extract and membrane filter.

| Extract/Filter       | A  | Ah | Fa | K  | Ak | Al | Ad | L  | Hh | Total |
|----------------------|----|----|----|----|----|----|----|----|----|-------|
| MeOH/PVDF            | 5  | 1  | 2  | 1  | 0  | 0  | 1  | 0  | 1  | 11    |
| MeOH/PTFE            | 8  | 2  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 12    |
| CHCl₃/PVDF           | 3  | 3  | 6  | 3  | 5  | 2  | 0  | 1  | 0  | 23    |
| CHCl₃/PTFE           | 1  | 4  | 1  | 0  | 0  | 1  | 0  | 0  | 0  | 7     |
| Total                | 17 | 10 | 9  | 5  | 5  | 2  | 2  | 1  | 1  | 53    |

Note:
Alkanes (A), Aromatic hydrocarbons (Ah), Esters (E), Ketones (K), Halogenated hydrocarbons (Hh), Alcohols (Al), Aldehydes (Ad), Alkenes (Ak), Lipids (L), Fatty acids (Fa).
phytochemical compounds with biological activities previously reported (Tables 6 and 7); their mass spectra resulting from the GC-MS analyses and chemical structures are presented in Figs. S1–S4.

Ten phytochemical compounds shown in Fig. 3 were the most prevailing in the two extracts (CHCl₃ and MeOH) and both membrane filters (PVDF and PTFE): Benzene, 1,3-bis(1,1-dimethylethyl) (4.19%) in MeOH/PVDF and (3.53%) in (MeOH/PTFE), hexadecanoic acid (4.67%) in MeOH/PVDF; 1,3-benzothiazole (5.33%), butyl hexadecanoate (4.83%), 2-methylpropyl octadecanoate (3.10%), (6E,10E,14E,18E)-2,6,10,14,18-pentamethylcicosa-2,6,10,14,18-pentaene (4.80%), and 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,7,8,9,11,12,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthrene (13.80%) in CHCl₃/PVDF; and 4-ethyl-1,2-dimethylbenzene (4.24%), 2,4-difert-butylphenol (5.28%) and 2-(2-ethylhexoxycarbonyl)benzoic acid (7.55%) in CHCl₃/PTFE (Fig. 3).

### Table 6 Phytochemical compounds with biological activity identified by GC-MS in MeOH extract.

| Peak No. | Name of the compound | Molecular weight (g/mol) | Peak area (%) | Molecular formula | Ions (m/z) | Compound nature | Biological activity |
|----------|-----------------------|--------------------------|---------------|-------------------|-----------|-----------------|---------------------|
| PVDF filter | | | | | | | |
| 1 | heptadecane | 240.46 | 1.03 | C₁₇H₃₆ | 57, 71, 85 | Alkane | Antifungal (Adeleye, Daniels & Omadime, 2010), antimicrobial (Rahbar, Shafagha & Salimi, 2012), anti-inflammatory and antioxidative (Kim et al., 2013) |
| 2 | hexadecanoic acid | 256.42 | 4.67 | C₁₆H₃₂O₂ | 57, 73, 129 | Fatty acid | Anti-inflammatory (Aparna et al., 2012) antialopecic, anti-androgenic, antioxidant, antipsychotic, hemolytic, hypocholesterolemic, nematicide, pesticide and 5-Alpha reductase inhibitor (USDA, 1992–2016 [U.S. Department of Agriculture, Agricultural Research Service]). Dr. Duke’s) |
| 3 | octadecanoic acid | 284.47 | 2.14 | C₁₈H₃₆O₂ | 73, 55, 129 | Fatty acid | Antifungal and antitumoral (Gehan et al., 2009; Hsouna et al., 2011) |
| PTFE filter | | | | | | | |
| 1 | heptacosane | 380.73 | 0.98 | C₂₇H₅₆ | 57, 71, 85 | Alkane | Antioxidant (Marrufo et al., 2013) |
| 2 | 2,4-di-tert-butylphenol | 206.32 | 2.13 | C₁₄H₂₂O | 191, 57, 206 | Aromatic hydrocarbon | Anti-inflammatory, antimicrobial and antioxidant USDA, 1992–2016 [U.S. Department of Agriculture, Agricultural Research Service]). Dr. Duke’s) |
| 3 | pentacosane | 352.68 | 1.66 | C₂₅H₅₂ | 57, 71, 85 | Alkane | Antimicrobial and antioxidant (Marrufo et al., 2013) |
| 4 | henicosane | 296.57 | 0.96 | C₂₁H₄₄ | 85,71,57 | Alkane | Antiasthmatics, urine acidifiers and antimicrobial (Usha Nandhini, Sangareshwari & Lata, 2015) |
| 5 | triacontane | 422.81 | 1.4 | C₃₀H₆₂ | 57, 85, 113 | Alkane | Antimicrobial and cytotoxic (Hsouna et al., 2011), antidiabetic, antitumor and antibacterial (Tiagy & Agarwal, 2017) |
| 6 | hentriacontane | 436.83 | 1.63 | C₃₁H₆₄ | 57, 85, 113 | Alkane | Antibacterial activity (Olabunmi et al., 2009), and anti-inflammatory (Kim et al., 2011) |
| 7 | octacosane | 394.76 | 1.28 | C₂₈H₅₈ | 57, 141, 239 | Alkane | Antimicrobial and antioxidant (Jin et al., 2018) |
Identity from five compounds that showed a similarity percentage above 95%, was supported by comparison of their retention times with pure commercial standards (Figs. S5–S9). 1,3-benzothiazole was found at RT of 14.61 min, with ions (m/z) of 135 and

| Peak No. | Name of the compound | Molecular weight (g/mol) | Peak area (%) | Molecular formula | Ions (m/z) | Compound nature | Biological activity |
|---------|----------------------|--------------------------|---------------|------------------|------------|----------------|-------------------|
| 1       | 1,3-benzothiazole    | 135.18                   | 5.33          | C₇H₅NS           | 135, 108, 69 | Aromatic Hydrocarbon | Anticonvulsant, anti-inflammatory, antileishmanial, antimicrobial and antitumor (Siddiqui, Khan & Rana, 2007) |
| 2       | (E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol | 296.53 | 0.45 | C₂₀H₄₀O | 55, 71, 81 | Alcohol | Antispasmodic (Pongprayoon et al., 1992), anticarcinogen USDA, 1992–2016 [U.S. Department of Agriculture, Agricultural Research Service]). Dr. Duke’s; Lee, Lee & Park, 1999; Hema, Kumaravel & Alagusundaram, 2011, antitubercular (Saikia et al., 2010), antibacterial, antifungal, antimalaria, analgesic and stimulant (Hema, Kumaravel & Alagusundaram, 2011), anticonvulsant (Costa et al., 2012), anti-inflammatory, antinociceptive (Ökici et al., 2009; Silva et al., 2014; Islam et al., 2018), anxiolytic, cell autophagy and apoptosis inducer metabolism-modulating, cytotoxic and immune-modulating (Islam et al., 2018), antimicrobial (Islam et al., 2018), and antioxidant (Mohammad, Omran & Hussein, 2016; Islam et al., 2018) |
| 3       | 7,9-diterter-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione | 276.37 | 1.61 | C₁₇H₂₄O₃ | 57, 175, 217 | Ketone | Antioxidant (USDA, 1992–2016 [U.S. Department of Agriculture, Agricultural Research Service]). Dr. Duke’s) |
| 4       | methyl hexadecanoate | 270.45 | 1.1 | C₁₇H₃₄O₂ | 74, 143, 227 74, 87, 43, 55, 143 | Fatty Acid | Antibacterial (Agoramoorthy et al., 2007), antifungal, anti-inflammatory, blood cholesterol decrease (Hema, Kumaravel & Alagusundaram, 2011), antioxidant (Agoramoorthy et al., 2007; Hema, Kumaravel & Alagusundaram, 2011) |
| 5       | methyl octadecanoate | 298.50 | 0.97 | C₁₉H₃₈O₂ | 143, 74, 55 | Fatty Acid | Antimicrobial (Abubakar & Majinda, 2016) |
| 6       | butyl hexadecanoate | 312.53 | 4.83 | C₂₀H₄₀O₂ | 257, 129, 56 | Fatty Acid | Antioxidant (Prakash, Gondwal & Pant, 2011), and antimicrobial (Sujatha et al., 2014) |
| 7       | icosane | 282 | 1.34 | C₂₀H₄₂ | 113, 85, 57 | Alkane | Antibacterial (Roussaada et al., 2008; Hsouna et al., 2011), antifungal, antitumor and cytotoxic (Hsouna et al., 2011) |

| PVDF filter | 1 | 2,4-diterter-butylphenol | 206.32 | 5.28 | C₁₄H₂₂O | 57, 191, 206 | Aromatic Hydrocarbon | Anti-inflammatory, antimicrobial and antioxidant USDA, 1992–2016 [U.S. Department of Agriculture, Agricultural Research Service]). Dr. Duke’s) |
| 2 | 2-(2-ethylhexyloxy carbonyl) benzoic acid | 278.34 | 7.55 | C₁₆H₂₂O₄ | 149, 167, 112 | Ester | Cytotoxic (Krishnan, Mani & Jasmine, 2014) |
107.9. henicosane at RT of 33.5 min, with ions (m/z) of 57, 113. hentriacontane at RT of 48.7 min, with ions (m/z) of 57, 85, and 113. methyl hexadecanoate at RT of 30.5 min, with ions (m/z) of 74, 143, 227, and 55. triacontane was detected at RT of 33.5 min, with ions (m/z) 57, 85 and 113.

Finally, 34 compounds with no identified biological activity were found, eight in the (CH$_3$)$_2$CO/MeOH extract with PVDF membrane filter (MeOH/PVDF), five in the (CH$_3$)$_2$CO/MeOH extract with PTFE (MeOH/PTFE), 16 in the (CH$_3$)$_2$CO/CHCl$_3$ extract with PVDF (CHCl$_3$/PVDF), and five in the (CH$_3$)$_2$CO/CHCl$_3$ extract with PTFE (CHCl$_3$/PTFE).

**DISCUSSION**

The use of *Opuntia megarrhiza* in traditional medicine in Mexico has been reported previously (Segura-Venegas & Rendón-Aguilar, 2016), however, this is the first study that...
demonstrate the presence of phytochemical compounds with biological activities. *Opuntia* species are used in the world as local medicinal interventions for chronic diseases and as food sources, mainly because they possess nutritional properties and biological activities that has been recently reviewed (Aruwa, Amoo & Kudanga, 2018). Here we report for the first time, the identification of several phytochemical compounds in *O. megarrhiza* with biological activities. Our findings highlight the relevance of this species in developing of new drugs, trough future chemical studies, and encourage of planting this species once this one is listed as endangered in the IUCN Red List.

Biotechnological methods are reliable and provide continuous sources of raw material and natural products for food, pharmaceutical, and cosmetic industries (Rao & Ravishankar, 2002; Nalawade et al., 2003; Julsing, Quax & Kayser, 2007). Previously, it has been indicated that more than 50,000 plant species are used in phytotherapy and medicine, and around 66% of them are harvested from nature leading to local extinction of many species or degradation of their habitats (Tasheva & Kosturkova, 2012). Alternatives to protect these useful plants, should be directed to both preservation of the plant populations and elevating the level of knowledge for sustainable utilization of these plants in medicine have been previously indicated (WHO, 2010, http://www.who.int/mediacentre/factsheets/fs134/en/). Biotechnological methods offer possibilities not only for faster cloning and conservation of the genotype of the plants (Verpoorte, Contin & Memelink, 2002; Tripathi & Tripathi, 2003) but for modification of their gene information, regulation, and expression for production of valuable substances in higher amounts or with better properties (Rao & Ravishankar, 2002; Khan et al., 2009).

GC-MS is one of the most precise methods to identify various metabolites present in plant extracts (Fiehn et al., 2000; Roessner et al., 2000; Roessner et al., 2001; Kopka, 2006a; Kopka, 2006b; Fiehn, 2006; Fernie, 2007; Saito & Matsuda, 2010; Tiago et al., 2016; Dinesh-Kumar & Rajakumar, 2018) since some of these chromatographs include preloaded libraries or databases (NIST and WILEY) that allows to know the possible identity of the metabolites by comparing the resulting mass spectra with those found as reference in these libraries (Kim et al., 2019; Wei et al., 2014). Several studies indicate that *Opuntia* plants contain different phytochemical groups such as phenolic acids, sterols, esters, coumarins, terpenoids, and alkaloids with several health benefits (Piattelli, Minale & Prota, 1965; Stintzing, Schieber & Carle, 2001; Strack, Vogt & Schliemann, 2003; Paiz et al., 2010; Osorio-Esquivel et al., 2011; Aruwa, Amoo & Kudanga, 2018). However, the nature of the compound extracted depends largely on their solubility in the extraction solvent, the degree of polymerization of the phenols, and the interaction of the phenols with other constituents of the plant (Choi et al., 2002; El Cadi et al., 2020). But the use of different membrane filters allows to identified chemical compounds with different hydrophobicity and molecule sizes. Previously, it has been indicated that PTFE has less hydrophobic adsorption but more size exclusion (Xiao et al., 2014).

In addition, identity of five of the compounds found was corroborated using pure commercial standards. The ions obtained from each of the standards corresponded to those found in the extracts according to the NIST base of the equipment. GC-MS has a library of mass spectra, which makes it easy to obtain compounds that have the most
similar mass to the library spectrum (Kim et al., 2019). However, the attribution of a GC-MS chromatographic peak should be confirmed whenever possible by comparison with a standard compound analyzed under the same experimental conditions (Sturaro, Parvoli & Doretti, 1994). We identified five compounds in the extracts performed through the use of standards. In this context, the analytical standard is used as a reference in the qualitative, quantitative and identity determinations of a compound, it must also have high purity and stability (Sun et al., 2015).

On the other hand, the major phytochemical compounds found in our study have been reported to possess several biological activities. Some alkanes like hentriacontane and triacontane have antibacterial activity (Boussaada et al., 2008; Olubunmi et al., 2009; Hsouna et al., 2011; Tiagy & Agarwal, 2017). Heptadecane has antifungal activity (Adelye, Daniels & Omadine, 2010). Icosane has both antibacterial and antifungal activity (Hsouna et al., 2011). Henicosane, heptadecane, octacosane, and pentacosane have antimicrobial activity (Rahbar, Shafagh & Salimi, 2012; Marrufo et al., 2013; Usha Nandhini, Sangareshwar & Lata, 2015; Jun et al., 2018). Heptadecane and hentriacontane have anti-inflammatory activity (Kim et al., 2011; Kim et al., 2013). Heptacosane, heptadecane, octacosane, and pentacosane have antioxidant activity (Kim et al., 2013; Marrufo et al., 2013; Jun et al., 2018). Icosane and triacontane have antitumor activity (Hsouna et al., 2011; Tiagy & Agarwal, 2017). Triacontane has anti-diabetic activity (Tiagy & Agarwal, 2017). Henicosane has been reported as an antiasthmatic, urine acidifier (Usha Nandhini, Sangareshwar & Lata, 2015). Icosane, octadecane, and hexadecanoic acid has been previously identified in Opuntia stricta (Izuegbuna, Otunola & Bradley, 2019).

Fatty acids like octadecanoic acid have antibacterial or antifungal activity (Gehan et al., 2009; Hsouna et al., 2011), and it has previously identified in Opuntia dillenii (Ben-Lataief et al., 2020). The hexadecanoic acid have antialopecic, anti-androgenic, antifibrinolytic, antioxidant, antipsychotic, hemolytic, hypocholesterolemic, nematicide, pesticide, 5-Alpha reductase inhibitor (USDA, 1992–2016 [U.S. Department of Agriculture, Agricultural Research Service]), and anti-inflammatory (Aparna et al., 2012). Octadecanoic acid have been reported as anticarcinogen or antitumoral (Hsouna et al., 2011; Gehan et al., 2009). Fatty acid like butyl hexadecanoate, methyl hexadecanoate, and methyl octadecanoate, has antimicrobial activity (Sujatha et al., 2014; Abubakar & Majinda, 2016). Benzenoids like 2-(2-ethylhexoxycarbonyl)benzoic acid ester has been reported as cytotoxic (Krishnan, Mani & Jasmine, 2014). The diterpene (E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol has been reported to have multiple activities like anticarcinogen, anticonvulsant, antifungal, anti-inflammatory, antimalaria, antimicrobial, antinoceceptive, antioxidant, antitubercular, antispasmodic, anxiolytic, autophagy and apoptosis inducing, cytotoxic, immune-modulating, metabolism-modulating, resistant to gonorrhea, and stimulant (USDA, 1992–2016 [U.S. Department of Agriculture, Agricultural Research Service]; Pongprayoon et al., 1992; Lee, Lee & Park, 1999; Okie et al., 2009; Saikia et al., 2010; Hema, Kumaravel & Alagusundaram, 2011; Costa et al., 2012; Silva et al., 2014; Mohammad, Omran & Hussein, 2016; Islam et al., 2018), and the 2-(2-ethylhexoxycarbonyl)benzoic
acid has anti-inflammatory, antimicrobial, antioxidant, antiviral, and cytotoxicity activities (Krishnan, Mani & Jasmine, 2014).

Additionally, phytochemical compounds we found in *Opuntia megarrhiza* with no reports of biological activity, have been previously identified in other *Opuntia* species. For example, (Z)-octadec-9-enoic acid and 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,7,8,9,11,12,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthrene was identified in *O. dillenii* (Ben-Lataief et al., 2020). Additionally, β-Sitosterol is the major sterol extracted from different parts of the fruit oils of *Opuntia ficus-indica* (Ramadan & Mörsel, 2003a, 2003b). Herein, we identify (3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5S)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol in *O. megarrhiza*.

**CONCLUSIONS**

The GC-MS analysis of cladode extracts of *Opuntia megarrhiza* conducted here proves the presence of several phytochemical compounds responsible for biological activities previously reported support the medicinal use of this plant in traditional medicine. In particular, the anti-inflammatory activity in compounds with a high similarity percentage in our results (e.g., hexadecanoic acid, 2,4-ditet-butylphenol, hentriacontane, 1,3-benzothiazole, and methyl hexadecanoate) supports its use for treating bone fractures. Hence, *O. megarrhiza* represents a source for finding phytochemical compounds with potential use in medicine and biotechnology. Our results represent an advance in the knowledge of an endangered plant, not previously studied, and with ethnical uses, and support future target studies through the identification of compounds with biotechnological potential using certified standard and additional tools.

**ADDITIONAL INFORMATION AND DECLARATIONS**

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Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Madeleyne Cupido conceived and designed the experiments, performed the experiments, analyzed the data, performed the computation work, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Arturo De-Nova conceived and designed the experiments, analyzed the data, performed the computation work, prepared figures and/or tables, authored or reviewed drafts of the paper, contributed materials and reagents, and approved the final draft.
- María L. Guerrero-González conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, contributed analysis tools, and approved the final draft.
- Francisco Javier Pérez-Vázquez conceived and designed the experiments, performed the experiments, analyzed the data, performed the computation work, prepared figures and/or tables, authored or reviewed drafts of the paper, contributed materials and reagents, and approved the final draft.
- Karen Beatriz Méndez-Rodríguez performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Pablo Delgado-Sánchez conceived and designed the experiments, analyzed the data, performed the computation work, prepared figures and/or tables, authored or reviewed drafts of the paper, contributed materials and reagents, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:

The raw data is available in the Supplemental File.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj-ochem.5#supplemental-information.

REFERENCES
Abubakar M, Majinda R. 2016. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* Schumach and *Pterocarpus angolensis* DC. *Medicines* 3(1):1–9 DOI 10.3390/medicines3010003.

Adeleye IA, Daniels FV, Omadime M. 2010. Characterization of volatile components of epa-ije: a native wonder cure recipe. *Journal of Pharmacology and Toxicology* 6(1):97–100 DOI 10.3923/jpt.2011.97.100.

Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu MJ. 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. *Brazilian Journal of Microbiology* 38(4):739–742 DOI 10.1590/S1517-83822007000400028.

Andrade-Cetto A, Wiedenfeld H. 2011. Anti-hyperglycemic effect of *Opuntia streptacantha* Lem. *Journal of Ethnopharmacology* 133(2):940–943 DOI 10.1016/j.jep.2010.11.022.
Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. 2012. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical Biology and Drug Design 80(3):434–439 DOI 10.1111/j.1747-0285.2012.01418.x.

Araújo F, Farias D, Neri-Numa I, Pastore G. 2021. Underutilized plants of the Cactaceae family: nutritional aspects and technological applications. Food Chemistry (362):130196 DOI 10.1016/j.foodchem.2021.130196.

Aruwa CE, Amoo SO, Kudanga T. 2018. Opuntia (Cactaceae) plant compounds, biological activities and prospects-A comprehensive review. Food Research International 112:328–344 DOI 10.1016/j.foodres.2018.06.047.

Banakar P, Jayaraj M. 2018. GC-MS analysis of bioactive compounds from ethanolic leaf extract of Waltheria indica Linn. and their pharmacological activities. International Journal of Pharmaceutical Sciences and Research 9(5):2005–2010 DOI 10.13040/IJPSR.0975-8232.9(5).2005-10.

Bargougui A, Le Pape P, Triki S. 2013. Antiplasmodial efficacy of fruit extracts and cladodes of Opuntia ficus-indica. Journal of Natural Sciences Research 3:31–37 DOI 10.7176/JNSR.

Barthlott W, Hunt D. 1993. Cactaceae. In: Kubitzki K, Rohmer JG, Bittridi V, eds. The Families and Genera of Vascular Plants. Berlin: Springer, 161–197.

Ben-Lataief S, Zourgui MN, Rahmani R, Najjaa H, Gharsallah N, Zourgui L. 2020. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of bioactive compounds extracted from Opuntia dillenii cladodes. Journal of Food Measurement and Characterization 15(1):782–794 DOI 10.1007/s11694-020-00671-2.

Bensadón S, Hervert-Hernández D, Sáyao-Ayerdi SG, Goñi I. 2010. By-products of Opuntia ficus-indica as a source of antioxidant dietary fiber. Plant Foods for Human Nutrition 65(3):210–216 DOI 10.1007/s11130-010-0176-2.

Boussaada O, Ammar S, Saidana D, Chriaa J, Chraif I, Daami M, Helal AN, Mighri Z. 2008. Chemical composition and antimicrobial activity of volatile components from capitula and aerial parts of Rhaponticum acaule DC growing wild in Tunisia. Microbiological Research 16(1):87–95 DOI 10.1016/j.micres.2007.02.010.

Bravo-Hollis H, Sánchez-Mejorada H. 1991. Las Cactáceas de México. México: Universidad Nacional Autónoma de México.

Bravo-Hollis H, Scheinvar L. 1999. El interesante mundo de las cactáceas. México D.F.: Fondo de Cultura Económica.

Caballero-Gutiérrez I, Gonzáles G. 2016. Alimentos con efecto anti-inflamatorio. Acta Medica Peruana 33(1):1–50 DOI 10.35663/amp.2016.331.18.

Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH, Kim SK. 2002. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Science 163(6):1161–1168 DOI 10.1016/S0168-9452(02)00332-1.

Costa JP, Ferreira PB, Sousa DP, Jordan J, Freitas RM. 2012. Anticonvulsant effect of phytol in a pilocarpine model in mice. Neuroscience Letters 523(2):115–118 DOI 10.1016/j.neulet.2012.06.055.

De Corato U, Maccioni O, Trupo M, Di Sanzo G. 2010. Use of essential oil of Laurus nobilis obtained by means of a supercritical carbon dioxide technique against post harvest spoilage fungi. Journal of Crop Protection 29(2):142–147 DOI 10.1016/j.cropro.2009.10.012.

Dinesh-Kumar G, Rajakumar R. 2018. GC-MS analysis of bioactive compounds from ethanolic leaves extract of Eichhornia crassipes (Mart) Solms. and their pharmacological activities. Journal of Pharmaceutical Innovation 7:459–462 DOI 10.22271/tpi.
El Cadi H, El Cadi A, Kounnoun A, Oulad El Majdoub Y, Lovillo MP, Brigui J, Dugo P, Mondello L, Cacciola F. 2020. Wild strawberry (Arbutus unedo): phytochemical screening and antioxidant properties of fruits collected in northern Morocco. Arabian Journal of Chemistry 13(8):6299–6311 DOI 10.1016/j.arabjc.2020.05.022.

Ennouri M, Fetoui H, Bourret E, Zeghal N, Attia H. 2006. Evaluation of some biological parameters of Opuntia ficus indica. 1. Influence of a seed oil supplemented diet on rats. Bioresource Technology 97(12):1382–1386 DOI 10.1016/j.biortech.2005.07.010.

Estrada-Castillon E, Soto-Mata B, Garza-López M, Villarreal-Quintanilla J, Jiménez-Pérez J, Pando-Moreno M, Sánchez-Salas J, Scott-Morales L, Cotera-Correà M. 2012. Medicinal plants in the southern region of the State of Nuevo León, México. Journal of Ethnobiology and Ethnomedicine 11(1):8–45 DOI 10.1186/1746-4269-8-45.

Fernie AR. 2007. The future of metabolic phytochemistry: larger numbers of metabolites, higher resolution, greater understanding. Phytochemistry 68(22–24):2861–2880 DOI 10.1016/j.phytochem.2007.07.010.

Feugang JM. 2006. Nutritional and medicinal use of Cactus pear (Opuntia spp.) cladodes and fruits. Frontiers in Bioscience 11(1):2574–2589 DOI 10.2741/1992.

Fiehn O, Kopka J, Dormann P, Altmann T, Trethewey RN, Willmitzer L. 2000. Metabolite profiling for plant functional genomics. Nature Biotechnology 18(11):1157–1161 DOI 10.1038/81137.

Fiehn O. 2001. Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks. Comparative and Functional Genomics 2(3):155–168 DOI 10.1002/cfg.82.

Fiehn O. 2002. Metabolomics the link between genotypes and phenotypes. Plant Molecular Biology 48(1/2):155–171 DOI 10.1023/A:1013713905833.

Fiehn O. 2006. Metabolite profiling in Arabidopsis. In: Totowa NJ, Salinas J, Sanchez-Serrano JJ, eds. Arabidopsis Protocols: Methods in Molecular Biology. Totowa: Humana Press, 439–447.

Fiehn O. 2016. Metabolomics by gas chromatography-mass spectrometry: combined targeted and untargeted profiling. Current Protocols in Molecular Biology (114):30.4.1–30.4.32 DOI 10.1002/0471142727.mb3004s114.

Gehan MA, Hanan AE, Hassan AH, Okbah MA. 2009. Marine natural products and their potential applications as antiinfective agents. World Applied Sciences Journal 7(7):872–880.

Ghasemzadeh A, Ghasemzadeh N. 2011. Flavonoids and phenolic acids: role and biochemical activity in plants and human. Journal of Medicinal Plant Research 5(31):6696–6703 DOI 10.5897/jmpr11.1404.

Gil-Chávez JG, Villa JA, Ayala-Zavala FJ, Heredia JB, Sepulveda D, Yahia EM, González-Aguilar GA. 2013. Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. Comprehensive Reviews in Food Science and Food Safety 12(1):5–23 DOI 10.1111/1541-4337.12005.

González-Durán A, Riojas L, Arreola N. 2001. El género Opuntia en Jalisco. Guía de campo. México: Universidad de Guadalajara-Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

Gurrieri S, Miceli L, Lanza CM, Tomaselli F, Bonomo RP, Rizzarelli E. 2000. Chemical characterization of Sicilian prickly pear (Opuntia ficus indica) and perspectives for the storage of its juice. Journal of Agricultural and Food Chemistry 48(11):5424–5431 DOI 10.1021/jf9907844.

Harlev E, Nevo E, Lansky EP, Lansky S, Bishayee A. 2012. Anticancer attributes of desert plants. Anti-Cancer Drugs 23(3):255–271 DOI 10.1097/cad.0b013e32834f968c.
Harrigan G, Goodacre R. 2012. Metabolic profiling: its role in biomarker discovery and gene function analysis. New York: Springer.

Hema R, Kumaravel S, Alagusundaram K. 2011. GC/MS determination of bioactive components of Murraya koenigii. American Journal of Science 7:80–83.

Hernández H, Godínez AH. 1994. Contribución al conocimiento de las cactáceas mexicanas amenazada. Acta Botánica Mexicana 26(26):33–52 DOI 10.21829/abm26.1994.690.

Hernández H, Gómez-Hinoostrosa C, Bárcenas R. 2001. Studies on Mexican Cactaceae. II. Opuntia megarrhiza, a poorly known endemic from San Luis Potosí, Mexico. Brittonia 53(4):528–533 DOI 10.1007/bf02809653.

Hernández HM, Gómez-Hinoostrosa C, Goettsch BK, Sotomayor M. 2013. Opuntia megarrhiza. The IUCN Red List of Threatened Species 2013 e.T41219A2952324 DOI 10.2305/IUCN.UK.2013-1.RLTS.T41219A2952324.

Hernández-Hernández T, Hernández HM, De-Nova JA, Puente R, Eguiarte L, Magallón S. 2011. Phylogenetic relationships and evolution lineages within Cactaceae. American Journal of Botany 98(1):44–61 DOI 10.3732/ajb.1000129.

Hsouna AB, Trigui M, Mansour RB, Jarraya RM, Damak M, Jaoua S. 2011. Chemical composition, cytotoxicity effect and antimicrobial activity of Ceratonia siliqua essential oil with preservative effects against Listeria inoculated in minced beef meat. International Journal of Food Microbiology 148(1):66–72 DOI 10.1016/j.ijfoodmicro.2011.04.028.

Hunt D, Taylor NP, Charles G. 2006. The new cactus lexicon. Milborne Port: DH Books.

Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, Chandra Shill M, Karmakar UK, Yarla NS, Khan IN, Billah MM, Pieczynska MD, Zengin G, Malainer C, Nicoletti F, Gulei D, Berindan-Neagoe I, Apostolov A, Banach M, Yeung A, El-Demerdash A, Xiao J, Dey P, Yele S, Jóźwik A, Strzalkowska N, Marchewka J, Rengasamy K, Horbańczuk J, Amjad-Kamal M, Mubarak M, Shilpi J, Atanasov A. 2018. Phytol: a review of biomedical activities. Food and Chemical Toxicology 121(6):82–94 DOI 10.1016/j.fct.2018.08.032.

Izuegbuna O, Otonola G, Bradley G. 2019. Chemical composition, antioxidant, anti-inflammatory, and cytotoxic activities of Opuntia stricta cladodes. PLOS ONE 14(1):e0209682 DOI 10.1371/journal.pone.0209682.

Julsing KM, Quax WJ, Kayser O. 2007. The engineering of medicinal plants: prospects and limitations of medicinal plant biotechnology. In: Kayser O, Quax WJ, eds. Medicinal Plant Biotechnology: from Basic Research to Industrial Applications. New York: Wiley, 1–8.

Jun M, Rui-Rui X, Yao L, Di-Feng R, Jun L. 2018. Composition, antimicrobial and antioxidant activity of supercritical fluid extract of Elsholtzia ciliata. Journal of Essential Oil Bearing Plants 21(2):556–562 DOI 10.1080/0972060X.2017.1409657.

Khan MY, Aliabbas S, Kumar V, Rajkumar S. 2009. Recent advances in medicinal plant biotechnology. Indian Journal of Biotechnology 8:9–22.

Kim DH, Park MH, Choi YJ, Chung KW, Park CH, Jang EJ, An HJ, Yu BP, Chung HY. 2013. Molecular study of dietary heptadecane for the anti-inflammatory modulation of NF-κB in the aged kidney. PLOS ONE 8(3):1–10 DOI 10.1371/journal.pone.0059316.

Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE. 2019. PubChem in 2021: new data content and improved web interfaces. Nucleic Acids Research 49:D1388–D1395 DOI 10.1093/nar/gkaa971.

Kim S, Chung W, Kim S, Ko S, Um J. 2011. Antiinflammatory effect of Oldenlandia diffusa and its constituent, hentriacontane, through suppression of Caspase-1 activation in mouse peritoneal macrophages. Phytotherapy Research 25(10):1537–1546 DOI 10.1002/ptr.3443.
Kopka J. 2006a. Current challenges and developments in GC-MS based metabolite profiling technology. *Journal of Biotechnology* 124(1):312–322 DOI 10.1016/j.jbiotec.2005.12.012.

Kopka J. 2006b. Gas chromatography mass spectrometry. In: Saito K, Dixon RA, Willmitzer L, eds. *Plant Metabolomics*. Vol. 57. Berlin: Springer, 3–20.

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Etherton TD. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine* 113:71–88 DOI 10.1016/s0002-9343(01)00995-0.

Krishnan K, Mani A, Jasmine S. 2014. Cytotoxic activity of bioactive Compound 1, 2-benzene dicarboxylic acid, mono 2-ethylhexyl ester extracted from a marine derived Streptomyces sp. VITSJK8. *International Journal of Molecular and Cellular Medicine* 3:246–254.

Kudanga T, Nemadziva B, Le Roes-Hill M. 2017. Laccase catalysis for the synthesis of bioactive compounds. *Applied Microbiology and Biotechnology* 101(1):13–33 DOI 10.1007/s00253-016-7987-5.

Lee KL, Lee SH, Park K. 1999. Anticancer activity of phytol and eicosatrienoic acid identified from Perilla leaves. *Journal of Ethnopharmacology* 28:1107–1112.

Mangas-Marín R, Montes de Oca PR, Herrera PM, Bello AA, Hernández BI, Menéndez S, Lopez M, Paz T, Rodeiro GI. 2018. GC/MS analysis and bioactive properties of extracts obtained from Clusia minor L. leaves. *Journal of the Mexican Chemical Society* 62(4):177–188 DOI 10.29356/jmcs.v62i4.544.

Marquet P. 2012. LC-MS vs. GC-MS, online extraction systems, advantages of technology for drug screening assays. In: Langman L, Snozek C, eds. *LC-MS in drug analysis*. Vol. 902. Totowa: Humana Press, 15–27.

Marrufo T, Nazzaro F, Mancini E, Fratianni F, Coppola R, De Martino L, Agostinho AB, De Feo V. 2013. Chemical composition and biological activity of the essential oil from leaves of *Moringa oleifera* Lam. cultivated in Mozambique. *Molecules* 18(9):10989–11000 DOI 10.3390/molecules180910989.

Martins S, Mussatto SI, Martínez-Avila G, Montañez-Saenz J, Aguilar CN, Teixeira JA. 2011. Bioactive phenolic compounds: production and extraction by solid-state fermentation. *Biotechnology Advances* 29(3):365–373 DOI 10.1016/j.biotechadv.2011.01.008.

Mohammad G, Omran AM, Hussein H. 2016. Antibacterial and phytochemical analysis of *Piper nigrum* using gas chromatography mass spectrum and Fourier-transform infrared spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research* 8:977–996.

Nalawade SM, Sagare AP, Lee CY, Kao CL, Tsay HS. 2003. Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. *Botanical Bulletin of Academia Sinica* 44(2):79–98 DOI 10.1079/IVP2003504.

Okiei W, Ogunlesi M, Ofor E, Osibote EA. 2009. Analysis of essential oil constituents in hydro-distillates of *Calotropis procera* (Ait.). *Research Journal of Phytochemistry* 33(3):44–53 DOI 10.3923/rjphyto.2009.44.53.

Olubunmi A, Gabriel OA, Stephen AO, Scott FO. 2009. Antioxidant and antimicrobial activity of cuticular wax from *Kigelia africana*. *Fabad Journal of Pharmaceutical Sciences* 34:187–194.

Osorio-Esquível O, Ortiz-Moreno A, Álvarez V, Dorantes-Álvarez I, Giusti M. 2011. Phenolics, betacyanins and antioxidant activity in *Opuntia joconostle* fruits. *International Food Research Journal* 44(7):2160–2168 DOI 10.1016/j.foodres.2011.02.011.

Paiz R, Juárez-Flores B, Rogelio J, Rivera JR, Cecilia N, Ortega C, Reyes-Agüero JA, García E, Alvarez G. 2010. Glucose-lowering effect of xoconostle (*Opuntia joconostle* A. Web., Cactaceae) in diabetic rats. *Journal of Medicinal Plants Research* 4:2326–2333 DOI 10.5897/JMPR.
Park EH, Kahng JH, Lee SH, Shin KH. 2001. An anti-inflammatory principle from cactus. *Fitoterapia* 72(3):288–290 DOI 10.1016/s0367-326x(00)00287-2.

Patra S, Nayak R, Patro S, Pradhan B, Sahu B, Behera C, Bhutia S, Jena M. 2021. Chemical diversity of dietary phytochemicals and their mode of chemoprevention. *Biotechnology Reports* (30):e00633 DOI 10.1016/j.btre.2021.e00633.

Pawar AV, Killeddar SG, Dhuri VG. 2017. *Opuntia*: medicinal plant. *International Journal of Advance Research, Ideas and Innovations in Technology* 3:148–154.

Perfumi M, Tacconi R. 1996. Antihyperglycemic effect of fresh *Opuntia dillenii* fruit from tenerife (Canary Islands). *International Journal of Pharmacognosy* 34(1):41–47 DOI 10.1076/phbi.34.1.41.13186.

Piattelli M, Minale L, Prota G. 1965. Pigments of Centrospermae III: Betaxanthins from *Beta vulgaris* L. *Phytochemistry* 4(1):121–125 DOI 10.1016/S0031-9422(00)86153-1.

Pichersky E, Gang D. 2000. Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends in Plant Science* 5(10):439–445 DOI 10.1016/s1360-1385(00)01741-6.

Pongprayoon U, Baeckström P, Jacobsson U, Lindström M, Bohlin L. 1992. Antispasmodic activity of beta-damascenone and e-phytol isolated from *Ipomoea pes-caprae*. *Planta Medica* 58(01):19–21 DOI 10.1055/s-2006-961381.

Prakash O, Gondwal M, Pant AK. 2011. Essential oils composition and antioxidant activity of water extract from seeds and fruit pulp of *Skimmia anquetilia* N.P. Taylor & Airy Shaw. *Journal of Asian Natural Products Research* 2:435–441.

Rahbar N, Shafagha A, Salimi F. 2012. Antimicrobial activity and constituents of the hexane extracts from leaf and stem of *Origanum vulgare* L. sp. *viride* (Boiss.) Hayek. Growing wild in Northwest Iran. *Journal of Medicinal Plants Research* 6:2681–2685 DOI 10.5897/JMPR11.1768.

Ramadan MF, Mörsel JT. 2003a. Oil cactus pear (*Opuntia ficus-indica* L.). *Food Chemistry* 82(3):339–345 DOI 10.1016/s0308-8146(02)00550-2.

Ramadan MF, Mörsel JT. 2003b. Lipid profile of prickly pear pulp fractions. *Journal of Food, Agriculture and Environment* 1:66–70.

Rao SR, Ravishankar GA. 2002. Plant cell cultures: chemical factories of secondary metabolites. *Biotechnology Advances* 20(2):101–153 DOI 10.1016/s0734-9750(02)00007-1.

Reyes-Agüero JA, Aguirre R, Carlín F. 2004. Análisis preliminar de la variación morfológica de 38 variantes mexicanas de *Opuntia ficus-indica* (L.). In: Miller en Esparza G, Valdez R, Méndez G, eds. *El nopal, Tópicos de actualidad*. Chapingo: Univ. Autónoma Chapingo - Colegio de Postgraduados, 21–47.

Reyes-Agüero JA, Aguirre-Rivera J, Hernández M. 2005. Notas sistemáticas y descripción detallada de *Opuntia ficus-indica* (L.) Mill. (Cactaceae). *Agrociencia* 39:395–408.

Robertson DG. 2005. Metabonomics in toxicology: a review. *Toxicological Sciences* 85(2):809–822 DOI 10.1093/toxsci/kfi102.

Roessner U, Luedemann A, Brust D, Fiehn O, Linke T, Willmitzer L, Fernie A. 2001. Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. *Plant Cell* 13(1):11–29 DOI 10.1105/tpc.13.1.11.

Roessner U, Wagner C, Kopka J, Trethewey RN, Willmitzer L. 2000. Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. *Plant Journal* 23(1):131–142 DOI 10.1046/j.1365-313x.2000.00774.x.
Saikia D, Parihar S, Chanda D, Ojha S, Kumar JK, Chanotiya CS, Shanker K, Negi AS. 2010. Antitubercular potential of some semisynthetic analogues of phytol. *Bioorganic & Medicinal Chemistry Letters* 20(2):508–512 DOI 10.1016/j.bmcl.2009.11.107.

Saito K, Matsuda F. 2010. Metabolomics for functional genomics, systems biology, and biotechnology. *Annual Review of Plant Biology* 61(1):463–489 DOI 10.1146/annurev.arplant.043008.092035.

Segura-Venegas D, Rendón-Aguilar B. 2016. *Opuntia megarrhiza* Rose (Cactaceae) en San Luis Potosí, México: Uso tradicional y distribución de nuevas poblaciones. *Cactáceas y Suculentas Mexicanas* 62:36–47.

Serra A, Poejo T, Matias J, Bronze A, Duarte C. 2013. Evaluation of *Opuntia* spp. derived products as antiproliferative agents in human colon cancer cell line (HT29). *Food Research International* 54(1):892–901 DOI 10.1016/j.foodres.2013.08.043.

Shedbalkar UU, Adki VS, Jadhav JP, Bapat VA. 2010. *Opuntia* and other cacti: applications and biotechnological insights. *Tropical Plant Biology* 3(3):136 DOI 10.1007/s12042-010-9055-0.

Siddiqui N, Khan SA, Rana A. 2007. Benzothiazoles: a new profile of biological activities. *Indian Journal of Pharmaceutical Sciences* 69:10 DOI 10.4103/0250-474x.32100.

Silva R, Sousa F, Damasceno S, Carvalho N, Silva V, Oliveira F, Sousa D, Aragão K, Barbosa A, Freitas R, Medeiros JV. 2014. Phytol a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. *Fundamental & Clinical Pharmacology* 28(4):455–464 DOI 10.1111/fcp.12049.

Sim KS, Sri-Nurestri AM, Sinniah SK, Kim KH, Norhanom AW. 2010. Acute oral toxicity of *Pereskia bleo* and *Pereskia grandifolia* in mice. *Pharmacognosy Magazine* 6(21):67–70 DOI 10.4103/0973-1296.59969.

Strack D, Vogt T, Schliemann W. 2003. Recent advances in betalain research. *Phytochemistry* 62(3):247–269 DOI 10.1016/S0031-9422(02)00564-2.

Sturaro A, Parvoli G, Doretti L. 1994. Standards and GC-MS analysis: an answer to the requirement of compound confirmation. *Chromatographia* 38(3–4):239–241 DOI 10.1007/BF02290344.

Sujatha M, Karthika K, Sivakamasundari S, Mariajancyrani J, Chandramohan G. 2014. GC-MS analysis of phytocomponents and total antioxidant activity of hexane extract of Sinapis alba. *International Journal of Pharmaceutical, Chemical and Biological Sciences* 4:112–117.

Sun W, Tong L, Li D, Huang J, Zhou S, Sun H, Bi K. 2015. Selection of reference standard during method development using the analytical hierarchy process. *Journal of Pharmaceutical and Biomedical Analysis* 107:280–289 DOI 10.1016/j.jpba.2015.01.006.

Tasheva K, Kosturkova G. 2012. The role of biotechnology for conservation and biologically active substances production of *Rhodiola rosea*: endangered medicinal species. *The Scientific World Journal* 2012:274942 DOI 10.1100/2012/274942.

Tiagty T, Agarwal M. 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. *Journal of Pharmacognosy and Phytochemistry* 6:195–206 DOI 10.22271/phyto.

Tripathi L, Tripathi JN. 2003. Role of biotechnology in medecinal plants. *Tropical Journal of Pharmaceutical Research* 2(2):243–253.
USDA. 1992. [U.S. Department of Agriculture, Agricultural Research Service] Dr. Duke’s Phytochemical and Ethnobotanical Databases. Available at https://phytochem.nal.usda.gov.

Usha Nandhini S, Sangareswari S, Lata K. 2015. Gas chromatography-mass spectrometry analysis of bioactive constituents from the marine Streptomyces. Asian Journal of Pharmaceutical and Clinical Research 8:244–246.

Velmurugan G, Anand P. 2017. GC-MS analysis of bioactive compounds on ethanolic leaf extract of Phyllodium pulchellum L. desv. International Journal of Pharmacognosy and Phytochemical Research 1(1):114–118 DOI 10.25258/ijppr.v9i1.8051.

Verpoorte R, Contin A, Memelink J. 2002. Biotechnology for the production of plant secondary metabolites. Phytochemistry Reviews 1(1):13–25 DOI 10.1023/A:1015871916833.

Wei X, Koo I, Kim S, Zhang X. 2014. Compound identification in GC-MS by simultaneously evaluating the mass spectrum and retention index. The Analyst 139(10):2507–2514 DOI 10.1039/C3AN02171H.

Weli A, Al-Kaabi A, Al-Sabahi J, Said S, Hossain MA, Al-Riyami S. 2019. Chemical composition and biological activities of the essential oils of Psidium guajava leaf. Journal of King Saud University - Science 31(4):993–998 DOI 10.1016/j.jksus.2018.07.021.

Xiao K, Sun J, Mo Y, Fang Z, Liang P, Huang X, Ma B. 2014. Effect of membrane pore morphology on microfiltration organic fouling: PTFE/PVDF blend membranes compared with PVDF membranes. Desalination 343:217–225 DOI 10.1016/j.desal.2013.09.026.

Yahia E, Mondragon-Jacobo C. 2011. Nutritional components and anti-oxidant capacity of ten cultivars and lines of cactus pear fruit (Opuntia spp.). Food Research International 44(7):2311–2318 DOI 10.1016/j.foodres.2011.02.042.

Yeddes N, Chérif J, Guyot S, Baron A, Trabelsi-Ayadi M. 2013a. Phenolic profile of Tunisian Opuntia ficus-indica thornless form flowers via chromatographic and spectral analysis by reversed phase-high performance liquid chromatography-UV-photodiode array and electrospray ionization-mass spectrometer. International Journal of Food Properties 17:741–751 DOI 10.1080/10942912.2012.665404.

Yeddes N, Chérif J, Guyot S, Sotin H, Ayadi M. 2013b. Comparative study of antioxidant power, polyphenols, flavonoids and Betacyanins of the peel and pulp of three Tunisian Opuntia forms. Antioxidants 2(2):37–51 DOI 10.3390/antiox2020037.

Yu M, Gouvinhas I, Rocha J, Barros A. 2021. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Scientific Reports (11):10041 DOI 10.1038/s41598-021-89437-4.