Research Article

***In Vitro* Antileishmanial Activity and GC-MS Analysis of Whole Plant Hexane Fraction of *Achillea wilhelmsii* (WHFAW)**

Jahangir Khan Achakzai¹, Muhammad Anwar Panezai¹, Abdul Manan Kakar¹, Basira Akhtar², Ali Akbar³, Shahabuddin Kakar⁴, Javed Khan⁵, Nazima Yousaf Khan¹, Ghulam Mustafa Khan⁶, Nizam Baloch⁶, Muhammad Hashim Jan Khoso¹, and Marina Panezai¹

¹Institute of Biochemistry, University of Balochistan, Quetta 87300, Pakistan

²Department of Botany, University of Balochistan, Quetta 87300, Pakistan

³Department of Microbiology, University of Balochistan, Quetta 87300, Pakistan

⁴Department of Zoology, University of Balochistan, Quetta 87300, Pakistan

⁵Department of Microbiology, Quaid-i-Azam University, Islamabad 45320, Pakistan

⁶Department of Chemistry, University of Balochistan, Quetta 87300, Pakistan

Correspondence should be addressed to Muhammad Anwar Panezai; anwarpaneza@yahoo.com

Received 5 July 2019; Accepted 6 August 2019; Published 1 October 2019

Academic Editor: Gabriel Navarrete-Vazquez

Copyright © 2019 Jahangir Khan Achakzai et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

In this study, GC-MS analysis has shown that whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW) consists of 66 compounds which exhibited antileishmanial activity. Antileishmanial bioassay was the method used for determining antileishmanial activity. The inhibitory concentration (IC_{50}) which was observed for whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW) against parasitic and vector-borne disease, leishmaniasis, is $58.27 \pm 0.52 \mu\text{g/mL}$. For leishmanicidal assay, *Leishmania major* is the species used for analysis. Whole plant methanol extract of *Achillea wilhelmsii* (WMEAW) and whole plant aqueous fraction of *Achillea wilhelmsii* (WFAFW) exhibited no antileishmanial activity.

1. Introduction

The genus *Achillea* consists of more than 100 species and belongs to the family Asteraceae, innate to Western Asia and Europe [1]. *Achillea* means Achilles which in Greek language refers to hero who with the usage of yarrow treated the wounds of soldiers during battles [2]. High mountains of the Mediterranean are the location for the occurrence of *Achillea* species [3]. The extracts and fractions of *Achillea* exhibited analgesic, antiulcerogenic, anti-inflammatory, antipyretic, antioxidant, cytotoxic, antibacterial, hypoglycemic, and immunosuppressive properties [4–12]. *Achillea wilhelmsii* exhibited antimycobacterial, antihypertensive abdominal pain, flatulence, and antihyperlipidemic properties [13–15]. Chemically, *Achillea wilhelmsii* consist of sesquiterpenoids, cineol, flavonoids, borneol, alkaloids, thujene, carvacrol, caryophyllene,

monoterpenoids, camphor, rutin, pinene, and linalool [16, 17]. The parasitic and vector-borne disease, leishmaniasis, is caused by 21 out of 30 species of *Leishmania*, and in the entire world, this is the 6th infectious disease predominantly in tropical and subtropical countries [18]. Leishmaniasis is classified into three forms, for instance, visceral, cutaneous, and mucocutaneous [19, 20]. In this research study, the extract and fractions of *Achillea wilhelmsii* are examined against leishmaniasis.

2. Materials and Methods

2.1. Plant Material. In this research study, whole plant of *Achillea wilhelmsii* was used.

2.2. Extraction. The plant *Achillea wilhelmsii* was collected from Killi Murda Karez Chaman, Balochistan, Pakistan,

and identified by Prof. Dr. Rasool Bakhsh Tareen, Department of Botany, University of Balochistan, Quetta, Pakistan. 0.5 kg whole plant of *Achillea wilhelmsii* was dried in shade and then grinded, soaked in 2 liters of methanol, kept for 7 days, and shaken daily. After 7-day period, methanol containing whole plant of *Achillea wilhelmsii* was filtered and vaporized with the help of rotary evaporator. Dried semisolid whole plant methanolic extract of *Achillea wilhelmsii* (WMEAW) was 53.2 g. This crude extract was examined for antileishmanial activity while the remaining extract was fractionated with solvents, for instance, *n*-hexane and aqueous.

2.3. Fractionation of Crude Extract. In a separatory funnel, with crude extract, two solvents were added such as *n*-hexane and aqueous. The separatory funnel was shaken thoroughly to create two layers such as *n*-hexane layer and aqueous layer. Both solvents were separately vaporized with the help of rotary evaporator to form whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW) 8.1 g and whole plant aqueous fraction of *Achillea wilhelmsii* (WAWAW) 17.3 g. Both fractions were examined for antileishmanial activity [21, 22].

2.4. Antileishmanial Bioassay. The extracts and fractions of medicinal plants were examined against *Leishmania major* (promastigotes) in culture by means of microplates. Using normal physiological saline, in bulk, promastigotes of *Leishmania major* (MHOM/Pk/88/DESTO) were grown in NNN biphasic medium. Then, for the culturing of promastigotes, RPMI 1640 medium (Sigma, St Louis, USA) supplemented with 10% heat-inactivated FBS (PAA Laboratory GmbH, Austria) was used. At log phase, the promastigotes were harvested, and then for 10 min at 2000 rpm, the leishmanial parasites were centrifuged. At same speed and time, the *Leishmania major* was washed three times with saline. Lastly, under microscope, by using Neubauer chamber, the promastigotes were counted and diluted with the addition of fresh culture medium to develop the leishmanial parasites with a final density of 10^6 cells/mL.

In a 96-well microtitre plate, culture medium with 180 μ L was added in different wells. PBS with pH 7.4 having 0.5% DMSO and 0.5% MeOH was used for the dissolution of extracts and fractions of medicinal plants to make 1000 mg/mL stock solution. The extracts and fractions with concentration of 20 μ L were introduced in wells, and this was further serially diluted to develop working solution with 1–100 μ g/mL range. The parasite culture with 100 μ L was introduced in all wells. During this protocol, two rows were left; one row was for negative control which received medium while the other for positive control that received the standard antileishmanial drugs such as amphotericin B (Fluka) and pentamidine (ICN). The 96-well microtitre plate was incubated for 72 h between 21 and -22°C . With the help of microscope, the culture was observed for cell viability; for instance, the number of motile cells was counted on an improved Neubauer counting chamber, and with the help of

software Ezfit 5.03 (Perella Scientific, USA), the IC_{50} values of extracts and fractions with antileishmanial activities were calculated [23].

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis Triple Quadrupole Acquisition Method MS Parameters. For identification and quantification of *Achillea wilhelmsii* compounds: 2 μ L of *Achillea wilhelmsii* extract or fraction was directly injected into the gas chromatograph mod. 6890N Network GC System (Agilent Technologies, Palo Alto, CA) together in the presence of mass spectrometer mod. 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, CA) and furnished in the presence of a column HP-5MS (30 m length, 0.25 mm interior diameter, 0.25 μ m film width; Agilent Technologies, Palo Alto, CA). Helium gas was off. Injection was made into a split-splitless injector (split ratio of 30:1) at 250°C . The oven program was the following: 70°C for 3 min then $6^{\circ}\text{C}/\text{min}$ to 180 for 5 min, then $6^{\circ}\text{C}/\text{min}$ to 280°C for 10 min, and then $8^{\circ}\text{C}/\text{min}$ to 290°C for 20 min. The MSD transfer line was set at a temperature of 250°C ; MSD temperature quadrupole was of 150°C and ionization temperature was 230°C . Mass spectra were seventy electronvolts, and scan achievement was accomplished in the series between 35 and 300 m/z . The identification of the components of the *Achillea wilhelmsii* extract or fraction was assigned by matching their mass spectra with those available in the libraries NIST 02 and WILEY [24].

3. Results and Discussion

Antileishmanial results have shown that whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW) exhibited antileishmanial activity. The inhibitory concentration (IC_{50}) which is observed for whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW) against parasitic and vector-borne disease, leishmaniasis, is $58.27 \pm 0.52 \mu\text{g/mL}$. For leishmanicidal assay, *Leishmania major* is the species used for analysis. In this bioassay, amphotericin B (IC_{50} : $0.29 \pm 0.05 \mu\text{g/mL}$) and pentamidine (IC_{50} : $5.09 \pm 0.09 \mu\text{g/mL}$) were used as positive control drugs to compare the parasite inhibition with that whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW). This activity was perceived under an incubation period of 72 h and an incubation temperature of 22°C . Whole plant methanolic extract of *Achillea wilhelmsii* (WMEAW) and whole plant aqueous fraction of *Achillea wilhelmsii* (WAWAW) exhibited no antileishmanial activity. The antileishmanial activities of extract and fractions of *Achillea wilhelmsii* are shown in Table 1.

Molecular formula, molecular mass, structure, and RT of compounds 1–66 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW) are shown in Tables 2–14 while mass spectra interpretation of compounds 1–66 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW) are presented in Tables 15–27. Mass spectra of compounds 1–66 are shown in Figures 1–66.

TABLE 1: Antileishmanial activities of extracts and fractions of whole plant of *Achillea wilhelmsii*.

S. no	Name of extracts/fractions/standard drugs	IC ₅₀ (μg/mL) ± S.D.
1	WMEAW	>100
2	WHFAW	58.27 ± 0.52 moderate activity
3	WAWAW	>100
4	Amphotericin B	0.20 ± 0.5
5	Pentamidine	5.09 ± 0.9

TABLE 2: Molecular formula, molecular mass, structure, and RT of compounds 1–5 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
1	Eucalyptol	C ₁₀ H ₁₈ O	154		7.606
2	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	C ₇ H ₁₀ O ₂	126		7.833
3	Terpineol, cis-β-	C ₁₀ H ₁₈ O	154		8.528
4	2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-	C ₁₀ H ₁₈ O ₂	170		8.660
5	1,6-Octadien-3-ol, 3,7-dimethyl-	C ₁₀ H ₁₈ O	154		9.363

TABLE 3: Molecular formula, molecular mass, structure, and RT of compounds 6–10 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
6	Butanoic acid, 2-methyl-, 2-methylbutyl ester	C ₁₀ H ₂₀ O ₂	172		9.458
7	<i>n</i> -Amyl isovalerate	C ₁₀ H ₂₀ O ₂	172		9.568
8	2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl-methanol	C ₁₁ H ₂₀ O	168		9.905

TABLE 3: Continued.

Compound	Name	Molecular formula	Molecular mass	Structure	RT
9	Isopinocarveol	C ₁₀ H ₁₆ O	152		10.476
10	Bicyclo[2.2.1]heptane-2,5-diol, 1,7,7-trimethyl-, (2-endo,5-exo)-	C ₁₀ H ₁₈ O ₂	170		10.622

TABLE 4: Molecular formula, molecular mass, structure, and RT of compounds 11–15 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
11	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	C ₁₀ H ₁₆ O	152		10.930
12	2(10)-Pinen-3-one	C ₁₀ H ₁₄ O	150		11.113
13	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	C ₁₀ H ₁₈ O	154		11.215
14	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	C ₁₀ H ₁₈ O	154		11.508
15	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	C ₁₀ H ₁₈ O ₂	170		11.801

TABLE 5: Molecular formula, molecular mass, structure, and RT of compounds 16–20 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
16	3-Cyclohexene-1-methanol, α,α 4-trimethyl-	C ₁₀ H ₁₈ O	170		11.867
17	1,7-Octadiene-3,6-diol, 2,6-dimethyl	C ₁₀ H ₁₈ O ₂	170		14.071

TABLE 5: Continued.

Compound	Name	Molecular formula	Molecular mass	Structure	RT
18	Thymol	C ₁₀ H ₁₄ O	150		14.620
19	Phenol, 2-methoxy-3-(2-propenyl)-	C ₁₀ H ₁₂ O ₂	164		16.319
20	7-Hexadecene, (Z)-	C ₁₆ H ₃₂	224		17.124

TABLE 6: Molecular formula, molecular mass, structure, and RT of compounds 21–25 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
21	Caryophyllene	C ₁₅ H ₂₄	204		17.959
22	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	C ₁₅ H ₂₆ O	222		21.385
23	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1α,4α,7β,7aβ,7bα)]-	C ₁₅ H ₂₄ O	220		21.803
24	Caryophyllene oxide	C ₁₅ H ₂₄ O	220		21.942
25	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	C ₁₅ H ₂₄ O	220		23.070

TABLE 7: Molecular formula, molecular mass, structure, and RT of compounds 26–30 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
26	Oxacyclotetradecan-2-one, 14-methyl	C ₁₄ H ₂₆ O ₂	226		23.465
27	Caryophyllene oxide	C ₁₅ H ₂₄ O	220		23.890

TABLE 7: Continued.

Compound	Name	Molecular formula	Molecular mass	Structure	RT
28	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	C ₁₅ H ₂₆ O ₂	238		25.493
29	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228		25.793
30	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	C ₁₅ H ₂₆ O	222		27.184

TABLE 8: Molecular formula, molecular mass, structure, and RT of compounds 31–35 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
31	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296		27.814
32	cis-9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254		28.305
33	2,6,10-Dodecatrien-1-ol, 12-acetoxy-2,6,10-trimethyl-(E,E,E)-	C ₁₇ H ₂₈ O ₃	280		31.570
34	<i>n</i> -Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256		32.486
35	Cholestan-3-ol, 2-methylene-, (3β,5α)-	C ₂₈ H ₄₈ O	400		34.463

TABLE 9: Molecular formula, molecular mass, structure, and RT of compounds 36–40 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
36	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	C ₁₅ H ₂₆ O ₂	238		36.806

TABLE 9: Continued.

Compound	Name	Molecular formula	Molecular mass	Structure	RT
37	trans-Z- α -Bisabolene epoxide	C ₁₅ H ₂₄ O	220		38.475
38	1-Hexadecanol	C ₁₆ H ₃₄ O	242		39.127
39	9,12-Octadecadienoic acid, methyl ester, (E,E)-	C ₁₉ H ₃₄ O ₂	294		39.903
40	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C ₁₉ H ₃₂ O ₂	292		40.349

TABLE 10: Molecular formula, molecular mass, structure, and RT of compounds 41–45 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
41	Phytol	C ₂₀ H ₄₀ O	296		41.118
42	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280		42.297
43	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	278		42.700
44	α -Santonin	C ₁₅ H ₁₈ O ₃	246		43.293
45	Limonen-6-ol, pivalate	C ₁₅ H ₂₄ O ₂	236		43.439

TABLE 11: Molecular formula, molecular mass, structure, and RT of compounds 46–50 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
46	Hexadecanoic acid, 1,1-dimethylethyl ester	C ₂₀ H ₄₀ O ₂	312		44.237
47	1-Eicosanol	C ₂₀ H ₄₂ O	298		46.851
48	Tetradecane, 2,6,10-trimethyl-	C ₁₇ H ₃₆	240		47.137
49	Butyl 9,12-octadecadienoate	C ₂₂ H ₄₀ O ₂	336		48.228
50	Butyl 9,12,15-octadecatrienoate	C ₂₂ H ₃₈ O ₂	334		48.360

TABLE 12: Molecular formula, molecular mass, structure, and RT of compounds 51–55 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
51	1-Docosene	C ₂₂ H ₄₄	308		50.476
52	Heptacosane	C ₂₇ H ₅₆	380		50.607
53	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	330		50.739
54	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	390		51.318
55	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₃₈ O ₄	354		53.097

TABLE 13: Molecular formula, molecular mass, structure, and RT of compounds 56–60 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
56	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	306		53.207
57	1-Hexadecanol, 2-methyl-	C ₁₇ H ₃₆ O	256		55.396
58	dl- α -Tocopherol	C ₂₉ H ₅₀ O ₂	430		57.842
59	Pregnan-20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-, (3 β ,5 α ,6 α ,16 α)-	C ₂₃ H ₃₂ O ₅	388		57.937
60	Campesterol	C ₂₈ H ₄₈ O	400		58.962

TABLE 14: Molecular formula, molecular mass, structure, and RT of compounds 61–66 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	Name	Molecular formula	Molecular mass	Structure	RT
61	Stigmasterol	C ₂₉ H ₄₈ O	412		59.357
62	β -Sitosterol	C ₂₉ H ₅₀ O	414		60.126
63	Quassin	C ₂₂ H ₂₈ O ₆	388		60.338

TABLE 14: Continued.

Compound	Name	Molecular formula	Molecular mass	Structure	RT
64	9,19-Cyclolanost-24-en-3-ol, (3 β)-	C ₃₀ H ₅₀ O	426		61.195
65	9,19-Cyclolanost-24-en-3-ol, acetate, (3 β)-	C ₃₂ H ₅₂ O ₂	468		62.908
66	Ethanol, 2-(9-octadecenyoxy)-, (Z)-	C ₂₀ H ₄₀ O ₂	312		63.626

TABLE 15: Mass spectra of compounds 1–5 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
1	154(M ⁺), 111(389), 108(482), 93(503), 84(443), 81(727), 71(506), 69(455), 55(403), 43(999), 41(320)
2	126(M ⁺), 111(999), 99(255), 98(210), 83(220), 71(410), 67(320), 56(310), 55(620), 43(510), 27(255)
3	154(M ⁺), 111(183), 93(316), 81(230), 79(171), 71(534), 69(240), 67(142), 55(335), 43(999), 41(500)
4	170(M ⁺), 94(224), 69(201), 68(330), 67(266), 59(999), 55(346), 43(668), 41(523), 39(233), 27(270)
5	154(M ⁺), 121(232), 93(760), 80(321), 71(999), 69(426), 67(206), 55(637), 43(598), 41(564), 39(179)

TABLE 16: Mass spectra of compounds 6–10 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
6	172(M ⁺), 103(133), 85(705), 71(224), 70(999), 57(818), 55(227), 43(427), 42(123), 41(348), 29(311)
7	172(M ⁺), 85(999), 71(263), 70(884), 57(626), 55(262), 43(589), 42(175), 41(349), 29(234), 27(154)
8	168(M ⁺), 95(700), 85(749), 84(849), 83(509), 81(794), 79(577), 69(842), 55(728), 43(550), 41(999)
9	152(M ⁺), 92(708), 91(543), 83(624), 79(350), 70(797), 69(561), 55(859), 41(999), 39(396), 27(336)
10	170(M ⁺), 108(722), 95(379), 93(669), 49(467), 67(561), 55(627), 43(450), 41(999), 39(596), 27(509)

TABLE 17: Mass spectra of compounds 11–15 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
11	152(M ⁺), 137(999), 109(311), 95(129), 93(155), 79(156), 69(342), 67(131), 55(159), 41(239), 29(118)
12	150(M ⁺ , 299), 108(759), 107(398), 81(999), 79(335), 69(424), 53(970), 41(673), 39(319), 27(307)
13	154(M ⁺), 110(196), 95(999), 93(111), 67(92), 55(150), 43(176), 41(323), 39(198), 29(160), 27(213)
14	154(M ⁺), 111(494), 93(505), 86(247), 71(999), 69(226), 67(184), 55(265), 43(544), 41(356), 27(175)
15	170(M ⁺), 85(80), 83(82), 82(999), 71(634), 67(365), 55(113), 43(572), 41(144), 39(60), 27(102)

TABLE 18: Mass spectra of compounds 16–20 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
16	170(M ⁺), 136(462), 121(487), 95(125), 93(602), 92(165), 81(331), 67(166), 59(999), 43(314), 41(155)
17	170(M ⁺), 82(526), 71(851), 69(172), 68(375), 67(999), 55(410), 43(864), 41(406), 39(176), 27(246)
18	150(M ⁺ , 248), 136(89), 135(999), 117(87), 115(91), 91(157), 77(80), 51(56), 41(55), 39(94)
19	164(M ⁺ , 999), 149(415), 137(210), 131(279), 121(245), 104(223), 103(344), 91(280), 77(378), 55(271)
20	224(M ⁺), 97(360), 83(517), 70(446), 69(628), 57(572), 56(547), 55(999), 43(664), 41(569), 29(242)

TABLE 19: Mass spectra of compounds 21–25 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
21	204(M ⁺), 133(646), 107(389), 105(372), 93(937), 91(551), 81(389), 79(614), 69(976), 55(432), 41(999)
22	222(M ⁺), 107(275), 93(560), 81(241), 79(192), 71(370), 69(999), 67(249), 55(272), 43(410), 41(586)
23	220(M ⁺), 205(440), 159(365), 119(524), 105(555), 93(449), 91(647), 79(379), 55(343), 43(999), 41(709)
24	220(M ⁺), 95(420), 93(661), 91(573), 81(373), 79(885), 69(407), 67(377), 55(393), 43(999), 41(927)
25	220(M ⁺), 136(999), 135(148), 105(132), 93(130), 91(260), 79(217), 69(190), 67(163), 55(174), 41(508)

TABLE 20: Mass spectra of compounds 26–30 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
26	226(M ⁺), 98(479), 97(375), 84(327), 83(448), 69(601), 56(376), 55(999), 43(491), 41(908), 29(354)
27	220(M ⁺), 95(420), 93(661), 91(573), 81(373), 79(885), 69(407), 67(377), 55(393), 43(999), 41(927)
28	238(M ⁺), 93(372), 85(316), 81(999), 71(623), 69(366), 67(292), 55(375), 43(919), 41(727), 29(314)
29	228(M ⁺), 185(292), 129(512), 73(999), 71(336), 69(353), 60(904), 57(652), 55(707), 43(721), 41(677)
30	222(M ⁺), 107(221), 93(402), 81(207), 71(384), 69(798), 67(242), 55(283), 53(153), 43(669), 41(999)

TABLE 21: Mass spectra of compounds 31–35 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
31	296(M ⁺), 123(892), 95(962), 82(986), 81(999), 71(748), 68(728), 57(811), 55(852), 43(965), 41(811)
32	254(M ⁺), 97(376), 96(251), 82(292), 83(461), 69(648), 67(290), 56(305), 55(999), 43(374), 41(587)
33	280(M ⁺), 107(176), 95(228), 94(138), 93(341), 81(186), 69(259), 68(314), 67(154), 43(999), 41(213)
34	256(M ⁺), 129(435), 83(267), 73(980), 71(373), 69(351), 60(999), 57(840), 55(767), 43(817), 41(574)
35	400(M ⁺), 105(344), 97(344), 95(666), 93(390), 83(528), 81(735), 79(413), 71(689), 69(999), 67(632),

TABLE 22: Mass spectra of compounds 36–40 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
36	238(M ⁺), 93(372), 85(316), 81(999), 71(623), 69(366), 67(292), 55(375), 43(919), 41(727), 29(314)
37	220(M ⁺), 109(320), 107(263), 93(291), 91(247), 79(234), 67(295), 57(311), 55(431), 43(999), 41(901)
38	242(M ⁺), 97(573), 83(760), 82(394), 70(425), 69(822), 57(653), 56(410), 55(999), 43(710), 41(758),
39	294(M ⁺), 95(450), 82(450), 81(670), 79(330), 69(350), 68(400), 67(999), 55(720), 54(380), 41(910)
40	292(M ⁺), 180(270), 95(400), 93(470), 81(430), 80(420), 79(999), 67(780), 55(570), 43(310), 41(610)

TABLE 23: Mass spectra of compounds 41–45 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
41	296(M ⁺), 123(184), 81(223), 71(999), 69(239), 68(199), 57(334), 56(169), 55(259), 43(381), 41(260)
42	280(M ⁺), 96(388), 95(617), 82(516), 81(827), 79(430), 68(437), 67(999), 55(680), 54(429), 41(523)
43	278(M ⁺), 108(391), 95(478), 93(503), 91(299), 81(373), 80(460), 79(999), 67(624), 55(463), 41(408)
44	246(M ⁺ , 667), 173(999), 172(429), 135(559), 91(793), 77(544), 69(491), 55(499), 45(351), 41(461)
45	236(M ⁺), 119(175), 109(214), 107(236), 93(398), 91(209), 85(206), 57(999), 55(287), 43(403), 41(493)

TABLE 24: Mass spectra of compounds 46–50 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
46	312(M ⁺), 73(303), 69(191), 61(153), 60(278), 57(622), 56(999), 55(317), 43(526), 41(444), 29(316)
47	298(M ⁺), 97(542), 83(620), 82(350), 71(330), 69(617), 57(743), 56(358), 55(893), 43(999), 41(799)
48	240(M ⁺), 155(134), 85(500), 84(179), 71(641), 69(147), 57(999), 56(256), 55(282), 43(782), 41(384)
49	336(M ⁺), 109(379), 96(439), 95(699), 82(489), 81(949), 79(409), 69(349), 68(389), 67(999), 55(669)
50	334(M ⁺), 121(299), 108(469), 95(679), 93(559), 91(309), 81(499), 80(409), 79(999), 67(689), 55(509)

TABLE 25: Mass spectra of compounds 51–55 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
51	308(M ⁺), 97(622), 83(617), 71(406), 70(362), 69(687), 57(902), 56(437), 55(999), 43(967), 41(859)
52	380(M ⁺), 99(140), 85(416), 71(622), 69(176), 57(999), 56(139), 55(283), 43(798), 41(275), 29(135)
53	330(M ⁺), 239(380), 98(710), 84(480), 74(630), 69(390), 57(850), 55(690), 43(999), 41(740), 29(480)
54	390(M ⁺), 167(350), 150(107), 149(999), 83(100), 71(224), 70(264), 57(341), 55(218), 43(200), 41(225)
55	354(M ⁺), 262(390), 95(490), 82(370), 81(840), 79(420), 69(390), 67(999), 55(780), 54(380), 41(690)

TABLE 26: Mass spectra of compounds 56–60 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
56	306(M^+), 93(470), 91(380), 81(630), 80(750), 79(780), 67(960), 57(400), 55(810), 43(810), 41(999)
57	256(M^+), 97(386), 83(460), 71(514), 70(341), 69(580), 57(999), 56(572), 55(670), 43(888), 41(633)
58	431($M + 1$), 84), 430(M^+), 261), 205(98), 166(114), 165(999), 164(322), 57(95), 55(77), 43(165), 41(84)
59	388(M^+), 328(999), 105(500), 95(378), 93(469), 91(730), 81(348), 79(545), 77(505), 55(580), 53(405)
60	400(M^+), 145(285), 107(403), 105(306), 95(414), 81(436), 71(312), 57(452), 55(617), 43(999), 41(470)

TABLE 27: Mass spectra of compounds 61–66 of whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW).

Compound	<i>m/z</i> (% relative abundance)
61	412(M^+), 159(418), 133(444), 105(427), 95(398), 91(427), 83(692), 81(638), 69(636), 55(999), 43(431)
62	414(M^+), 107(378), 105(392), 95(387), 91(377), 81(427), 69(348), 57(447), 55(639), 43(999), 41(561)
63	388(M^+), 302(346), 165(310), 152(642), 127(369), 91(399), 77(298), 69(806), 55(510), 43(999), 41(735)
64	426(M^+), 135(320), 121(350), 109(440), 107(400), 95(430), 93(370), 81(440), 69(999), 55(549), 41(579)
65	468(M^+), 121(278), 109(324), 107(311), 95(456), 93(260), 81(316), 69(752), 55(485), 43(999), 41(572)
66	312(M^+), 96(708), 95(546), 83(670), 82(968), 81(627), 69(770), 67(664), 55(999), 43(577), 41(621)

FIGURE 1: Mass spectrum of compound eucalyptol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW). FIGURE 2: Mass spectrum of compound 2(3H)-furanone, 5-ethenyldihydro-5-methyl- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW). FIGURE 3: Mass spectrum of compound terpineol, cis-β- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 4: Mass spectrum of compound 2-furanmethanol, 5-ethenyltetrahydro- $\alpha,\alpha,5$ trimethyl-, cis- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 5: Mass spectrum of compound 1,6-octadien-3-ol, 3,7-dimethyl- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 6: Mass spectrum of compound butanoic acid, 2-methyl-, 2-methylbutyl ester of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 7: Mass spectrum of compound *n*-amil isovalerate of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 8: Mass spectrum of compound 2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl-methanol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 9: Mass spectrum of compound isopinocarveol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 10: Mass spectrum of compound bicyclo[2.2.1]heptane-2,5-diol, 1,7,7-trimethyl-, (2-endo,5-exo)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 11: Mass spectrum of compound 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 12: Mass spectrum of compound 2(10)-pinen-3-one of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 13: Mass spectrum of compound bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 14: Mass spectrum of compound 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 15: Mass spectrum of compound 3,7-octadiene-2,6-diol, 2,6-dimethyl- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 16: Mass spectrum of compound 3-cyclohexene-1-methanol, α,α4-trimethyl- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 17: Mass spectrum of compound 1,7-octadiene-3,6-diol, 2,6-dimethyl of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 18: Mass spectrum of compound thymol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 19: Mass spectrum of compound phenol, 2-methoxy-3-(2-propenyl)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 20: Mass spectrum of compound 7-hexadecene, (Z)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 21: Mass spectrum of compound caryophyllene of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 22: Mass spectrum of compound 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 23: Mass spectrum of compound 1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1α,4α,7β,7aβ,7bα)]- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 24: Mass spectrum of compound caryophyllene oxide of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 25: Mass spectrum of compound tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 26: Mass spectrum of compound oxacyclotetradecan-2-one, 14-methyl of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 27: Mass spectrum of compound caryophyllene oxide of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 28: Mass spectrum of compound 9-(3,3-dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 29: Mass spectrum of compound tetradecanoic acid of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 30: Mass spectrum of compound 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 31: Mass spectrum of compound 3,7,11,15-tetramethyl-2-hexadecen-1-ol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 32: Mass spectrum of compound cis-9-hexadecenoic acid of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 33: Mass spectrum of compound 2,6,10-dodecatrien-1-ol, 12-acetoxy-2,6,10-trimethyl-(E,E,E)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 34: Mass spectrum of compound *n*-hexadecanoic acid of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW). FIGURE 35: Mass spectrum of compound cholestan-3-ol, 2-methylene-, (3 β ,5 α)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW). FIGURE 36: Mass spectrum of compound 9-(3,3-dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW). FIGURE 37: Mass spectrum of compound *trans*-Z- α -bisabolene epoxide of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW). FIGURE 38: Mass spectrum of compound 1-hexadecanol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 39: Mass spectrum of compound 9,12-octadecadienoic acid, methyl ester, (E,E)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 40: Mass spectrum of compound 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 41: Mass spectrum of compound phytol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 42: Mass spectrum of compound 9,12-octadecadienoic acid (Z,Z)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 43: Mass spectrum of compound 9,12,15-octadecatrienoic acid, (Z,Z,Z)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 44: Mass spectrum of compound α -santonin of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 45: Mass spectrum of compound limonen-6-ol, pivalate of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 46: Mass spectrum of compound hexadecanoic acid, 1,1-dimethylethyl ester of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 47: Mass spectrum of compound 1-eicosanol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 48: Mass spectrum of compound tetradecane, 2,6,10-trimethyl- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 49: Mass spectrum of compound butyl 9,12-octadecadienoate of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 50: Mass spectrum of compound butyl 9,12,15-octadecatrienoate of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 51: Mass spectrum of compound 1-docosene of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 52: Mass spectrum of compound heptacosane of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 53: Mass spectrum of compound hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 54: Mass spectrum of compound 1,2-benzenedicarboxylic acid, diisooctyl ester of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 55: Mass spectrum of compound 9,12-octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 56: Mass spectrum of compound 8,11,14-eicosatrienoic acid, (Z,Z,Z)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 57: Mass spectrum of compound 1-hexadecanol, 2-methyl- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 58: Mass spectrum of compound dl-α-tocopherol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 59: Mass spectrum of compound pregnan-20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-, (3 β ,5 α ,6 α ,16 α)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 60: Mass spectrum of compound campesterol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 61: Mass spectrum of compound stigmasterol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 62: Mass spectrum of compound β -sitosterol of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 63: Mass spectrum of compound quassin of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 64: Mass spectrum of compound 9,19-cyclolanost-24-en-3-ol, (3β)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 65: Mass spectrum of compound 9,19-cyclolanost-24-en-3-ol, acetate, (3β)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

FIGURE 66: Mass spectrum of compound ethanol, 2-(9-octadecenyloxy)-, (Z)- of whole plant hexane fraction of *Achillea wilhelmsii* (WHFAW).

4. Conclusion

In this research study, whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW) exhibited antileishmanial activity and consists of 66 compounds which was analyzed by GC-MS. Whole plant methanol extract of *Achillea wilhelmsii* (WMEAW) and whole plant aqueous fraction of *Achillea wilhelmsii* (WFAW) exhibited no antileishmanial activity. Therefore, the compounds present in the whole plant *n*-hexane fraction of *Achillea wilhelmsii* (WHFAW) which has shown antileishmanial activity will be the drugs for the treatment of leishmaniasis with least side effects and toxicity.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are thankful to Hussain Ebrahim Jamal (HEJ), Research Institute of Chemistry, University of Karachi, Karachi, Pakistan, for providing antileishmanial, GC-MS analysis, and Institute of Biochemistry, University of Balochistan, Quetta, Pakistan, for providing lab facilities for extraction and fractionation.

References

- [1] S. Dokhani, T. Cottrell, J. Khajeddin, and G. Mazza, "Analysis of aroma and phenolic components of selected *Achillea* species," *Plant Foods for Human Nutrition*, vol. 60, no. 2, pp. 55–62, 2005.
- [2] S. Saeidnia, A. R. Gohari, N. Mokhber-Dezfuli, and F. Kiuchi, "A review on phytochemistry and medicinal properties of the genus *Achillea*," *DARU*, vol. 19, no. 3, pp. 173–186, 2011.
- [3] S. Trifunovic, V. Vajs, Z. Juranic et al., "Cytotoxic constituents of *Achillea clavennae* from Montenegro," *Phytochemistry*, vol. 67, pp. 887–893, 2006.

- [4] R. Rezaeiipoor, M. Saeidnia, and M. Kamalinejad, "Immunosuppressive activity of *Achillea talagonica* on humoral immune responses in experimental animals," *Journal of Ethnopharmacology*, vol. 65, no. 3, pp. 273–276, 1999.
- [5] S. Konyalioglu and C. Karamenderes, "The protective effects of *Achillea* L. species native in Turkey against H₂O₂-induced oxidative damage in human erythrocytes and leucocytes," *Journal of Ethnopharmacology*, vol. 102, no. 2, pp. 221–227, 2005.
- [6] R. Kotan, A. Cakir, F. Dadasoglu et al., "Antibacterial activities of essential oils and extracts of Turkish *Achillea*, *Satureja* and *Thymus* species against plant pathogenic bacteria," *Journal of the Science of Food and Agriculture*, vol. 90, no. 1, pp. 145–160, 2009.
- [7] G. Stojanovic, N. Radulovic, T. Hashimoto, and R. Palic, "In vitro antimicrobial activity of extracts of four *Achillea* species: the composition of *Achillea clavennae* L. (Asteraceae) extract," *Journal of Ethnopharmacology*, vol. 101, no. 1–3, pp. 185–190, 2005.
- [8] R. Yazdanparast, A. Ardestani, and S. Jamshidi, "Experimental diabetes treated with *Achillea santolina*: effect on pancreatic oxidative parameters," *Journal of Ethnopharmacology*, vol. 112, no. 1, pp. 13–18, 2007.
- [9] C. Karamenderes and S. Apaydin, "Antispasmodic effect of *Achillea nobilis* L. subsp. *sipylea* (O. Schwarz) Bässler on the rat isolated duodenum," *Journal of Ethnopharmacology*, vol. 84, no. 2–3, pp. 175–179, 2003.
- [10] F. B. Potrich, A. Allemand, L. M. da Silva et al., "Antiulcerogenic activity of hydroalcoholic extract of *Achillea millefolium* L.: involvement of the antioxidant system," *Journal of Ethnopharmacology*, vol. 130, no. 1, pp. 85–92, 2010.
- [11] A. M. Cavalcanti, C. H. Baggio, C. S. Freitas et al., "Safety and antiulcer efficacy studies of *Achillea millefolium* L. after chronic treatment in Wistar rats," *Journal of Ethnopharmacology*, vol. 107, no. 2, pp. 277–284, 2006.
- [12] C. I. G. Tuberoso, P. Montoro, S. Piacente et al., "Flavonoid characterization and antioxidant activity of hydroalcoholic extracts from *Achillea ligustica* All," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 50, no. 3, pp. 440–448, 2009.
- [13] A. Kaliora and G. Dedoussis, "Natural antioxidant compounds in risk factors for CVD," *Pharmacological Research*, vol. 56, no. 2, pp. 99–109, 2007.
- [14] F. Tosun, Ç. A. Kızılay, B. Şener, M. Vural, and P. Palittapongarnpim, "Antimycobacterial screening of some Turkish plants," *Journal of Ethnopharmacology*, vol. 95, no. 2–3, pp. 273–275, 2004.
- [15] G. Honda, E. Yesilada, M. Tabata et al., "Traditional medicine in Turkey VI. Folk medicine in west Anatolia: Afyon, Kütahya, Denizli, Mugla, Aydin provinces," *Journal of Ethnopharmacology*, vol. 53, no. 2, pp. 75–87, 1996.
- [16] S. Niazmand, E. Khooshnood, and M. Derakhshan, "Effect of *Achillea wilhelmsii* on rats gastric acid output at basal, vagotomized and vagal-stimulated conditions," *Pharmacognosy Magazine*, vol. 6, no. 24, pp. 281–285, 2010.
- [17] K. Javidnia, R. Miri, and H. Sadeghpour, "Composition of the volatile oil of *Achillea wilhelmsii* C. koch from Iran," *DARU*, vol. 12, no. 2, pp. 63–66, 2004.
- [18] World Health Organization, *Leishmaniasis*, WHO, Geneva, Switzerland, 2015.
- [19] S. Kaur, H. Patel, V. Sharma, P. Garg, and Roy. N, "*Leishmania* major structural database," *International Journal of Integrative Biology*, vol. 7, no. 2, pp. 63–68, 2009.
- [20] N. C. Hepburn, "Cutaneous leishmaniasis. clinical dermatology. Review article," *Clinical and Experimental Dermatology*, vol. 25, no. 5, pp. 363–370, 2000.
- [21] B. Jehan, K. Shehla, and S. Mohammad, "Antimicrobial potentials of fresh *Allium cepa* against gram negative bacteria and fungi," *Pakistan Journal of Botany*, vol. 45, pp. 1–6, 2013.
- [22] J. K. Achakzai, M. A. Panezai, M. A. Kakar et al., "In vitro anticancer MCF-7, anti-inflammatory, and brine shrimp lethality assay (BSLA) and GC-MS analysis of whole plant butanol fraction of *Rheum ribes* (WBFRR)," *BioMed Research International*, vol. 2019, Article ID 3264846, 8 pages, 2019.
- [23] R. Atta-ur, M. I. Chaudhary, and W. Thompson, *Bioassay Techniques for Drug*, vol. 60, Harwood Academic publishers, Amsterdam, The Netherlands, 2001.
- [24] E. Ahmed El-Wakil, M. Mohamed El-Sayed, and E. E. Abdel-Lateef, "GC-MS investigation of essential oil and antioxidant activity of Egyptian white onion (*Allium cepa* L.)," *International Journal of Pharmaceutical Sciences and Research IJPSR*, vol. 6, no. 3, 2015.