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Isolation, identification, and antimycotic activity of plumbagin from *Plumbago europaea* L. roots, leaves and stems

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Abstract: *Plumbago europaea* L. is a plant utilized in Palestinian ethnomedicine for the treatment of various dermatological diseases. The current investigation was designed to isolate plumbagin from *P. europaea* leaves, roots and for the first time from the stems. Moreover, it aimed to evaluate the antimycotic activity against three human fungal pathogens causing dermatophytosis, also against an animal fungal pathogen. The qualitative analysis of plumbagin from the leaves, stems, and roots was conducted using HPLC and spectrophotometer techniques, while the structure of plumbagin was established utilizing Proton and Carbon-13 Nuclear Magnetic Resonance (NMR) and Infrared (IR) techniques. The entire plant constituents were determined by GC-MS. Moreover, the antimycotic activity against *Ascosphaera apis, Microsporum canis*, *Trichophyton rubrum,* and *Trichophyton mentagrophyte*s was assessed utilizing the poison food technique method. The percentage of plumbagin recorded in the leaves, stems, and roots was found to be 0.51±0.001%, 0.16±0.001%, and 1.65±0.015%, respectively. The GC-MS examination declared the presence of 59 molecules in the plant extract. The plant extract and pure plumbagin exhibited complete inhibition against all tested dermatophytes at 6.0mg/mL for the extracts and 0.2mg/mL for plumbagin. *P. europaea* root is the best source of plumbagin and the plant extract could represent a potential drug candidate for the treatment of dermatophytosis infections. Further studies required to design suitable dosage forms from the natural *P. europaea* root extracts or plumbagin alone, to be utilized for the treatment of dermatological and veterinary ailments.

Keywords: Antifungal, GC-MS, HPLC, NMR, plumbagin, *Plumbago europaea*

INTRODUCTION

Traditional herbal medicine in Palestine has a longstanding history of use and continues to provide essential values for the treatment of countless diseases (Jaradat *et al.*, 2016). However, scientific investigations to explore the therapeutic and chemical properties of medicinal herbs used in Palestinian folk medicine are petty (Alkowni *et al.*, 2018). It has been reported that numerous Palestinian traditional medicinal plants enjoy notable bioactivities, that might contribute towards improving community health and lifestyle (Abu-Qaoud *et al.*, 2018).

Plumbago genus (Plumbaginaceae) represents only 12 species that are spread in warm temperate and tropical regions. Several *Plumbago* species, such as *Plumbago europaea*, displayed decent biological activity such as antiviral, diuretic, antitumor, leishmanicidal, trypanocidal, antimalarial, and insecticide properties (Jayanthi *et al.*, 2020, Rahhal *et al.*, 2018, Singh *et al.*, 2018, Sobhani *et al.*, 2018). *Plumbago europaea* L. is a shrubby climber that is native to the Mediterranean and Central Asian regions. Two flavonol glucosides (europetin 2 and myricetin 3 glucosides) and plumbagin 1 (5-hydroxy-2-methyl-1,4-naphthoquinone) were reported to be the major bioactive components in *P. europaea* L (Chung *et al.*, 2004, Jaradat *et al.*, 2016). *P. europaea* plant is utilized in the Palestinian ethnomedicine for the medication of various ailments such as skin hardness, calluses, injury, blisters, warts, toothache, scabies, hepatitis, inflammations, leprosy, edema, and respiratory disorders, (Sezik *et al.*, 2001, Shawahna and Jaradat, 2017). *P. europea* leaves have been exploited for curing calluses, injuries, blisters, skin hardness and warts in Spanish folk medicine (Passalacqua *et al.*, 2007), while its roots were utilized for the healing of herpes and dermatitis in Italian folk medicine (Benítez *et al.*, 2010). In fact, *P. europea* roots and leaves are up to the present time are used in the Indian, Iranian, Iraqi and Chinese folk medicines as a therapeutic agent for warts, cancer, dysmenorrhea and rheumatoid arthritis (Chaplot *et al.*, 2006, Muhammad *et al.*, 2017). Moreover, the entire *P. europea* plant is used in veterinary medicine as an anti-vermin and as a cicatrizant agent (Palmese *et al.*, 2001).

The current investigation aims to determine the phytochemical constituents of *P. europea* ethanolic extract by GC-MS and to quantitatively and qualitatively analyze the main active constituents in the plant leaves,

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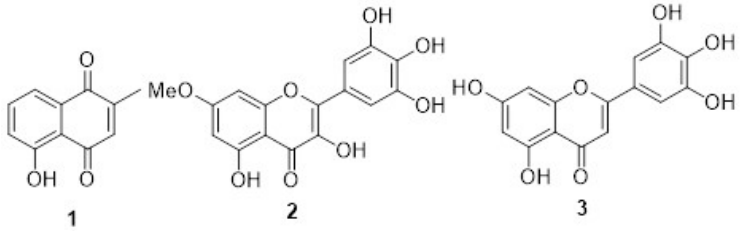

stems, and roots extracts using a spectrophotometer, HPLC, and NMR techniques. Moreover, it aimed to evaluate the antimycotic activities against three human fungal pathogens causing dermatophytosis and against a fungal pathogen that causes chalkbrood infection of bees.

MATERIALS AND METHODS

Chemicals and instruments

Analytical grade plumbagin was purchased from Sigma Aldrich, Germany. Melting point (m.p.) was determined on an apparatus Büchi 530, Switzerland. Thin-layer chromatography (TLC) was performed using precoated F254 silica gel plates purchased from Merck, USA. HPLC determinations were carried out using a Waters 1525 Binary HPLC Pumb, dynamic mixer model 811, manual sample injector Rheodyne 7125, HM/Holochrome UV-visible detector with 190-600 nm. HPLC pre-packed (250 x 4.0 mm ID, 5 mum C18) steel column protected with an inlet filter and guard column was used. The analysis was performed on a Breeze 2 HPLC System. HPLC-grade water and methanol were used as eluents. The UV-Vis measurements were recorded on Jenway 6800 (UK) UV-Vis spectrophotometer. Gentamicin, sabouraud dextrose agar (Difco) and econazole, were purchased from Merck (Darmstat, Germany). NMR spectroscopy (Agilent Technologies 400-MR (California, USA) (^{1}H, 400 MHz; ^{13}C 100.62 MHz). Ethanol, sodium carbonate, methanol, chloroform, ethyl acetate and all other chemicals and reagents were of analytical grade.

Plant materials

The *P. europea* plant materials were collected from the Nablus region during May-April 2017, authenticated by Prof. Dr. Mohammed Saleem Ali-Shtayeh at Biodiversity and Environmental Research Center (BERC)-Til/ Palestine. A voucher specimen (BERC-C-0065) was deposited in the BERC Herbarium.

Extraction of the active constituents

The plant material was collected, dried in the shadow for seven days, divided into different parts (leaves, stems, and roots), and grounded separately using a Mullenix (PHILIPS HR 2167, France). Three grams of the obtained materials from each part were soaked separately in 20mL of ethanol for 72 hrs. The extracts were filtered using Whatman filter paper grade 1 (11µm) and the solvents were removed under reduced pressure affording a dark green residue (0.054g) from leaves, an orange-yellow residue (0.145g) from the root, and a dark orange color residue (0.011g) from stems. The crude samples were preserved at 4°C for further studies.

Development of thin-layer chromatography plates

A hundred mg of whole plant extract was dissolved in ethyl acetate (0.5mL) and deposited in a horizontal line at a preparative thin layer silica gel plate using a capillary tube. The major band, which was detected visually and under UV 366, was scraped off. The powdered silica gel was sonicated in chloroform: Ethanol (3:1), filtered, and the solvents were removed under reduced pressure. ^{1}H- and ^{13}C-NMR revealed that the red crystalline residue was plumbagin.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS examination was established utilizing a "Perkin Elmer Clarus 500 Gas Chromatography" connected with Elite-5MS fused silica column (0.25µm df, 30m) and interfaced with a detector of the variant ion trap. The temperature of the injector was adjusted at 260°C with an initial temperature of 60°C, initial hold 5 min, and ramp 5.0°C/min to 280°C. The transfer line and injector temperatures were set at 290 and 280°C, respectively. The helium flow rate was kept at 1mL/min with a linear velocity of 31.0cm/s. The split ratio was 1/60, ionization energy was 70 eV, scan time was 1s and the mass range was 50-400 amu. "Perkin Elmer Clarus 560D mass spectrometer" was utilized in the current examination. 20µL Diluted samples (10mg/mL) of the whole plant ethanolic extract were injected in the GC-MS spectrophotometer. The compounds were identified by GC-MS based on the molecular formula, the mass spectrum for each in addition to comparison with the literature data from previous studies (Šamec and Zeljković, 2020).

Calibration curve of plumbagin

Part of a solution containing plumbagin within the range of 4.5-224µg was placed in a 5mL volumetric flask. The volume was completed with a buffer solution pH 12 and the absorbance was determined after 2min at 510 nm against a reference buffer solution (Adusei *et al.*, 2019).

High-Performance Liquid Chromatography analysis

High-Performance Liquid Chromatography (HPLC) technique was utilized for the quantitative determination of plumbagin in the extracts obtained from *P. europea*

leaves, stems, and root, separately. Methanol: Water (70:30) was used as eluent with a flow rate of 0.75mL/min. The injection volume was 20µL and the wavelength of the detector was set at 270nm. Several reports described the use of the HPLC technique for quantitative analysis of plumbagin in other species of the genus *Plumbago* (Naqishbandi, Saour *et al.*, 2009). During this study, plumbagin was quantitatively determined in *P. europaea* leaves, stems, and roots extracts by comparing the retention time of each extract with that of the standard plumbagin trial (rt=6.6 min). The concentrations of plumbagin were determined based on the calibration curve of the standard plumbagin. The percentages of plumbagin in roots, leaves and stems extracts were found to be 1.65±0.015%, 0.51±0.001%, and 0.16±0.001%, respectively.

Nuclear magnetic resonance spectroscopy (NMR)

NMR spectra were recorded on an Agilent 400-MR (^{1}H, 200 MHz; ^{13}C 100.62 MHz) spectrometer at ambient temperature. Chemical shifts for 1H NMR spectra are reported in parts per million (ppm) from tetramethylsilane with the solvent resonance as the internal standard (chloroform: d7.26). Coupling constant values (*J*) are given in Hz. Chemical shifts for ^{13}C NMR spectra are reported in parts per million (ppm) from tetramethylsilane with the solvent as the internal standard (chloroform: d 77.26 ppm) (Yen *et al.*, 2019).

Microorganisms

Three human fungal pathogens causing dermatophytosis, namely, *Trichophyton rubrum* (castellani) Sabouraud, *Trichophyton mentagrophyte*s (Robin) Blanchard, and *Microsporum canis* Bodin) and one animal pathogen, *Ascosphaera apis* (Olive Spiltoir) Maassan ex Claussen, a fungus that causes bees' chalkbrood disease were used in this investigation. All the above-mentioned fungal strains were a generous gift from Prof. Ali-Shtayeh's fungal collection at the Biodiversity and Environmental Research Center, Til, Nablus.

Antifungal testing

The plant extract and pure plumbagin were screened at several concentrations for their antifungal potential against the four strains mentioned above employing the poisoned food technique. Different concentrations of the whole plant extract (0.8, 1.6, 3.2, 6.4mg/mL) and pure plumbagin at concentration of (0.025, 0.05, 0.1, 0.2 mg/mL) were incorporated in pre-sterilized SDA medium to prepare a series of concentrations. A mycelial agar disk of 5 mm diameter of the pathogen was cut from a 12 days old culture and inoculated onto the freshly prepared agar plates. In controls, distilled sterile water was utilized in place of the examined samples and for each dilution three replicate plates were conducted. The inoculated plates were incubated at 24˚C in the dark and the results were obtained after 10 days. The mycelial inhibition activity was measured utilizing the following formula:

Mycelial inhibitory activity, % = (ccd-scd/ccd) X 100%; where ccd is the control colony diameter and scd is sample colony diameter (Dikshit and Husain, 1984).

STATISTICAL ANALYSIS

All the conducted experiments were determined in triplicates, and their results were calculated as means ± standard deviation (± SD).

RESULTS

Identification of chemical constituents of P. europaea

The percentage of plumbagin in *P. europaea* roots leaves and stems extracts were determined *via* spectrophotometric and HPLC techniques. As indicated in table 1, the roots extract contained the highest concentration of plumbagin (1.65±0.015%), while the stems extract possessed the lowest percentage (0.16±0.001%). The data obtained using spectrophotometry techniques have slightly deviated from that obtained by the HPLC technique.

Fifty-nine compounds were determined using GC-MS, amounting to 100% of *P. europaea* extract, of which plumbagin was found to be the major component followed by *β*-bisabolene, nonanal, limonene, and 1-octen-3-yl acetate as shown in table 2. Plumbagin was isolated using the preparative TLC technique. The IR spectrum and ^{1}H- and ^{13}C- NMR (figs. 1 and 2, respectively) of the isolated plumbagin were identical to those reported in the literature.

FT-IR (KBr, cm^1) n_{max}: 3384 (O-H), 2927.5 (CH_3), 1662 and 1642 (C=O), ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 2.178 (s, 3H, CH_3), 6.68 (d, *J*=Hz, H-7), 6.68 (d, *J*=Hz, H-7), 7.31 (d, *J*=Hz, H-7), 13.59 (br s, 1H, OH), ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 16.1 (CH_3), 116 (C-3), 119 (C-), 124 (C-), 133 (C-), 136. (C-), 138 (C-), 148 (C-), 162 (C-), 184. (C), 190 (C-); MS m/z 188 [M^+], 173 [M^+-15], 131, 92.

Antifungal activity

As revealed in fig. 3, *P. europea* ethanolic extract inhibited the mycelial growth of *Microsporum canis (Mc)*, *Trichophyton mentagrophyte*s (*Tm*), *Trichophyton rubrum (Tr),* and *Ascosphaera apis (Aa)* in a dose-dependent manner. Plumbagin inhibited the growth of all the tested fungi, significantly (fig. 4), where a complete inhibition was attained at concentrations of 0.1-0.2mg/mL. At 0.05mg/mL concentration, plumbagin completely inhibited the growth of *Trichophyton mentagrophyte*s (*Tm*), whereas 0.2mg/mL concentration was needed to prevent the growth of the animal pathogen, *Ascosphaera apis* completely.

Table 1: The percentage of plumbagin in *P. europaea* plant parts by the two methods

Plant parts	Spectrophotometric determination, %	HPLC, %
Leaves	0.56±0.01	0.51±0.001
Stems	0.1±0.009	0.16±0.001
Roots	1.5±0.087	1.65±0.015

Table 2: Constituents of *P. europeae* entire plant found by GC-MS

No.	Compound Name	Formula	Molecular Weight
1	Methylchromone	$C_{10}H_8O_2$	160
2	1-(1-Propynyl) cyclohexene	C_9H_{12}	120
3	2-(2-Prpanyloxy)- benzaldehyde	$C_{10}H_8O_2$	160
4	5-Hydroxy-2-methyl-1,4-naphthalenedione	$C_{11}H_8O_3$	188
5	8-Methoxy-2,2,4-trimethyle-1,2-dihydro-quinoline	$C_{13}H_{17}ON$	203
6	2,3,6,7-Tetrahydro-1H,5H-benzo[IJ]quinolizin-8-ol	$C_{12}H_{15}ON$	189
7	3-(Hydroxyamino)-6-methylindolin-2-one	$C_9H_8O_2N_2$	176
8	15B-Methyl-18B-nor-8-aza-16-oxaestera-1,3,5(10)-triene-17-on	$C_{16}H_{19}O_2N$	257
9	8-Methylindolizine	C_9H_9N	131
10	3,4,5,6,7,8,10,10A,-Octahydro-2(4AH)-anthracenone	$C_{14}H_{18}O$	202
11	2,3-Dimethylchromone	$C_{11}H_{10}O_2$	174
12	3-(2-Methylallyl)salicylic acid	$C_{11}H_{12}O_3$	192
13	8-Methoxy-2,2,4-trimethyl-1,2-Dihydro-quinoline	$C_{13}H_{17}ON$	203
14	1-[Trifuroromethyl)phenyl]- piperazine	$C_{11}H_{13}N_2F_3$	230
15	Nalidixic acid	$C_{12}H_{12}O_3N_2$	232
16	8-Hydroxy-2-methyl-1,4-naphthalenedione	$C_{11}H_8O_3$	188
17	4-Chromanol	$C_9H_{10}O_2$	150
18	2-Ethoxy-N-(1-phynylethylideno)-benzhydrazide	$C_{17}H_{18}O_2N_2$	282
19	2-Ethoxybenzhydrazide	$C_9H_{12}O_2N_2$	180
20	2-Ethoxyacetophenone	$C_{10}H_{12}O_2$	164
21	3,5-Dihydroxy-2-methyl 1,4-naphthalenedione	$C_{11}H_8O_4$	204
22	2-Propnoic acid, 3-(2-nitrophenyl)-, ethyl ester	$C_{11}H_{11}O_4N$	221
23	4-Methyl-7-ethoxycoumarin	$C_{12}H_{12}O_3$	204
24	7-Hydroxy-2-hydroxymethylene-1-indanone	$C_{10}H_8O_3$	176
25	2-Chloro-2-methylindan-1,3-dione	$C_{10}H_7O_2Cl$	194
26	6-Hydroxy-2-methylcyclohepta(B)pyridine-one	$C_{11}H_9O_2N$	187
27	6-Methyl-3-pyridyl styrylketone	$C_{15}H_{13}ON$	223
28	6-Ethoxyquinaldine	$C_{12}H_{13}ON$	187
29	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
30	4-hexen-1-ol, 5-methyl-2-(1-methylethenyl)- acetate, (R)	$C_{12}H_{20}O_2$	196
31	z,z-6,28-heptatriacetontadien-2-one	$C_{37}H_{70}O_2$	530
32	Butanoic acid, 3-methyl- 3,7-dimethyl-6-octenyl ester	$C_{15}H_{28}O_2$	240
33	4-Methoxy-6-methyl-6,7-dihydro-4H-Furo[3,2-C] pyran	$C_9H_{12}O_3$	168
34	2-Pentacosanone	$C_{25}H_{50}O$	366
35	2-Heptacosanone	$C_{27}H_{54}O$	394
36	6,10,14-Trimethyl 2-pentadecanone	$C_{18}H_{36}O$	268
37	1,2-Benzenedicarboxylic acid, bis (2-Methylpropyl ester)	$C_{16}H_{22}O_4$	278
38	1,2-Benzenedicarboxylic acid, butyl 2-Methylpropyl ester	$C_{16}H_{22}O_4$	278
39	1,2-Benzenedicarboxylic acid, mono butyl ester	$C_{12}H_{14}O_4$	222
40	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	$C_{18}H_{24}O_4$	304
41	Hexanoic acid ethyl ester	$C_{18}H_{36}O_2$	284
42	10-Bromodecanoic acid, ethyl ester	$C_{12}H_{23}O_2Br$	278
43	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256

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44	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	270
45	Butylated hydroxytoluene	$C_{15}H_{24}O$	220
46	Phenol,4,6-di(1,1-dimethylethyl)-2-methyl	$C_{15}H_{24}O$	220
47	2,4,6-tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one	$C_{15}H_{24}O$	276
48	Phenol 2,4,6-tris(1-methylethyl)-	$C_{15}H_{24}O$	220
49	Bicyclo[3.1.10]hexan-2-one,1,5-bis (1,1-dimethylethyl)-3,3-dimethyl	$C_{16}H_{28}O$	236
50	2,3-Benzofurandione	$C_8H_4O_3$	148
51	4H-1-Benzopyran-4-one,2,3-dihydro-2-methyl	$C_{10}H_{10}O_2$	162
52	2-Hydroxyethyl salicylate	$C_9H_{10}O_4$	182
53	Fosfosal	$C_7H_7O_6P$	218
54	Beta-1,5-o-dibenzoil-ribofuranose	$C_{19}H_{18}O_7$	358
55	2-Chloroethyl benzoate	C_9H_9OCl	184
56	(+)-Dibenzoyl-L-tartaric acid anhydride	$C_{18}H_{12}O_7$	340
57	Benzoic acid 2-bromoethyl ester	$C_9H_9O_2Br$	228
58	Pyrimidine,2-amino-4-(3-fluorophenyl)	$C_{10}H_8N_3F$	189
59	2-Quinollinecarboxaldehyde,8-hydroxy-,oxime	$C_{10}H_8O_2N_2$	188

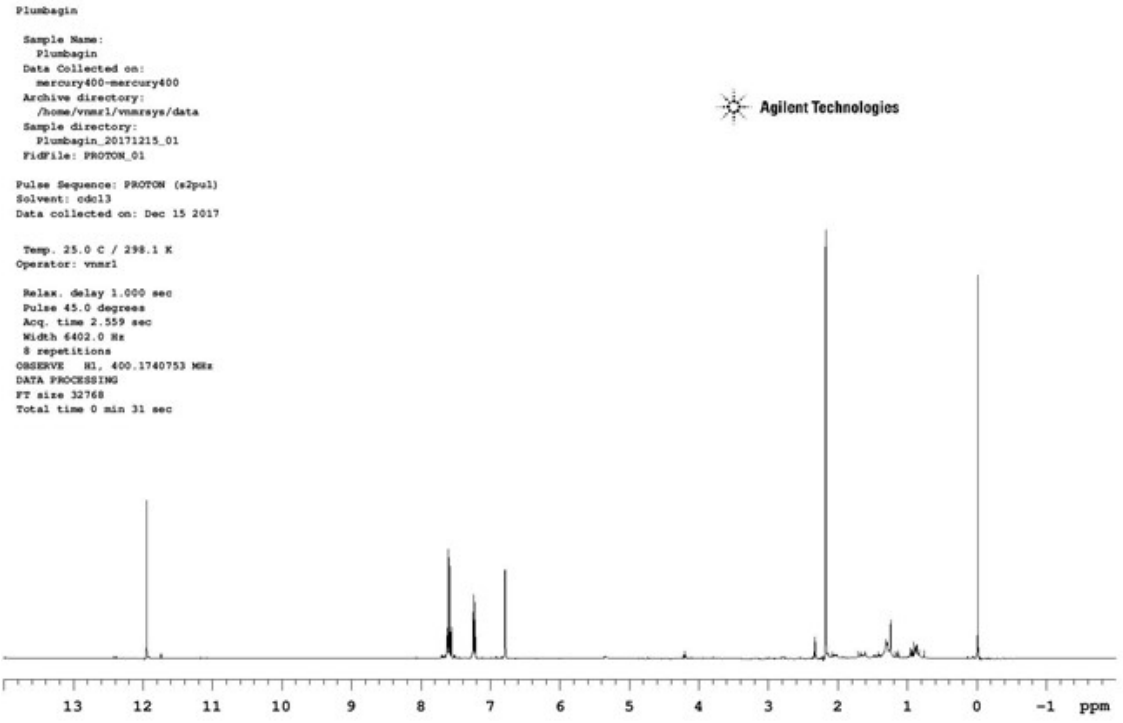


Fig. 1: ^{1}H- NMR of the isolated plumbagin

DISCUSSION

From ancient times, *P. europaea* leaves and roots have been utilized in Arabian traditional medicine to cure different dermatological infectious diseases and non-infectious diseases such as rheumatoid arthritis, dysmenorrhea, and cancer (Muhammad *et al.*, 2017). Plumbagin, a marker biological and pharmacological active compound isolated from different *Plumbago* plant species, displayed anti-inflammatory (Zheng *et al.*, 2017), anticancer (Gowda *et al.*, 2017, Rahman and Anwar, 2007), antifungal (Zheng *et al.*, 2017) and antibacterial effects (Gupta *et al.*, 2017) activities. Plumbagin has been previously separated from the roots and leaves of *P. europaea* and for the first time in the current study from *P. europaea* stems. It was found that *P. europaea* roots extract contains the highest percentage of plumbagin (1.65±0.015%) followed by leaves (0.51%), whereas stems have the lowest percentage (0.16±0.001%). This is too little, when compared with the high concentration of plumbagin (50%) found in *Plumbago scandens* roots extract, which considered to be the best source of plumbagin (Paiva *et al.*, 2004). GC-MS examination of the total plant extract revealed that plumbagin was the chief component.

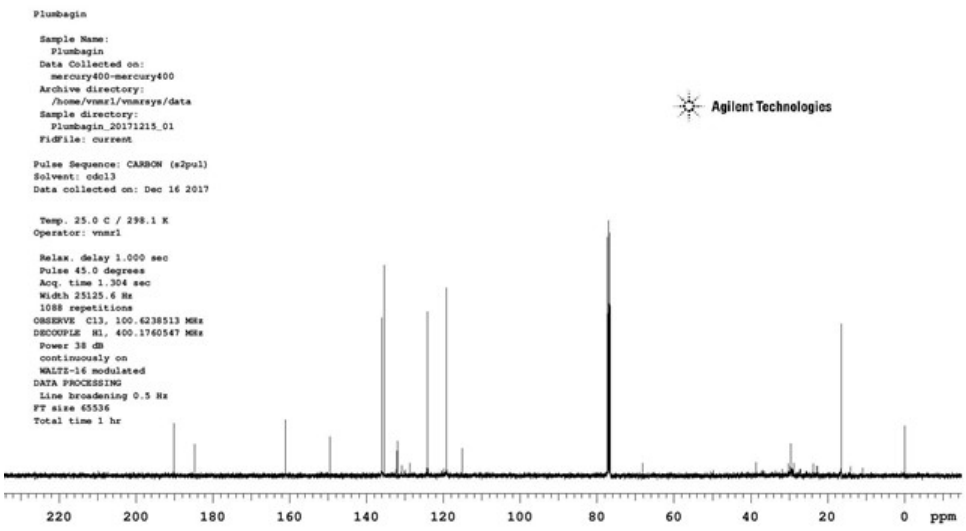
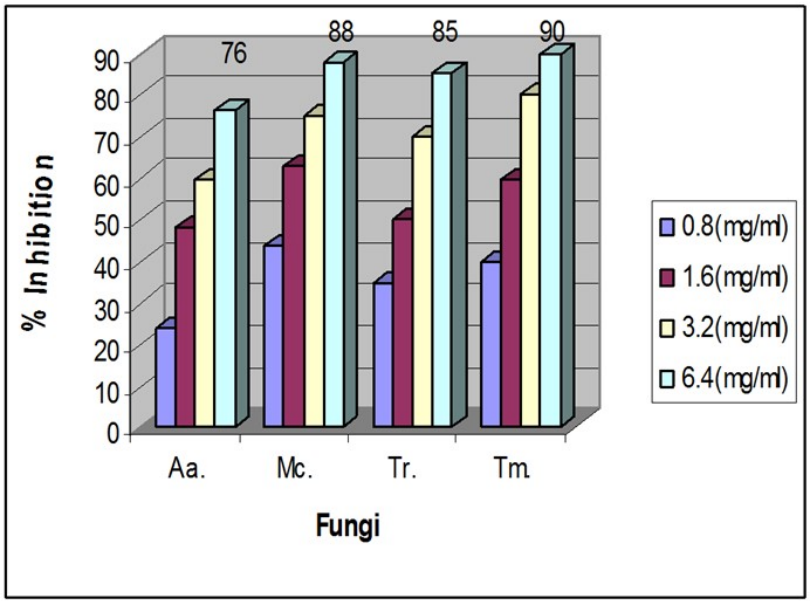


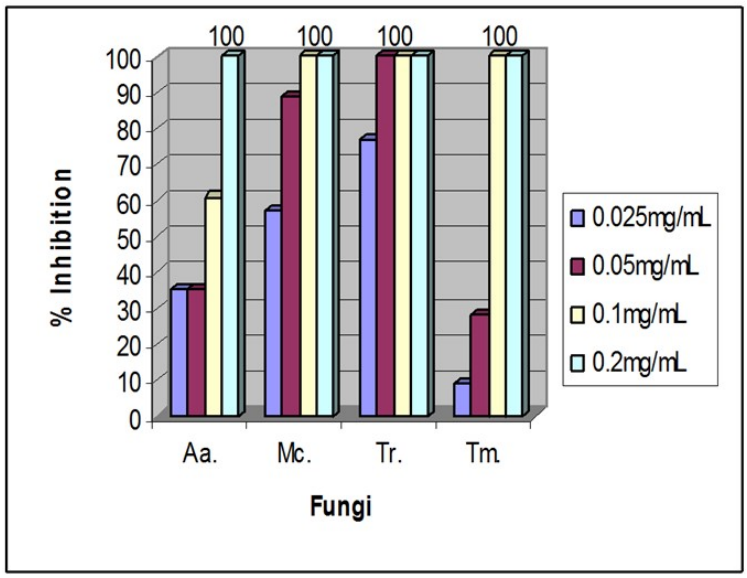
Fig. 2: ^{13}C- NMR of the isolated plumbagin



Where; Aa: *Ascophaera apis*, Mc: *Microsporum canis,* Tr: *Trichophyton rubrum* and Tm *Trichophyton mentagrophytes*.

Fig. 3: Antifungal activity of *P. europea* ethanolic extract

Fungal infections have displayed resistance against several types of antifungal drugs (Khan *et al.*, 2019). Chemical agents, such as azole derivatives, used to cure human and animal dermatological illnesses, possess the potential to develop resistance in harmful fungal strains and cause environmental pollution, giving rise to severe health hazards to humans and animals (Fisher *et al.*, 2018). Besides, the Chalkbrood disease of bee's brood that caused by *Ascosphaera apis* fungi was blamable for the death of several hundreds of bee colonies annually and considered one of the most harmful agricultural and economic issues worldwide (Ansari *et al.*, 2017, Sarwar 2016). During this investigation, it was observed that *P. europea* ethanolic extract exhibited potent fungal toxicity (% mycelial inhibition), with 76% and 90% inhibition for *A. apis* and *T. mentagrophytes* at a concentration of 6.4 mg/mL, respectively. A positive correlation was found between the concentration of *P. europea* ethanolic extract and the inhibitory effects on the mycelial growth of the four fungal strains tested. The results were in agreement with that described by Shafiqur *et al*. stating that the extract of the root of *Plumbago zeylanica* inhibited the growth of *Curvularia lunata, Colletotrichum corchori* and *Fusarium equiseti* fungi (Rahman and Anwar, 2007).



Where; Aa: *Ascophaera apis*, Mc: *Microsporum canis,* Tr: *Trichophyton rubrum* and Tm *Trichophyton mentagrophytes*.

Fig. 4: Antifungal activity of ethanolic extract of Plumbagin

The Antifungal activity of *P. europea* is mainly attributed to the presence of plumbagin, even though other active antifungal compounds may also present. The antifungal activity may be referred to plumbagin ability to form hydrogen bonding with the DNA of bacteria or fungi and consequently stopping their growth.

Plumbagin inhibited the growth of all examined fungi, significantly, where a complete inhibition was achieved at a concentration range of 0.1-0.2mg/mL. This finding is in agreement with that observed by Vichkanova *et al*., (Vichkanova, Izosimova *et al.*, 1979), who also found that plumbagin displayed inhibition activity against *Microsporum lanesum* and *Candida albicans* at 0.24-162 µg/mL level. The concentration of 0.05mg/mL of plumbagin completely inhibited the growth of *Trychphyton mentagrphytes*, whereas the concentration of 0.1mg/mL was required to completely inhibit the growth of *Micosporum canis*, *Trichphyton rubrum*, which is higher than MIC80 (the minimum inhibitory concentration 80% of microbial growth) of the extracts which considered good antifungal activity results of natural products. However, 6.4mg/mL of the *P. europea* extracts caused a growth inhibition ranging from 76-89.7% of the four tested fungi strains.

CONCLUSION

In the present work, plumbagin was isolated from the roots, leaves, and for the first time from the stems of *P. europea*, of which the roots extract possessed the highest concentration. In addition, 59 different compounds were identified from the whole plant using GC-MS. The *P. europea* extract as well as plumbagin exhibited antifungal activity against *T. mentagrphytes*, *M. canis*, *T. rubrum* and against the animal pathogen, *A. apis*. Based on the obtained results of this investigation, plumbagin and *P. europea* extract could be potentially used as antifungal tools. To the best of our knowledge, this is the first report on the inhibitory effect of plumbagin on *T. mentagrphytes*, *M. canis*, *T. rubrum* and *A. apis* fungi.

REFERENCES

Abu-Qaoud H, Shawarb N, Hussen F, Jaradat N and Shtaya M (2018). Comparison of qualitative, quantitative analysis and antioxidant potential between wild and cultivated *Borago officinalis* leaves from palestine. *Pak. J. Pharm. Sci.*, **31**(3): 953-959.

Adusei E, Adosraku RK, Oppong-Kyekyeku J and Amengor CD (2019). Investigation of Acid-Base Indicator Property of Plumbagin from *Plumbago zeylanica* Linn. *Int. J. Anal. Chem.*, **2019**(13): doi: 10.1155/2019/4061927.

Alkowni R, Jodeh S, Hussein Fa nd Jaradat N (2018). Phytochemical screening and antibacterial activity of Cyclamen persicum Mill tuber extracts. *Pak. J. Pharm. Sci.*, **31**(1): 187-192.

Ansari MJ, Al-Ghamdi A, Usmani S, Khan KA, Alqarni AS, Kaur M and Al-Waili N (2017). *In vitro* evaluation of the effects of some plant essential oils on Ascosphaera apis, the causative agent of Chalkbrood disease. *Saudi J. Biol. Sci.*, **24**(5): 1001-1006.

Benítez G, González-Tejero M and Molero-Mesa J (2010). Pharmaceutical ethnobotany in the western part of Granada province (southern Spain): ethno-pharmacological synthesis. *J. Ethnopharmacol.*, **129**(1): 87-105.

Chaplot BB, Dave AM and Jasrai YT (2006). A valued medicinal plant-Chitrak (Plumbago zeylanica Linn.): Successful plant regeneration through various explants and field performance. *Plant Tissue Cult. Biotechnol.*, **16**(2): 77-84.

Chung S-K, Kim Y-C, Takaya Y, Terashima K and Niwa M (2004). Novel flavonol glycoside, 7-O-methyl mearnsitrin, from Sageretia theezans and its antioxidant effect. *J. Agr. Food Chem.,* **52**(15): 4664-4668.

Dikshit A and Husain A (1984). Antifungal action of some essential oils against animal pathogens. *Fitoterapia*, **3**: 171-176.

Fisher MC, Hawkins NJ, Sanglard D and Gurr SJ (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science*, **360**(6390): 739-742.

Gowda R, Sharma A and Robertson GP (2017). Synergistic inhibitory effects of Celecoxib and Plumbagin on melanoma tumor growth. *Cancer Lett*, **385**: 243-250.

Gupta P, Sarkar A, Sandhu P, Daware A, Das MC, Akhter Y and Bhattacharjee S (2017). Potentiation of Antibiotic against *Psedomonas aeruginosa* biofilm: A study with Plumbagin and Gentamicin. *J. Appl. Microbiol*, **123**: 246-260.

Jaradat NA, Damiri BandAbualhasan MN (2016). Antioxidant evaluation for *Urtica urens, Rumex cyprius* and *Borago officinalis* edible wild plants in Palestine. *Pak. J. Pharm. Sci.*, **29**(1): 325-330.

Jaradat NA, Zaid AN and Hussein F (2016). Investigation of the antiobesity and antioxidant properties of wild *Plumbago europaea* and *Plumbago auriculata* from North Palestine. *Chem. Biol. Technol. Agricult.*, **3**(1): 31-40.

Jayanthi M, Gokulanathan A, Haribalan P, Ashakiran K, Kumar CD, Kamla D, Girija S and Renukadevi BS (2020). Plumbagin from two Plumbago species inhibits the growth of stomach and breast cancer cell lines. *Ind. Crops Prod.*, **146**: 112147.

Khan Altaf IU, Hussain MM and Rahim A (2019). Phytochemical and antimicrobial study of *Alstonia scholaris* leaf extracts against multidrug resistant bacterial and fungal strains. *Pak. J. Pharm. Sci.*, **32**(4): 1655-1661.

Muhammad HM, Saour KY and Naqishbandi AM (2017). Quantitative and qualitative analysis of Plumbagin in the leaf and root of *Plumbago europaea* growing naturally in Kurdistan by HPLC. *Iraqi J. Pharm. Sci.*, **18**: 54-59.

Naqishbandi AM, Saour KY and Muhammad HM (2009). Quantitative and qualitative analysis of Plumbagin in the leaf and root of *Plumbago europaea* growing naturally in Kurdistan by HPLC. *Iraqi J. Pharm. Sci.*, **18**(1): 54-59.

Paiva SRd, Lima LA, Figueiredo MR and Kaplan MAC (2004). Plumbagin quantification in roots of *Plumbago scandens* L. obtained by different extraction techniques. *Anais da Academia Brasileira de Ciências*, **76**(3): 499-504.

Palmese MT, Manganelli REU and Tomei PE (2001). An ethno-pharmacobotanical survey in the Sarrabus district (south-east Sardinia). *Fitoterapia*, **72**(6): 619-643.

Passalacqua N, Guarrera P and De Fine G (2007). Contribution to the knowledge of the folk plant medicine in Calabria region (Southern Italy). *Fitoterapia*, **78**(1): 52-68.

Rahhal B, Jaradat N, Shraim WBM, Zyoud A and Hattab S (2018). The diuretic activity of *Ephedra alata* and plumbago europaea in mice using an aqueous extract. *Mor. J. Chem.*, **6**(4): 2569-2576.

Rahman MS and Anwar MN (2007). Antimicrobial activity of crude extract obtained from the root of *Plumbago zeylanica*. *Bangladesh J. Microbiol.*, **24**(1): 73-75.

Šamec D and Zeljkovic SC (2020). Analytical methods focused on studying phytonutrients in food. Phytonutrients in Food, Elsevier, pp.237-244.

Sarwar M (2016). Fungal diseases of honey bees (Hymenoptera: Apidae) that induce considerable losses to colonies and protocol for treatment. *Int. J. Zool. Res.*, **1**: 8-13.

Sezik E, Yeşilada E, Honda G, Takaishi Y, Takeda Y and Tanaka T (2001). Traditional medicine in Turkey X. Folk medicine in central Anatolia. *J. Ethnopharmacol.*, **75**(2): 95-115.

Shawahna R and Jaradat NA (2017). Ethno-pharmacological survey of medicinal plants used by patients with psoriasis in the West Bank of Palestine. *BMC Complement. Altern. Med*, **17**(1): 4-11.

Singh K, Naidoo Y and Baijnath H (2018). A Comprehensive review on the genus Plumbago with focus on *Plumbago auriculata* (Plumbaginaceae). *Afr. J. Tradit. Complement. Altern. Med.*, **15**(1): 199-215.

Sobhani M, Abbas-Mohammadi M, Ebrahimi SN and Aliahmadi A (2018). Tracking leading anti-Candida compounds in plant samples; Plumbago europaea. *Iran. J. Microbiol.*, **10**(3): 187-193.

Vichkanova SA, Izosimova SB, Adgina VV and Shipulina LD (1979). Search for antimicrobial drugs among quinones of plant origin. *Rastit Resur*, **15**: 67-177.

Yen DTH, Thu VK, Tai BH, Yen PH, Nhiem NX and Kiem PV (2019). Pregnane glycosides from *Gymnema sylvestre*. *Vietnam J. Chem.*, **57**(2): 208-212.

Zheng W, Tao Z, Chen C, Zhang C, Zhang H, Ying X and Chen H (2017). Plumbagin prevents IL-1β-Induced inflammatory response in human osteoarthritis chondrocytes and prevents the progression of osteoarthritis in mice. *Inflammation*, **40**(3): 849-860.