Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 07 | 2022
DOI 10.17605/OSF.IO/XPMVG

IN VITRO ANTIBACTERIAL, ANTIFUNGAL, ANTILEISHMANIAL, ANTICANCER, ANI-INFLAMMATORY, BSLA AND GC-MS STUDY OF WHOLE PLANT PEUCEDANUM FERULAEFOLIUM AQUEOUS FRACTION (WPFAF)

NAZIMA YOUSAF KHAN[1], MUHAMMAD ANWAR PANEZAI[1], MUDASSIR ASSRAR ZAIDI[1], MARINA PANEZAI[1], JAHANGIR KHAN ACHAKZAI[2], ABDUL MANAN KAKAR[1] and AJAB KHAN TAREEN[1]

[1]University of Balochistan, Faculty of Life Sciences, Institute of Biochemistry, Quetta, Pakistan.

[2]Disipline of Biochemistry, Department of Natural and Basic Sciences, University of Turbat, Kech, Turbat 92600, Balochistan, Pakistan.

Abstract

Recently, various plant species and different extracts obtained from them got more attention analyzing their biological activities. The in-vitro study wasdone for the first time to determine anticancer, anti-inflammatory, cytotoxicity, antifungal, antibacterial, leishmanicidal in Peucedanum ferulafolium whole plant (stem, root, flower and leaves).Our investigations revealed that, the antifungal activity in whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF) anf butanol fraction (WPFBF) was significantly active against C. albicans with 89% of inhibition and C. glabarata with 89% of inhibition. For antibacterial activity, whole plant Peucedanum ferulaefolium methanol extract (WPFME) exhibited remarkable activity against S. aureus with 74% of inhibition and aqueous fraction (WPFAF) showed moderate activity with 60%of inhibition against S. aureus. After getting remarkable antifungal and antibacterial activity results from the aqueous fraction of Peucedanum ferulafolium we have done GC-MS of same fraction (WPFAF) and isolated 34 compounds, which were identified by GC-MS. Whole plant Peucedanum ferulaefoliumextract and fractions showed very low cytotoxic effect. Unfortunately whole plant Peucedanum ferulaefolium exhibited inactive against cancer cell line (HeLa and 3T3), antileishmanial and anti-inflammatory. In conclusion, Peucedanum ferulafolium aqueous fraction showed astounding amount of effective antifungal and antibacterial activity.

Keywords: Peucedanum ferulaefolium, Antibacterial, Antifungal, Antileishmanial, Anticancer, Anti-inflammatory, Brine Shrimp Letahlity.

INTRODUCTION

Medicinal plants are known to be an important part of their tradition and culture, 80% of population directly depends on traditional medicines as primary health cure (Ekhaise & Okoruwa, 2001). Peucedanum genus belongs to Umbelliferae family; it is the largest genus with 120 known species, distributed mainly in Asia and Europe (Nasir, 1972; Tutin et al., 1968). In Pakistan there are only three species found; Peucedanum baluchistanicm,

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Peucedanum ferulafolium and Peucedanum aucheri (Inam ul Haq, 1990). Peucedanum ferulafolium is the native to Pakistan and Afghanistan (Nasir, 1972). It grows wild in Balochistan at different places. It's known as "jungali sowa" and used instead of dill as traditional medicine by local people (Inam ul Haq, 1990). Some species of Peucedanum genus have been traditionally used in treatment of cough, cold, phlegm accumulation, anti-asthma, anti-tussive and for angina (Gan WS, 1965; Kong L Y et al., 1996; Tang W, 1992).Seven couramins isolated from the methanolic extract of plantP. zenkeri, which manifested significant antimicrobial activity (Nagunde et al., 2003).P. nebrodense acetone extract exhibited notable antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, ,E.coli, Candidaalbicans, Pseudomonas aeruginosa, , C. tropicalis and Streptococcusagalacti (Schillacri et al., 2003).P. chenur specie methanolic extract examined for anticancer activity on human colorectal cancer cell line and the results were very effective (Saeed et al., 2020). P. japonicum plant that belongs to Korean coast was examined for anti-inflammatory and anticancer activity against A549, MCF07 and HL-60, hyuganin C compound exhibited significant activity against HL-60 with13.2µg/l IC_{50}value and all fractions were inactive against LPS-induced model in mice for inflammation (Jang et al., 2008).P. beluchistanicum methanol extract have shown maximum activity as antileishminal with 32.65µg/ml value as IC_{50} (Baloch et al., 2013).Previous study had done only on the essential oil of Peucedanum ferulafolium, showed significant activity as antimicrobial activity (Inam ul Haq, 1990).Therefore Peucedanum ferulafolium whole plant (root, leaves, stem and flower) extracts and further fractions were subjected for the investigation of anticancer, antifungal, antibacterial, antileishminal, cytotoxicity and anti-inflammatory and GC-MS analysis. The investigation of Peucedanum ferulaefolium has been done for the very first time for all the above stated activities, which may vindicate the use of this genus in traditional folk medicine and mark the importance of bioactive ethanobotanical approach, for the discovery of new therapeutic substances.

MATERIAL & METHODS

Plant Material

Whole plant of Peucedanum ferulaefolium (Stem, Leaves, flower and roots) was collected from Ziarat, Balochistan, Pakistan. Plant was identified by taxonomist from Botany department, University of Balochistan, Quetta, Pakistan.

Extraction step for methanol extract

Peucedanum ferulaefolium plant was dried in shade for two months, later on, converted into powder in grinder. Macerated plant's powder in 7.5L methanol for one week. Filtered methanolic supernatant by what man filter paper No.1, concentrated the mixture below 50^0C temperature under lower pressure in rotary evaporator. Semisolid Peucedanum

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ferulaefolium methanolic crude extract (WCTME) was obtained as 133.03g (Jehan et al., 2013).

Fractionation step from crude methanol extract

Semisolid Peucedanum ferulaefolium methanolic crude extract were divided into two portions, one for analysis and other for fractionation. Dissolved 120.66g methanol extract (WPFME) into 1500ml distilled water and 1500ml n-hexane solvents. After separation and evaporation step, we obtained 90.16g of whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF) and 20.66g of whole plant Peucedanum ferulaefolium n-hexane fraction (WPFHF). For butanol fraction, dissolved 69.78g whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF) into 1000ml of distilled water and 1000ml of butanol solvents to obtained whole plant Peucedanum ferulaefolium butanol fraction (WPFBF) (Jehan et al., 2013).

Antibacterial activity

Standard five bacterial strains which are Salmonella typhi (ATCC 14028), Pseudomonas aeruginosa (ATT 10145), Staphylococcus aureus (NCTC 6571), Bacillus subtilis (ATCC 23857) and Escherichia coli (ATCC 25922), obtained from PCMD microbial bank, International Center for Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

Microplate Alamar Blue Assay

Organisms were grown in muller hinton medium. McFarland turbidity index was used. Dissolved plant extract and fractions in DMSO for stock solution preparation. Prepared media was first transferred in all wells, except control well. Wells volume made up to 200µl. In 96 wells plate included control and test added cells of 5×10^6. Parafilm used to seal the plates and incubated them for 18-20 h in incubator. Added Alamar blue dye in all wells and shaken the plate for 2-3 h, at 80 RPM. Bacterial growth indicated by the change in color from blue to pink of Alamar blue dye. ELISA reader used at 570nm absorbance (Pettit et al., 2005 & Sarker et al., 2007).

Antifungal assay

Standard six selected fungal strain, Fusarium lini, Microsporum canis, Aspergillus niger, Trichphyton rubrum, Candida glabarata and Candida albicans used for antifungal activity. These strains were purchased from Northern Regional Research Laboratories (NRRL) Karachi.

Agar tube dilution method

1ml DMSO (Merck) used to dissolve the 24mg plant extracts. Prepared SDA solution by mixing 4 gm agar-agar, 32.5gm sabouraud and 4% glucose agar, and dissolved them in distilled water (500ml), mixed the mixture thoroughly. Steamed the growth media for

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complete dissolution, in screw cap tubes poured 4ml of it and kept it in autoclave for 15 min at 121^0C. Allowed to cool the tubes at 15^0C, loaded non-solidified SDA with 66.6 µl plant crude extract. Allowed the tubes at 25^0C for solidifying in slant position. 4mm fungus piece was inoculated in all tubes. In other media, DMSO for negative control and reference drug (antifungal) for positive control were used. For incubation step kept the tubes in incubator at 27^0C temperature for 7 days. Examined the fungal cultures twice a week during incubation (Choudhaery et al., 1995 & Atta ur Rehman et al., 2001).

Antileishmanial Activity

L. major (promastigotes) cultured in microplates. Normal saline was used in bulk for the growth ofL. Major promastigotes in NNN. Promastigotes were cultured in RPMI 1640 were supplemented by 10% FBS which was heat-inactivated. Harvested the promastigotes at log phase, centrifuge at 2000 rpm in 10 min. Washed 3 times L. major with saline. At last in Neubauce chamber under microscope, counted the permastigotes and then diluted them by adding fresh culture medium for development of leishmanial parasites up to 10^6 cell/ml density.

In microtitre plate of 96 wells, added 180µl culture medium in different wells. 7.4 pH of PBS which has 0.5% MeOH, 0.5% DMSO in it, used for plant extract and fractions in wells. Developed working solution of further serially diluted within range of 1-100µg/ml. Introduced 100µl parasite culture in all wells. Two rows for -ve and +ve control. In negative control received medium and in positive control received antileishmanial standard drug which was pentamidine (ICN) and amphotericin B (Fluka). The microtitre plate of 96 wells was kept in incubator at 21-22 ^{0}C for 72 hrs. Used microscope, to observe the culture for viability of cell, counted the motile cells by counting Neubaur chamber. Ezfit 5.03 (Percella Scientific, USA) software used for IC_{50} value (Atta ur Rehman et al., 2001).

Anti-inflammatory assay

Oxidative brust assay

25µl whole blood in diluted form, $HBSS^{++}$ consist of $CaCl_2$ and $MgCl_2$ (Sigma, St. Louis, USA), added 25µl plant extract and fractions, kept them in luminometer (Labsystem, Helsink, Finland), further in 96 wells plate (Coster, NY, USA) mixture was plated. Blank wells had $HBSS^{++}$, whereas control wells had cells plus $HBSS^{++}$. Then added Serum Opsonied Zymosan in each well about 25µl (Sigma Chemical Co., St, Louis, Mo, USA) and Luminol about 25µl (Sigma Chemical Co., St, Louis, Mo, USA). In presence of RLU, ROS level was recorded in luminometer. Standard drug (Ibuprofen) with IC_{50} 11.2 ± 1.9 used (Helfand et al., 1982; Choudhary et al., 2006).

MTT Assay (HeLa and 3T3 cell lines)

The cancer cell lines (HeLa and 3T3) were obtained from American Type Culture Collection (ATCC). Cancer cell line culture formed by, Dulbecco's Eagle medium modified

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by 10% FBS, 2% antibioticsis (Streptomycin) about 100µg/ml and Penicilline about 100IU/ml and kept them in 5% CO_2. Incubated the mixture at 37^0C. Developed the confluency, harvested the cell lines. In all 96 wells plated 5×10^4 cells. After 24 hours, added plant extract and fractions about 50µg/ml. Again incubated them in 96 wells flat for 48 hrs,added 100µl of 0.5mg/ml MTT in each well, incubated for 4 hrs at 37^0C. After MTT reduction formanzan crystals were formed. Dissolved them in 100µl DMSO. Used micro-plate (Spectr Maxplus, Molecular Devices, CA, and USA) at 570nm. Doxorubicin was standard drug for HeLa and Cyclohexamide for 3T3 cell line. The formula was used for the calculation of % inhibition, as follow:

% Inhibition = 100 – (mean of O.D of test compound – mean of O.D negative control) / (mean of O.D positive control – mean of O.D of negative control) × 100)

For IC_{50} calculation, prepared plant extract and fractions stock solutions (20mM), diluted them in 50µM working solution to achieve less than 50 % inhibition. IC_{50} calculation done by software EZ-fit5 (Scudiere, 1988; Mosmann et al., 1983; Choudhary et al., 2010).

Brine Shrimp Lethality Assay

B-hatching Technique

The hatching tray had filtered brine solution, scattered 50 mg eggs of brine shrimp. Incubated at 37^0C for 2 days. Dissolved the plant extract and fractions about 20 mg in 2 ml methanol. Transferred the solution of 5, 50, 500 µl in 3 vials and made up to 10, 100, and 1000 µg/ml. Transferred 30 larvae in each vials and added 5 ml seawater in to them. After 24 hrs incubation at 27^0C under illumination, added cytotoxic drug for +ve and -ve control. 7.4625 µg/ml of Etoposide used as standard drug, Finney computer program used for LD_{50} value (Alves, 2000; Kivack, 2001; Carballo, 2002).

GC-MS

Triple quadrupole acquisition method was used for compounds identification. Gas-chromatography (Aglient technology, Mod. 6890N, CA) coupled to mass-spectrometer (Mod.5973N). Column of HP-5MS (length 30m, width 0.25µm, diameter 0.25m). Injector (split-splitless), at 250^0C ratio and splited (30:1). Oven temperature was like: 3 min (70^0C), 1 min (6^0C), 5min (180^0C); then 6^0C/min - 10 min on 280^0C, 8^0C/min - 20 min on 290^0C. Detector (Aglient technology, CA) transfer line was arranged at 250^0C, the quardpole temp was arranged on 150^0C, on 230^0C arranged ionization. Scan achievement was placed in 35-300m/z at 70eV mass spectra. Libraries for instance NISTO2, and WILEY were used (E. Ahmed et al., 2015).

RESULTS

Peucedanum ferulaefolium of Balochistan crude extract and fractions were analyzed for its medicinal purpose. Infection spread by protozoa L. major is responsible for the notable

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burden disease in developing countries. The effect of leishmanias is rise due to lack of vaccines. Peucedanum beluchitanicum methanol extract have shown antileisminal activity with IC_{50} (32.65 µg/ml) (Baloch et al., 2013). To examine antiprotozoal activity inPeucedanum ferulaefolium whole plant methanol extract and fractions as; n-hexane, aqueous and butanol, Pentamidine and Amphoterian B were taken as standard drug with IC_{50} value as; 3.15 ± 0.005 and 0.39 ± 0.05.Whole plant Peucedanum ferulaefolium methanolic crude extract (WPFME) and all fractions included whole plant Peucedanum ferulaefolium n-hexane (WPFHF), whole plant Peucedanum ferulaefolium aqueous (WPFAF) and whole plant Peucedanum ferulaefolium butanol (WPFBF) expressed inactive against the inhibition of L. major growth, results of antileishmanial activity are shown in table no 1.

Table 1: Antileishmanial activity in whole plant Peucedanum ferulaefolium

Extract/ Fractions	IC_{50}(µg/ml) ± S. D.
WPFME	> 100
WPFHF	> 100
WPFAF	> 100
WPFBF	> 100

Table 2: Antibacterial activity in whole plant Peucedanum ferulaefolium

% Inhibition					
Extract/ Fraction	**E. coli**	**B. subtilis**	**S. aureus**	**P. aerginosa**	**S. typhi**
WPFME	-	14%	74%	-	7%
WPFHF	-	25%	30%	-	-
WPFAF	-	-	60%	-	-
WPFBF	-	-	9%	-	8%

- No inhibition

The initial inspection of plant for antibacterial activity, usually obtained new bioactive compounds. In growing world the rate of infections are increasing due to antibiotic resistant by microorganisms. Therefore new and very effective therapeutic agents are required. Peucedanum ruthenium non-polar extract have shown significant antibacterial activity because of the presence of coumarins compound (Noor et al., 2009). Previous investigation has been done on essential oil of Peucedanum ferulaefolium in which methyl eugenol extracted as major component which showed antimicrobial activity (Syed et al., 1988). Antibacterial activity was done by microplate alamar blue assay, olfoxacin was taken as standard drug for each bacterial strain and its % of inhibition for each bacterial strain was; E. coli – 90%, B. subtilis- 96%, S. aureus- 93%, P. aeruginosa- 95% and with S. typhi- 95%. Whole plant Peucedanum ferulaefolium methanol extract (WPFME) antibacterial activity exhibited significant against Staphylococcus aureus with 74%

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inhibition, while 14% against B. subtilis and 7% inhibition against S. typhi, while non-significant activity against other bacteria. Whole plant Peucedanum ferulaefolium n-hexane fraction (WPFHF) showed 25% inhibition against B. subtilis and 30% inhibition against S. aures which was very low inhibition,non-significant activity against remain bacterial strains. In whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF) showed moderate activity against S. aureus with 60% inhibition, non-significant activity against remain bacterial strains. Whole plant Peucedanum ferulaefolium butanol fraction (WPFBF) showed 9% inhibition with S. aures and 8% inhibition with S. typhi, rest of bacterial strain were in activity, all results are listed in table no 2.

Table 3: Antifungal activity in whole plant Peucedanum ferulaefolium

Extract/ Fractions	% inhibition					
	T. rubrum	C. albican	A. niger	M. canis	F. lini	C. glabarata
WPFME	-	-	-	-	-	-
WPFHF	-	-	-	-	-	-
WPFAF	-	89%	-	-	-	89%
WPFBF	-	89%	-	-	-	89%

- No inhibition

Medicinal plants secondary metabolites, that usually posses' antifungal activities. Medicinal plants as herbal formulated drugs are commonly used worldwide because they are safe, accessible and affordable as compare to synthetic drugs. In this research Miconazole used as standard drug for fungal strain with MIC (µg/ml) value as; T. rubrum-70, C. albican- 110, M. canis- 98.4, F. lini- 73.25, C. glabarata- 110.8. Whereas Amphotericin B as standard drug used for A. niger with MIC (µg/ml) value 20.The antifungal activity in whole plant Peucedanum ferulaefolium methanol extract (WPFME) has shown non-significant activity against all organisms. Whole plant Peucedanum ferulaefolium n-hexane fraction (WPFHF) has shown non-significant activity against all organisms. Whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF) shown noticable activity against Candida albicans with 89% inhibition and Candida glabarata with 89% inhibition with rest of strain it have not shown inhibition. Whole plant Peucedanum ferulaefolium butanol fraction (WPFBF) was remarkable active against Candida albicans with 89% inhibition and Candida glabarata with 89% inhibition and marked inactive with other strains. Antifungal activity results of whole plant Peucedanum ferulaefolium extract and fractions are shown in table no 3.

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Table 4: Anti-inflammatory activity in Whole plant Peucedanum ferulaefolium

Extract/Fractions and Std. Drug	Conc. (µg/ml)	IC_{50} ± S.D
WPFME	250,50,10	Inactive
WPFHF	250,50,10	Inactive
WPFAF	250,50,10	Inactive
WPFBF	250,50,10	Inactive
Ibuprofen		11.2 ± 1.9µg/ml

Anti-inflammatory activity was done by chemiluminescence technique, in oxidative brust assay. Ibuprofen was taken as standard drug with IC_{50} value (11.2 ± 1.9).Peucedanum ferulaefolium extract and fraction were analyzed against reactive oxygen species (ROS) which are key signaling molecules in progression of inflammatory disorders. Whole plant Peucedanum ferulaefolium methanol extract (WPFME), Whole plant Peucedanum ferulaefolium n- hexane fraction (WPFHF), Whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF) and Whole plant Peucedanum ferulaefolium butanol fraction (WPFBF) shown no inhibition against reactive oxygen species (ROS). Anti-inflammatory activities results of whole plant Peucedanum ferulaefolium extract and fractions are listed in table no 4.

Table 5: Anti-cancer (HeLa) activity in whole plant Peucedanum ferulaefolium

Extract/Fractions/ Std. Drug	Conc.(µM)	IC_{50} ± S.D
WPFME	30	Inactive
WPFHF	30	Inactive
WPFAF	30	Inactive
WPFBF	30	Inactive
Doxorubicin	30	0.9 ± 0.1

Table 6: Anti-cancer (3T3) activity in whole plant Peucedanum ferulaefolium

Extract/Fractions/Std. Drug	Conc. (µM)	IC_{50} ± S.D
WPFME	30	Inactive
WPFHF	30	Inactive
WPFAF	30	Inactive
WPFBF	30	Inactive
Cyclohexamide	30	0.8 ± 0.14

Compounds like polyphrnols, taxols and brassinosteroid extracted from medicinal plants considered as cancer cell line inhibitors (Azmi et al., 2006). Anticancer activity was done

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by MTT assay on 3T3 and HeLa cell lines. In which Doxorubicin (IC_{50} value 0.9 ± 0.01) was used as standard drug for HeLa and Cyclohexamide (IC_{50} value 0.8 ± 0.14) for 3T3. The results whole plant Peucedanum ferulaefoliummethanol extract (WPFME), whole plant Peucedanum ferulaefolium n-hexane fraction (WPFHF), whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF) and whole plant Peucedanum ferulaefolium butanol fraction (WPFBF) showed inactive against HeLa and 3T3 cell line. Anticancer activity results of whole plant Peucedanum ferulaefolium extract and fractions are shown in table no 5 for HeLa and table no 6 for 3T3.

Table 7: Brine Shrimp Lethality activity in whole plant Peucedanum ferulaefolium

Extract/Fractions	Dose µg/ml	% Mortality
WPFME	10	3.33
	100	16.66
	1000	20
WPFHF	10	3.33
	100	6.66
	1000	13.33
WPFAF	10	3.33
	100	13.33
	1000	16.66
WPFBF	10	3.33
	100	6.66
	1000	10

The larvae of brine shrimp used in bioassay to examine cytotoxic agent in plants. In this research bioassay Etoposide used as standard drug against mortality of brine shrimp larvae, with 46.66% mortality. The results of whole plant Peucedanum ferulaefolium methanol extract (WPFME) shown 20% mortality at 1000µg/ml dose which was moderate mortality percentile as compare to standard drug, whole plant Peucedanum ferulaefolium n-hexane fraction (WPFHF) shown 13.33 % mortality at 1000µg/ml dose, whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF) shown 16.66% mortality at 1000µg.ml dose and whole plant Peucedanum ferulaefolium butanol fraction (WPFBF) shown 10% mortality at 1000µg/ml which was very low mortality percentile as compare to standard drug mortality percentile.. The results are shown in table 7.

Plants extracts structural elucidation further validates the qualitative phytochemical data. GC-MS analysis of Peucedanum ferulaefoliumhad done and 11 more compounds were identified other than methyl eugenol (Inam ul Haq, 1990). The GC-MS analysis of whole plant Peucedanum ferulaefolium aqueous fraction was done because aqueous fraction

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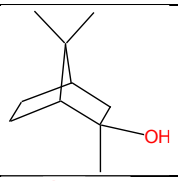
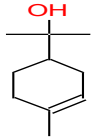
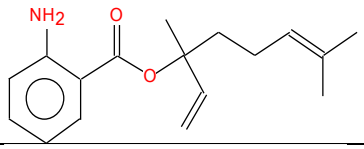
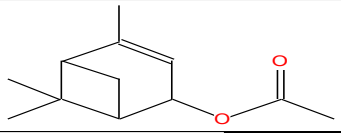
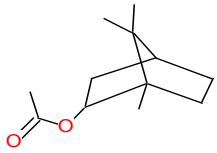
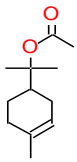
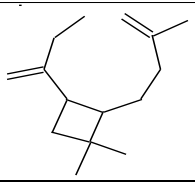
gives very significant results as antifungal, antibacterial and as cytotoxic agent. 34 compounds identified with their molecular formula, retension time, their structure and mass spectra. Results are shown in table no 8 and 9.

Table 8: Molecular mass, Molecular formula, RT and Structure of compounds 1-34 from whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF)

Compounds	Molecular Mass	Molecular Formula	RT	Structure
1	154	$C_{10}H_{18}O$	15.67	
2	154	$C_{10}H_{18}O$	16.15	
3	273	$C_{17}H_{23}NO_2$	17.36	
4	194	$C_{12}H_{18}O_2$	17.61	
5	196	$C_{12}H_{20}O_2$	18.17	
6	196	$C_{12}H_{20}O_2$	19.4	
7	204	$C_{15}H_{24}$	21	

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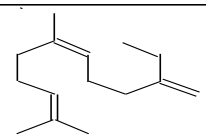
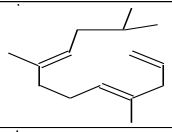
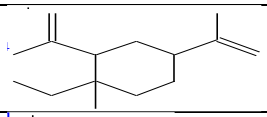
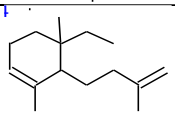
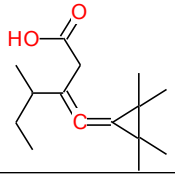
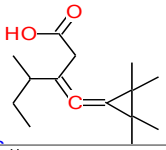
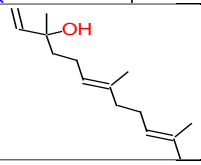
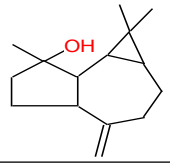
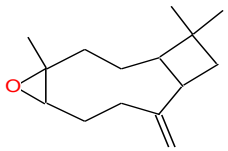
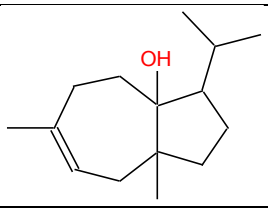
8	204	$C_{15}H_{24}$	21.33	
9	204	$C_{15}H_{24}$	21.64	
10	204	$C_{15}H_{24}$	22.25	
11	204	$C_{15}H_{24}$	22.39	
12	236	$C_{15}H_{24}O_2$	22.75	
13	236	$C_{15}H_{24}O_2$	23..02	
14	222	$C_{15}H_{26}O$	23.28	
15	220	$C_{15}H_{24}O$	23.86	
16	220	$C_{15}H_{24}O$	24	
17	222	$C_{15}H_{26}O$	24.21	

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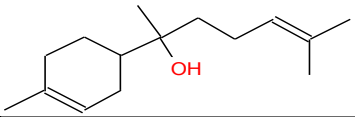
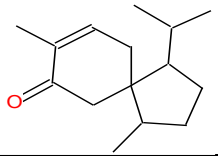
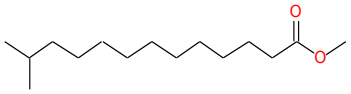
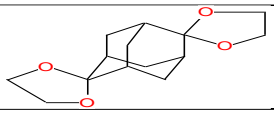
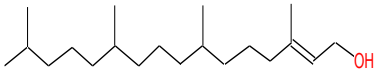
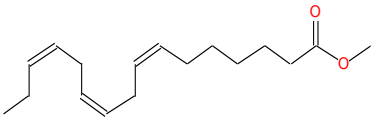
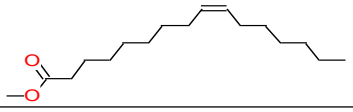
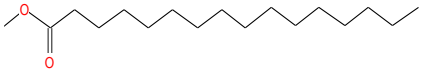
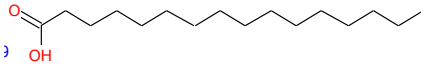
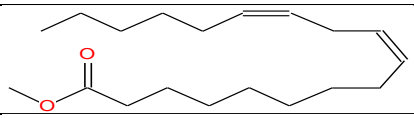
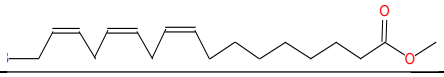
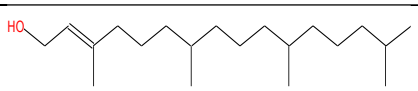
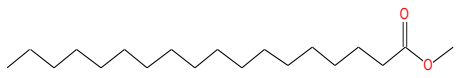
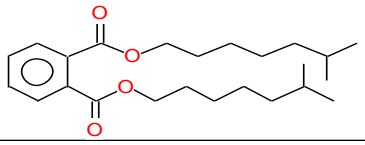
18	222	$C_{15}H_{26}O$	25.55	
19	220	$C_{15}H_{24}O$	25.87	
20	242	$C_{15}H_{30}O_2$	26	
21	252	$C_{14}H_{20}O_4$	27.42	
22	296	$C_{20}H_{40}O$	28.76	
23	264	$C_{17}H_{28}O_2$	30.67	
24	268	$C_{17}H_{32}O_2$	31.33	
25	270	$C_{17}H_{34}O_2$	31.51	
26	256	$C_{16}H_{32}O_2$	32.92	
27	294	$C_{19}H_{34}O_2$	39.91	
28	292	$C_{19}H_{32}O_2$	40.41	
29	296	$C_{20}H_{40}O$	41.12	
30	298	$C_{19}H_{38}O_2$	41.99	
31	390	$C_{24}H_{38}O_4$	60.24	

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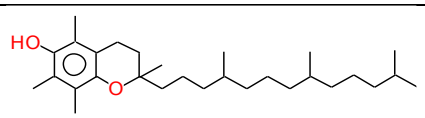
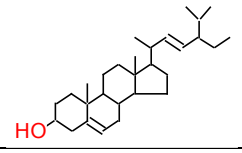
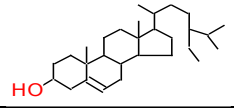
32	430	$C_{29}H_{50}O_2$	69.27	
33	412	$C_{29}H_{48}O$	72.54	
34	414	$C_{29}H_{50}O$	74.1	

Table 9: Mass spectra of compounds 1-34 whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF)

Compounds	m/z (% relative abundance)
1	154(M^+) 95(999), 41(179), 110(163), 93(118), 55(109),67(98), 139(95), 121(89), 96(83), 69(76)
2	154(M^+) 59(999), 93(584), 121(576), 136(524), 81(348),43(240), 92(179), 95(163), 67(143), 79(127)
3	273(M^+) 93(999), 43(938), 41(503),80(410), 69(340), 55(256), 71(236), 121(227), 67(186), 79(179)
4	194(M^+) 119(999), 43(634), 109(331), 134(283), 91(281), 93(248), 41(222), 59(149), 81(131), 77(126)
5	196(M^+) 95(999), 93(450), 121(423), 43(384), 136(351), 41(197), 108(146), 80(131), 92(131), 110(118)
6	196(M^+) 121(999), 93(697), 136(636), 43(378), 79(177), 107(168), 91(149), 92(137), 67(134), 81(127)
7	204(M^+) 93(999), 133(921), 91(858), 41(769),79(763), 69(754), 105(623), 107(483), 120(447), 77(439)
8	204(M^+) 41(999), 69(735), 93(368), 39(254), 67(217),79(187), 81(186), 91(171), 53(169),133(162)
9	204(M^+) 93(999), 80(351), 121(265), 41(226), 92(168),107(141), 147(141), 79(135), 94(135), 91(119)
10	204(M^+) 93(999), 105(938), 41(915), 107(894), 81(864), 79(749), 121(628), 91(608), 67(607), 55(570)
11	204(M+) 189(999), 204(761), 93(746), 81(641), 41(635), 107(635), 105(495), 133(480), 55(459), 91(451)
12	236(M+) 41(999), 161(713), 57(675), 73(537), 119(504), 221(472), 105(470), 133(458), 91(452),77(343)
13	236(M^+) 41(999),161(713), 57(675), 73(537),119(504),221(472), 105(470), 133(458), 91(452), 77(343)
14	222(M^+) 69(999), 41(586), 93(560),43(410),71(370),107(275), 55(272), 67(249), 81(241), 79(192)

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15	220(M^+) 43(999), 41(627), 205(614), 119(569), 91(503),93(482), 159(427), 105(400), 162(391),107(359)
16	220(M^+) 43(999), 41(927), 79(885), 93(661), 91(573), 95(420), 69(407), 55(393), 67(377), 81(373)
17	222(M^+) 161(999), 204(373), 69(323), 43(268), 119(268), 123(263), 81(254), 97(249), 105(244), 41(234)
18	222(M^+) 109(999), 119(920), 69(796), 43(572), 93(442),41(407), 95(372), 121(313), 67(303), 71(267)
19	220(M^+) 109(999), 82(957), 41(642), 69(591), 93(590),135(590), 55(545), 79(474), 81(468), 108(433)
20	242(M^+) 74(999), 87(639), 55(249), 199(249), 69(159),143(159), 57(139), 75(139), 59(100), 83(100)
21	252(M^+) 252(999), 99(688), 55(421), 73(328), 191(268), 207(238), 190(198), 41(181), 253(152), 113(150)
22	296(M^+) 81(999), 82(986), 43(965), 95(962), 123(892), 55(852), 41(811), 57(811), 71(748), 68(728)
23	264(M^+) 79(999), 93(559), 67(479), 95(429), 108(379),91(369), 121(359), 80(339), 81(269), 94(269)
24	268(M^+) 55(999), 69(668), 41(631), 74(566), 83(467), 43(437), 67(414), 96(412), 97(387), 81(380)
25	270(M^+) 74(999), 87(720),43(325), 55(310),41(228), 143(208), 75(188), 57(183), 69(132), 227(110)
26	256(M^+) 60(999), 73(980), 57(840), 43(817), 55(767), 41(574), 129(435), 71(373), 69(351), 83(267)
27	294(M^+) 67(999), 81(956), 95(720), 55(650), 82(534),79(474), 96(472), 41(456), 68(423),109(372)
28	292(M^+) 79(999), 67(603), 95(583), 93(567), 108(457), 80(430), 81(422), 55(414), 41(354), 91(332)
29	296(M+) 71(999), 43(381), 57(334), 41(260),55(259), 69(239), 81(223), 68(199), 123(184), 56(169)
30	298(M) 74(999), 87(744), 43(338), 55(303), 75(240),143(235), 41(228), 57(202), 69(164), 298(140)
31	390(M^+) 149(999), 167(350), 57(341), 70(264), 41(225), 71(224), 55(218), 43(200), 150(107), 83(100)
32	430(M^+)165(999), 430(494), 164(332), 43(178), 431(155), 166(122), 57(104), 205(91),55(75),121(71)
33	412(M^+) 55(999), 83(692), 81(638), 69(636), 133(444), 43(431), 91(427), 105(427), 159(418), 95(398)
34	414(M^+) 43(999), 55(667), 57(563), 41(507), 81(481), 95(449), 107(440), 69(362), 414(354), 145(321)

CONCLUSION

The thoroughly investigation of whole plant Peucedanum ferulaefolium extract and fractions was done to investigate in vitro anticancer, antifungal, antibacterial, antileishmanial, anti-inflammatory and its cytotoxicity. The whole plant Peucedanum ferulaefolium aqueous fraction (WPFAF) and whole plant Peucedanum ferulaefolium

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butanol fraction (WPFBF) showed significant activity with highly percentile of inhibition against Candida albicans and Candida glabarata. Whole plant Peucedanum ferulaefolium methanol extract (WPFME) exhibited highly percentile of inhibition against Staphylococcus aureus. Further investigation will lead to find active compounds which have potential as antimicrobial agent with least side effects.

Authors' Contribution

NYK, MAP and MAZ conceptualized the manuscript. NYK wrote the manuscript. MAP and MAZ supervised the manuscript. MP, JKA, AMK and AKT edited final version.

Declaration of competing interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Authors are very grateful to HEJ (Hussain Ebrahim Jamal), Research Institute of Karachi, University of Karachi, Pakistan for facilitating us for GCMS analysis and biological activities and Institute of Biochemistry, University of Balochistan, Quetta, Pakistan for providing lab facilities for extraction and fractionation.

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ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 07 | 2022
DOI 10.17605/OSF.IO/XPMVG

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