Analysis of Phytochemical Composition in *Washingtonia filifera* Leaves Using GC-MS

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DOI: https://doi.org/10.36077/kjas/2026/v18i3.17454

Received date : 15/9/2024

Accepted date: 19/3/2025

Abstract

The study aimed at identify the phytochemical compounds in the leaves of *Washingtonia filifera* using Gas Chromatography-Mass Spectrometry (GC-MS). *Washingtonia filifera* was selected for this study due to several key factors that enhance its importance as a research topic. First, it is an ideal model for studying the adaptation of plants to arid and semi-arid environments. It has a unique ability to withstand high temperatures and drought, which makes it suitable for studying the physiological mechanisms and environmental strategies. Its widespread use in landscaping and environmental reclamation, understanding its biological characteristics may contribute to improving its cultivation and management practices in urban and desert environments. It was conducted at the shade house of the College of Agriculture, University of Basrah, on *Washingtonia filifera* trees belonging to the *Arecaceae* family. This study aimed to identify the phytochemical compounds in the leaves of *Washingtonia filifera* using Gas Chromatography-Mass Spectrometry (GC-MS). The results of the GC-MS analysis revealed the presence of more than forty phytochemical compounds in the ethanolic extract of *Washingtonia filifera* leaves. The identified phytochemicals with their relative abundance were as follows: Pelletierine 1.513%, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- 4.275%, Pentadecanoic acid, 14-methyl-, methyl ester 1.034%, beta-D-Glucopyranoside methyl 14.672%, Loliolide 2.92%, n-Hexadecanoic acid 4.634%, Phytol 20.834%, 9,12,15-Octadecatrienoic acid, (Z, Z, Z-) 3.053%, and Vitamin E 16.61%.The present study revealed that the extract of *Washingtonia filifera* leaves consists of a variety of secondary metabolites and bioactive compounds, some of which are novel and may be isolated, evaluated, and verified for their medicinal and biological activities, thus potentially being used as a therapeutic botanical source.

Keywords: *Washingtonia filifera*, Arecaceae , GC-MS , Ethanolic, Phytol

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Introduction

The *Washingtonia filifera* tree belongs to the Arecaceae family, which is well known for its diverse range of medicinal plants. The bioactive compounds identified in the roots, leaves, fruits, and seeds of *W. filifera* have been linked to digestive health and other therapeutic applications, highlighting the medicinal significance of this plant family (1).In recent years, scientific and medical interest in *Washingtonia* species, particularly *W. filifera*, has significantly increased. This species is widely distributed across various regions and is valued as an ornamental tree due to its unique biological properties, making it an important subject of research. Several studies have focused on the phytochemical composition and potential medicinal benefits of *W. filifera* leaves, flowers, and other plant parts, particularly their antioxidant capacity. These studies revealed that different plant parts exhibit strong antioxidant effects, which play a crucial role in inhibiting or preventing diseases associated with oxidative stress (2). Research has shown that *W. filifera* leaves contain biologically active compounds such as alkaloids and polyphenolic compounds, which are known for their antimicrobial properties and medical significance (3). Additionally, *W. filifera* leaves contain the phloroglucinol compound *filiferol*, which was first isolated from this plant and demonstrated potent inhibitory effects against Human Immunodeficiency Virus (*HIV-1*), effectively suppressing viral replication (4). Moreover,(5) found that leaf extracts of *W. filifera* and *W. robusta*, particularly *W. robusta*, help alleviate oxidative stress. *W. robusta* leaves have been shown to contain four major phenolic compounds: *naringenin*, *kaempferol*, *quercetin*, and *gallic acid*, all of which exhibit significant antioxidant activity. Furthermore, *Washingtonia* trees contain a variety of medicinally active compounds that are believed to have promising therapeutic benefits. Chemical analyses of *W. filifera* leaves, flowers, and seeds have demonstrated their antibacterial potential. Extracts of *W. filifera* have been evaluated for their ability to combat human pathogens using the agar diffusion assay and minimum inhibitory concentration (MIC) tests to assess their effectiveness (6). Additionally, *Washingtonia* palm oil is rich in fatty acids, particularly oleic acid and lauric acid, both of which possess high medicinal value (7). Given the widespread distribution of *W. filifera* across Iraq and its easy availability, studying its bioactive compounds is of particular significance. If proven to contain antioxidant and antimicrobial properties, this plant could serve as a valuable natural source for therapeutic applications. Therefore, this study aims to identify and analyze the phytochemical compounds present in *W. filifera* leaves, contributing to the exploration of its potential medicinal uses. This study not only deepens our understanding of the phytochemical composition and medicinal potential of Washingtonia filifera leaves, but will also serve as a basis for future research, drug development, and sustainable use of this plant, especially in areas such as Iraq where it is abundant.

Material and Methods

Plant Sample Collection

Fresh *Washingtonia filifera* leaves were collected from the shade house of the College of Agriculture, University of Basrah, during the (2023-2024) agricultural season. The leaves were washed with running water for 5 minutes and then with distilled water, air-dried, and ground into powder using a laboratory blender. The samples were stored in airtight glass containers at 4°C until use.

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Preparation of Ethanolic Extract of Washingtonia Leaves

Twenty-five grams of dry samples (leaves) were dissolved in 500 ml of ethanol (1 g in 20 ml of ethanol) as described by (8) and left for 24 hours. The extract was filtered using Whatman No. 41 filter paper, and the filtrate was concentrated using a rotary evaporator at 45°C under low pressure.

GC-MS Analysis

GC-MS analysis was conducted at the laboratories of the College of Agriculture, University of Basrah to identify the chemical components in the ethanolic extract of *Washingtonia filifera* leaves. The analysis was performed on an Agilent 7890B GC system coupled with a 5977A MSD system (with an autosampler and a gas chromatograph-mass spectrometer) using an EI detector.

Column Conditions

An HP-5ms column (5% phenyl, 95% methyl siloxane, 30 m length, 250 µm diameter, 0.25 µm film thickness) was used. The oven temperature was set at 40°C hold for 5 minutes, then ra 10°C per minute iced to 8 ml. min $^{-1}$ to 300°C for 20 minutes. The carrier gas (helium) flow rate was 1 ml. min^{-1} and the purge flow rate were 3 ml.min^{-1}.

Injection

Pulsed spitless injection was done at 290°C with an injection volume of 1 µl. The mass spectrometer ion source temperature was 230°C, with a scan rate of 1562 (N2), and a mass range of 44-750 m/z.

Identification of Phytochemical Compounds in Washingtonia Leaves Extract

The bioactive compounds in the ethanolic extract of Washingtonia leaves were identified based on retention time. The GC-MS mass spectrum was interpreted using the National Institute of Standards and Technology (NIST) 2014, 2020 library data base, and the compound identities were confirmed using the Wiley 9 library. The compound name, molecular weight, molecular formula, and structure were verified.

Results and Discussion

GC-MS is one of the most accurate methods for identifying various chemical components in plant extracts. The ethanolic extract of *Washingtonia filifera* was analyzed by GC-MS to identify the different compounds using the NIST library. The GC-MS analysis revealed more than 40 chemical compounds (Table 1) and (Figure 1), with the most medically important compounds listed in (Table 2). Table 1 details the biologically active chemical components, including peak, retention time, molecular formula, and peak area percentage, comprising phenols, alkaloids, glycosides, fatty acids, and their esters. The key medical compounds identified in the ethanolic extract of Washingtonia leaves were as follows: Pelletierine 1.513% Pct 7.606 R.T. , 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- 4.275% Pct 11.943 R.T. , Pentadecanoic acid, 14-methyl-, methyl ester 1.034% Pct 21.518 R.T. , beta-D-Glucopyranoside methyl 14.672% Pct 18.902 R.T. , Loliolide 2.92% Pct 20.034 R.T. , n-Hexadecanoic acid 4.634% Pct 21.887 R.T. , Phytol 20.834% Pct 23.317 R.T. , 9,12,15-Octadecatrienoic acid, (ZZZ)- 3.053% Pct 23.561 R.T. , and Vitamin E 16.61% Pct 31.07 R.T. .

The results of the GC-MS analysis (Table 1) indicated that the compound Dihydro-3-

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(2H)-thiophenone had the lowest retention time 4.409 minutes with a relative abundance of 0.371%, while the compound with the highest retention time was Arsenous acid, tris(trimethylsilyl) ester 34.228 minutes with a relative abundance of 1.059%. The current study also revealed the presence of several significant and active compounds in the ethanolic extract of Washingtonia leaves (Table 2). The Washingtonia leaf extract was distinguished by its content of Dimethyl Sulfoxide (DMSO), a sulfur-containing organic compound, used to preserve hematopoietic cells and thus can be infused into patients undergoing stem cell transplantation (9). Additionally, it is used as a solvent for chemotherapy drugs, and due to its anti-inflammatory properties, it has been successfully used to treat rheumatism in humans (10), as well as for pulmonary treatment (11), and for the gastrointestinal, nervous, urinary, and skin diseases (12).

Table 1. Chemical components identified by GC-MS in Washingtonia leaves

Peak	R.T.	Peak area	Library/ID
1	4.409	0.371	Dihydro-3-(2H)-thiophenone
2	5.784	1.92	Dimethyl Sulfoxide
3	7.551	0.325	1-Butanol, 3-methyl-, carbonate (2:1)
4	7.606	1.513	Pelletierine
5	8.541	0.221	1-Heptene, 4-methyl-
6	8.793	1.281	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
7	9.075	0.213	Phenol
8	10.654	1.133	1-Ethyl-2-pyrrolidinone
9	11.84	0.352	2-Propanamine, N-methyl-N-nitroso-
10	11.943	4.275	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
11	12.972	0.282	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose
12	13.192	0.359	Benzofuran, 2,3-dihydro-
13	14.48	0.944	2-Methoxy-4-vinylphenol
14	16.805	0.24	Pentadecane, 2,6,10-trimethyl-
15	16.915	0.392	Hexanoic acid, 3,5,5-trimethyl-, tridec-6-yl ester
16	17.355	0.519	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-
17	17.622	0.407	Dodecanoic acid
18	17.708	0.525	Phenol, 4-ethenyl-2,6-dimethoxy-
19	17.795	0.491	2-Methyl-5-oxohexanethioic acid, S-t-butyl ester
20	18.046	0.233	Diethyl Phthalate
21	18.447	0.348	3-(4-Isopropylphenyl)-2-methylpropionaldehyde
22	18.902	14.672	.beta.-D-Glucopyranoside, methyl
23	19.295	0.268	Heptadecane
24	19.837	0.477	Pentadecanoic acid
25	20.034	2.92	Loliolide
26	20.253	0.282	4,5-Dimethyl-2-pyrimidone
27	20.654	0.722	Neophytadiene
28	20.725	0.82	2-Pentadecanone, 6,10,14-trimethyl-
29	20.992	0.276	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
30	21.086	0.473	1,19-Eicosadiene
31	21.518	1.034	Pentadecanoic acid, 14-methyl-, methyl ester
32	21.887	4.634	n-Hexadecanoic acid

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33	21.927	4.286	Dibutyl phthalate
34	22.186	0.246	Hexadecanoic acid, ethyl ester
35	23.199	0.453	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
36	23.317	20.834	Phytol
37	23.49	0.522	10E,12Z-Octadecadienoic acid
38	23.561	3.053	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
39	23.733	0.423	Octadecanoic acid
40	23.804	0.307	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-
41	26.624	0.21	15-Hydroxypentadecanoic acid
42	26.938	0.23	Bis(2-ethylhexyl) phthalate
43	28.965	0.744	Squalene
44	30.497	1.053	.gamma.-Tocopherol
45	31.07	16.61	Vitamin E
46	32.115	0.925	Stigmasterol
47	32.626	1.927	.gamma.-Sitosterol
48	33.364	1.123	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-
49	33.725	0.966	Tris(tert-butyldimethylsilyloxy)arsane
50	33.992	2.107	A'-Neogammacer-22(29)-en-3-one
51	38.203	1.059	Arsenous acid, tris(trimethylsilyl) ester

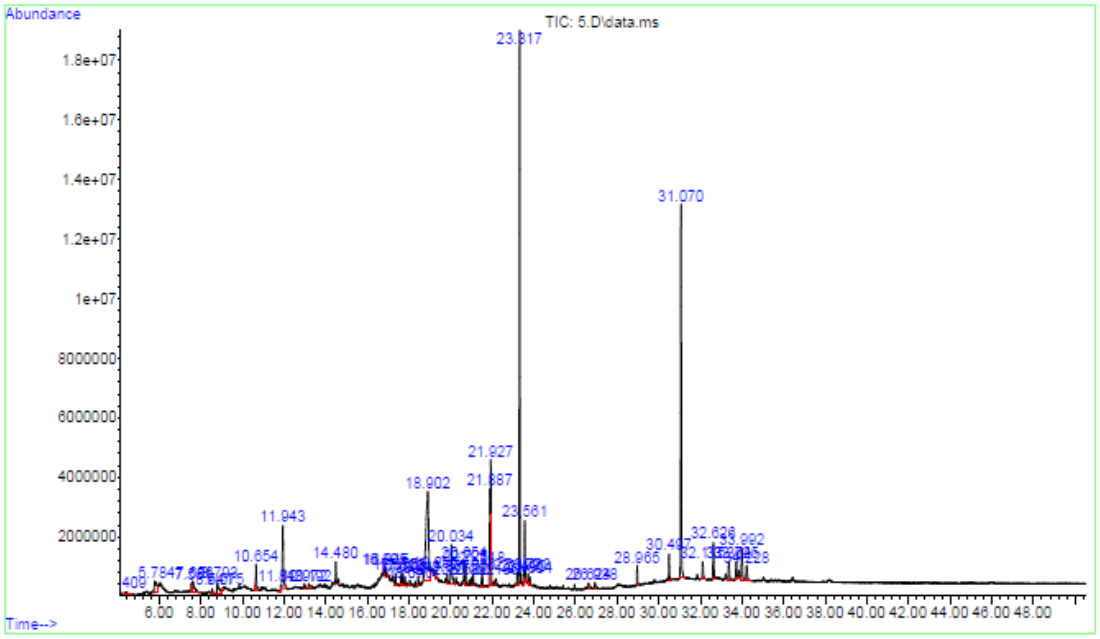
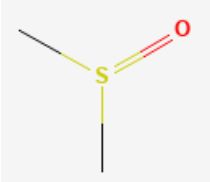
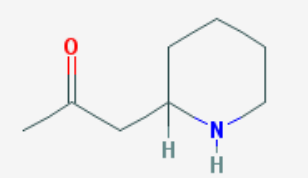
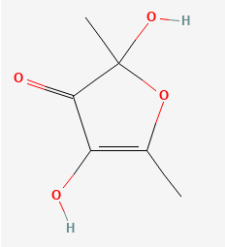
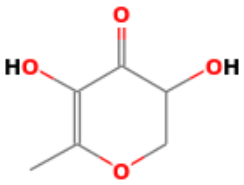
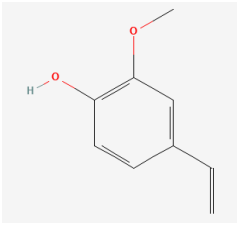
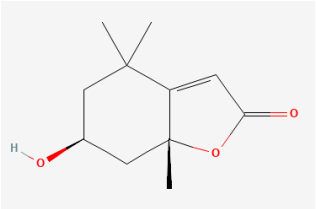
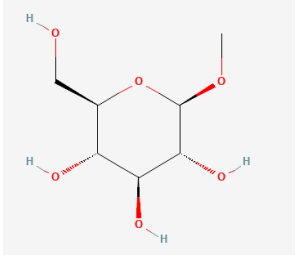


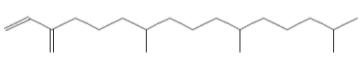
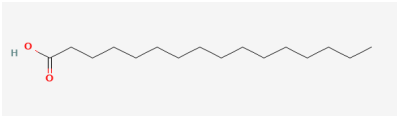
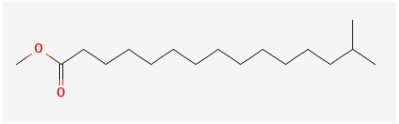
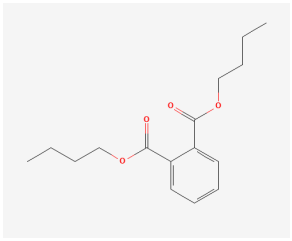
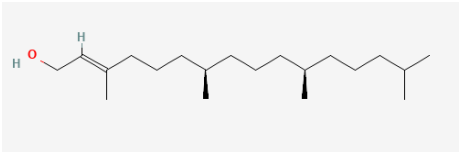
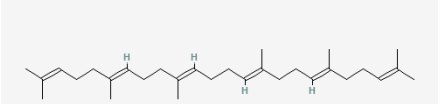
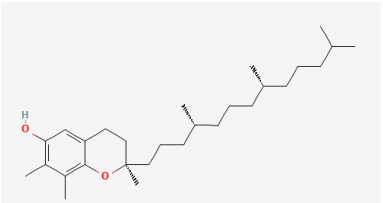
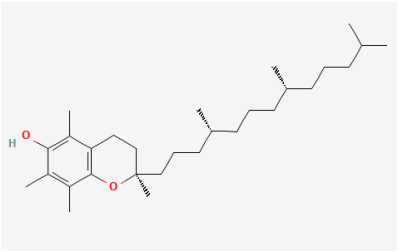
Figure 1. The GC-MS analysis of *Washingtonia filifera*

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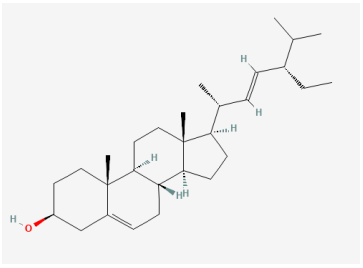
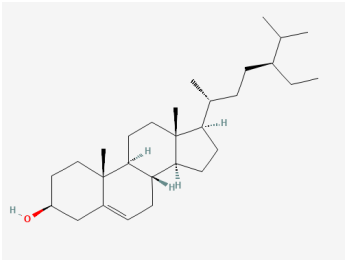
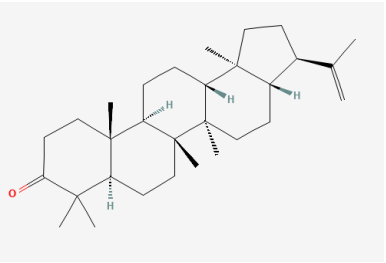
Table2. Phytochemical compounds identified by GC-MS in *Washingtonia filifera* leaves

Sequence	Compound	Molecular Formula	Chemical Structure	Classification
1	Dimethyl Sulfoxide	C2H6OS		Sulfur-containing organic compound
2	Pelletierine	C8H15NO		Alkaloid
3	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C6H8O4		Aromatic furanone
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4		oxazine
5	2-Methoxy-4-vinylphenol	C9H10O2		Phenols
6	Loliolide	C11H16O3		Sesquiterpene lactone
7	beta-D-Glucopyranoside, methyl	C7H14O6		Glycosides

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Sequence	Compound	Molecular Formula	Chemical Structure	Classification
8	Neophytadiene	C20H38		Terpenes, specifically diterpene
9	n-Hexadecanoic acid	C16H32O2		Fatty acid
10	Pentadecanoic acid, 14-methyl-, methyl ester	C17H34O2		Fatty acid
11	Dibutyl phthalate	C16H22O4		Esters
12	Phytol	C22H42O2		Terpenes, specifically diterpene
13	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2		Fatty acid
14	Squalene	C30H50		Terpenes, specifically triterpenes
15	gamma-Tocopherol	C28H48O2		Vitamin
16	Vitamin E	C29H50O2		Vitamin

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Sequence	Compound	Molecular Formula	Chemical Structure	Classification
17	Stigmasterol	C29H48O		steroid
18	gamma-Sitosterol	C29H50O		steroid
19	A'-Neogammacer-22(29)-en-3-one	C30H48O		Terpenes, specifically triterpenes

Pelletierine, an alkaloid compound, was also discovered, widely used in medicine to treat diarrhea and dysentery, and to eradicate tapeworms and intestinal worms. It is also used as an antibacterial and antifungal agent when mixed with iron salts (13). Pelletierine is also known for its anti-inflammatory and anti-tumor properties (14). The results showed the presence of 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, known as furaneol, an aromatic compound found in various fruits and foods, which acts as an antimicrobial agent against infection-causing microbes in human microbial infections (15). The compound 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- also has medical importance as it is an anti-cough, antibacterial, antioxidant (16), anti-inflammatory, anti-asthmatic (17), anticancer, and laxative agent (18).

The compound 2-Methoxy-4-vinylphenol functions as an antioxidant and antifungal (19). Loliolide, a terpenoid compound, acts as an anti-aging, antioxidant, anti-inflammatory, neuroprotective, anti-Parkinsonian, anti-cholinesterase, and antidepressant agent (20). The compound beta-D-Glucopyranoside, methyl, a glycoside, exhibited antioxidant, anti-inflammatory, anticancer, antibacterial, and antifungal activities, and plays a role in carbohydrate metabolism and enzymatic reactions within plants (21).

Neophytadiene was also identified, known for its analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant properties (22). Washingtonia leaf extract contains several Hexadecanoic acid compounds, known as Palmitic acid, including n-Hexadecanoic acid, which acts as an antioxidant, antimicrobial, cholesterol-lowering agent, nematicide, insecticide, and antiandrogen (23). Pentadecanoic acid, 14-methyl-, methyl ester, a saturated fatty acid known as isopalmitic acid, functions as an

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antioxidant, antimicrobial (24), and anticancer agent (25). Dibutyl phthalate, discovered in the extract, exhibits antifungal, antimicrobial, and antimalarial activities (26).

Phytol, an essential part of chlorophyll, was identified in the ethanolic extract of Washingtonia leaves, with medicinal properties such as analgesic, antipyretic, anti-inflammatory, antioxidant, antiallergic, lipid-lowering, anticancer, antimicrobial, antidiabetic, anti-teratogenic, antispasmodic, and antiseptic effects (27). The study results indicate the presence of compounds 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)-, 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-, and 9,12-Octadecadienoic acid (Z, Z)- in the extract. The highest identified compound was 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-, known as Linolenic acid, an omega-3 fatty acid with therapeutic roles in reducing cardiovascular disease risk, anti-inflammatory, anticancer, antimicrobial, antioxidant, cholesterol-lowering, anthelminthic, anti-acne, anti-arthritic, anti-atherogenic, insecticidal, cancer-preventive, hepatoprotective, and anthelmintic properties (28). Octadecatrienoic acid, (Z, Z, Z)- is an unsaturated fatty acid acting as a strong antioxidant and precursor to jasmonic acid, which stimulates anti-stress responses(29), and enhances Photosystem II repair by accelerating protein synthesis and relieving PSII inhibition (30). GC-MS analysis revealed the presence of Squalene, a key compound contributing to biological activities such as antioxidant, antimicrobial, anticancer, antidiabetic, and anti-inflammatory properties (31,32). Vitamin E and gamma-Tocopherol, belonging to the fat-soluble vitamin family, are known for their strong antioxidant and anti-inflammatory properties, beneficial for neuroprotection, cardiovascular health, skin, bone health, and immune enhancement (32,33).

Sterols are crucial components in eukaryotic organisms, playing vital roles in plant cell membranes. Phytosterols' biological role in human and animal health focuses on their immune activity. GC-MS analysis of Washingtonia leaves revealed Stigmasterol and gamma-Sitosterol, which boost immunity, prevent cancer, and treat heart diseases and high cholesterol (34), as well as osteoporosis (35), anti-inflammatory, antidiabetic, antiparasitic, antifungal, antibacterial, and antioxidant properties (36). The analysis also detected A'-Neogammacer-22(29)-en-3-one, exhibiting significant biological activities such as antioxidant, cholesterol-lowering (37), and antibacterial effects (38).

Conclusion

The GC-MS analysis of the ethanolic extract of *Washingtonia filifera* leaves revealed the presence of a diverse range of biologically and pharmacologically active compounds. Among the most significant identified compounds are Pelletierine, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Pentadecanoic acid, 14-methyl-, methyl ester, beta-D-Glucopyranoside, methyl, Loliolide, n-Hexadecanoic acid, Phytol, 9,12,15-Octadecatrienoic acid (Z,Z,Z), and Vitamin E. The presence of these compounds enhances the medicinal value of the plant extract due to their multiple therapeutic properties. Specifically, beta-D-Glucopyranoside, methyl has exhibited antioxidant and anti-inflammatory activities, along with anticancer, antibacterial, and antifungal effects, suggesting its potential role in promoting health and protecting the body from various diseases. Additionally, squalene, one of the key identified compounds, plays a crucial role in biological activity, acting as a potent antioxidant that helps prevent oxidative

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damage caused by free radicals. It also exhibits antimicrobial and anticancer properties. Moreover, stigmasterol and gamma-sitosterol have demonstrated significant pharmacological importance, contributing to immune system enhancement, cancer prevention, and cholesterol reduction, making the ethanolic extract of *Washingtonia filifera* leaves a promising candidate for cardiovascular and metabolic disease prevention. Considering that many of the compounds identified in this extract are also found in other medicinal plants, such as papaya (39) and pomegranate (40), which are well known for their therapeutic efficacy, it is plausible that the bioactive compounds in *Washingtonia filifera* exhibit similar or even greater pharmacological effects. This underscores the potential of this plant extract as a natural source of bioactive compounds, which could be further explored for applications in pharmaceuticals, dietary supplements, cosmetics, and agricultural industries. Despite the promising results obtained, further advanced studies are required to isolate, characterize, and purify these bioactive compounds to gain a more precise understanding of their mechanisms of action. This necessitates the use of modern analytical techniques, such as high-performance liquid chromatography (HPLC) for compound separation and nuclear magnetic resonance (NMR) for molecular structure elucidation. Additionally, biological evaluations through in vitro and in vivo assays are essential to assess their antioxidant capacity, anticancer potential, antibacterial and antifungal efficacy, and anti-inflammatory properties. Investigate how the chemical structure of isolated compounds correlates with their biological activity to optimize their therapeutic potential. Study the optimal conditions for cultivating Washingtonia filifera to maximize the yield of bioactive compounds while ensuring sustainability.

Conflict of interest

The authors declare no conflict of interest.

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