Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

First record of phytochemical and the antifungal activity of methanol extracts from vegetative parts of *Juncus rigidus* plants in Iraq

Neepal Imtair AlGaraawi[1]*, Sarab Fadhil AlAmery[1], Manal Abd Alwahed Alsirrag[2]

1. Department of Biology, College of Education for Pure Science, Kerbala University, Kerbala, Iraq
2. College of Agricultural University, Kerbala, Iraq

** Corresponding author's Email:* neepal.i@uokerbala.edu.iq

ABSTRACT

In this study, we worked in the various naturally-appearing compounds isolated from Juncus rijidus which exhibit phyto-constituents from leaves and stems against four dermatophytes: Microsporum canis, Microsporum gypseum, Trichophyton rubrum and Trichophyton mentagrophytes. The phytochemical of the stems and leaves were exposed to GC-MS analysis. The results showed highest activity against all reviewed fungal (dermatophytes). In the cases of T. mentagrophytes and T. rubrum all three concentrations of the extract (2.5, 5 and 10 mg mL-1) exhibited a results of 0.00 mm in the diameter of colonies, while in the case of M. canis, once using 2.5, 5 and 10 mg mL-1, the diameter of colonies were 1.85, 1.25 and 1.00 mm respectively. In the case of M. gypseum, the diameter of colonies were 1.5, 1.00 and 1.00 mm for concentrations of 2.5, 5, 10 mg mL-1 respectively. The GC-MS analysis of the J. rijidus parts showed the presence of 20 components, the list of them are presented in this report.

Keywords: *Juncus rijidus*, Gas chromatography– mass spectrometry, Bioactive phytochemical, Antifungal activity.
Article type: Research Article.

INTRODUCTION

Family Juncaceae consists of 9 genera and 300-400 species with multinational distribution in the temperate and arctic region, tropical mountains, and in cold damp place. *Juncus rigidus* species were marsh herbs usually with thick, creeping rhizomes no intravaginal shoots formed, stems thick, rigid, erect, 75-150 cm in height, leaves terete, pungent, inflorescence with two leafy pungent bracts, lax (Townsen & Guest 1985) grows in marshes, shallow brackish water, and semi-saline soil and can be found in a variety of moist, wet and temperate climate (Hamza 2020). The *Juncus* plants contains important bioactive compounds such as phenolic acids, flavonoids, sterols, terpenes, coumarins, phenanthrenes, stilbenes, sterols, Luteolin-5-glucosid carotene (Abdelsamed *et al.* 2020) which are encompassed in the extracts of *J. acutus* and *J. subulatus.* The discovered active compounds could play an essential role in the antifungal effects of the extracts, which can contain different pharmacological procedures (Kúsz *et al.* 2016). Thuerig *et al.* indicates that a methyl acetate extract of *J. effusus* L. medulla, and dehydroeffusol (DHEF) were recognized as its main active components against *Venturia* spp. and *Plasmopara* spp. (Thuerig *et al.* 2016). Yoshihito et al. investigated the *Juncus* powder against *C. albicans.* They discovered that "the tissue conditioner involving *Juncus* powder has a high significant growth inhibitory effect against *C. albicans*" (Yoshihito *et al.* 2018).

Three new phenanthrenes were isolated from the stem of *J. effuses* by Zhao *et al.* which demonstrated remarkable antifungal activities against six agricultural pathogenic fungi (*Verticillium dahliae* Kleb, *Phytophthora parasitica*, *Bipolaris zeicola*, *Sclerotinia sclerotiorum*, *Gibberella saubinetii* and *Rhizoctonia solani*; Saeed *et al.* 2019). So, Al-Amery & Al-Garaawi studied the methanol extract of the *J. maritimus* leaves and stems against four

Caspian Journal of Environmental Sciences, Vol. 20 No. 5 pp. 1011-1021 Received: June 16, 2022 Revised: Sep. 07, 2022 Accepted: Nov. 23, 2022
DOI: 10.22124/CJES.2022.6058

Publisher: University of Guilan,

dermatophytes (*Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum canis* and *M. gypseum*), which showed highest activity against fungi (Shuang shuang *et al.* 2020). Conversely, the chemical composition of *J. rigidus* were not reviewed carefully". Dermatophytosis is "a term used to express mycotic infections caused by a group of fungi that mostly remain limited to the artificial layers of the skin, nails and hair, It is an notable public health problem because of its high dominance and associated indisposition.

The important two species, *T. mentagrophytes* and *T. rubrum,* are the prevalent pathogens. "Therapeutic worth of antifungals can be restricted because of their costs and side effects. Plant extracts symbolize a potential source of active antimicrobial agents, by the content of a chemical bioactive compounds (Porusia & Septiyana 2021; Naser AL-Isawi 2022; Salih *et al.* 2022; Al-Shurait & Al-Ali 2022).

There are many analyses such as the antifungal activity of *Lawsonia* spp. clarified against Candida isolates (AlAmery & AlGaraawi 2020). The leaf extracts of *Cynodon dactylon*, *Ocimum tenuiflorum*, and bark extract of *Ocimum tenuiflorum* have anti-fungal cures for dermatophytosis (*Epidermophyton*, *Microsporum*, and *Trichophyton*; Lee *et al.* 2019). So the extracts of *Coccinia indica* was assessed to exhibit antifungal activity using dermatophytes like *Candida albicans*, *Microsporum canis* and other mycotic fungi" (Lin *et al.* 2013). The biochemical components are taxonomically and chemically awfully diverse composites with in-comprehensible function. They are used in agriculture, scientific research and the human therapy (Ma *et al.* 2016). So, this study aimed to explain a syntheticdrugs from herbal plant extract and propolis and their effects on aforementioned fungi.

MATERIALS AND METHODS

Study area and sampling

Dermatophytes were isolated from patients (n = 90) visited the Al-Hussein Hospital in Karbala. The samples were clinically spotted by dermatologists from the hospital and samples were collected from skin, nails and head hair.

Microscopic assessment

The samples were examined using methodology of (Adem *et al.* 2020). The area was cleaned with a cotton saturated swab with 70% alcohol to get rid of a bacteria and saprophytic fungi, followed by taking a scrape from the influenced parts by a loop fertilization. Thereafter, the sample was placed on a pure glass slide with a drip of 10% KOH and then put the glass slide cover and heat the sample on a benzene flame and examined by a microscope for the occurrence of dermatophytes spores or hypha. Mentioned fungi were diagnosed according to: (Szepietowski & Schwart 2005; Ayman *et al.* 2013). The phenotypic characteristics of spores and fungal colonies along with their microscopic properties were accompanied by identifying the appearance and colour of the colony from the bottom of the dish.

Plant extract preparation

Wahid & Jafar methodology (Wahid & Jafar 2005) was followed in the extraction process.

Cultivated method of alcoholic extract of *Juncus rigidus* on dermatophyte growth

"El-Kady *et al.* (1993) method were chased: The alcoholic extract of *J. maritimus* was merged with SDA cultivated media using three concentrations including 5, 10 and 15 mg mL^{-1} (three replicates for each concentration). After solidifying medium, a hole was made at a centre of each dish by a cork borer piercing (5 mm) in a diameter with a control treatment. The dishes were inoculated with experimented fungus inoculum and grown on the SGA medium for three weeks each by fixing a disk with a diameter of 5 mm in the centre of the dish. The dishes were incubated at 25 °C for 2 weeks, then the diameter of the growing colony was measured. Results were recorded, and the inhibition ratio was calculated using the following (El Kady *et al.* 1993)":

$$\text{Inhibition ratio} = \frac{Average\ diameter\ of\ fungus\ in\ control\ dish(1) - Average\ diameter\ of\ fungus\ in\ tretment\ dish}{Average\ diameter\ of\ fungus\ in\ control\ dish(1)} \times 100$$

Collection and preparation of plant materials

J. rigidus leaves and stem were provided from various spots in Iraq, then washed and dried at room temperature. Eighteen grams of plants was powdered and taken in 40-mL ethanol and then filtered.

Constituents identification of extract by gas chromatography – mass spectroscopy (GC-MS)

Phytochemical identification of *J. maritimus* were carried out by GC-MS analysis in a QP 2015 Plus SHIMADZU instrument under computer designed control at 60 eV. About 1 µL of ethanol extract was injected into the GC-MS column using a micro syringe and the scanning was performed for 45 minutes (Gahukar 2012; Abu-Serag *et al.* 2019).

RESULTS AND DISCUSSION

Antifungal activity

The ethanolic extract of *J. rigidus* stems and leaves exhibited a high antifungal activity against *T. mentagrophytes*, *T. rubrum*, *M. gypseum* and *M. canis.* All studied fungi (dermatophytes), at three extract concentrations (2.5, 5 and 10 mg mL^{-1}) exhibited 0.00 mm in the diameter including the colonies of *T. mentagrophyte* and *T. rubrum.* In the cases of *M. canis* and *M. gypseum*, we found that the diameters of colonies at the concentrations of 2.5, 5, 10 mg mL^{-1} were 1.85, 1.25 and 1.00 mm as well as 1.5, 1.00 and 1.00 mm respectively (Table 1). The results of Al-Amery & Al-Garaawi on *J. maritimus* against the same fungi was in agreement with the present study, while (Sa Allaith *et al.* 2019) registered four new phenanthrenes as antifungal agents against seven pathogenic fungi with minimum inhibitory concentration (MIC) values ranging from 3.123 to 12.6 µg mL^{-1}. (Sahli *et al.* 2018) found that phenanthrene derivatives showed a clear antifungal activity against the studied pathogen.

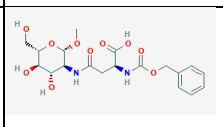
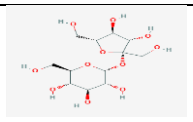
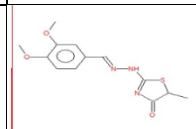
Table 1. Antifungal activity of ethanol extracts of *J. rigidus.*

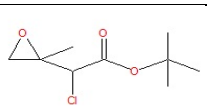
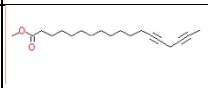
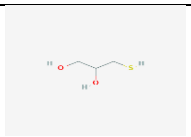
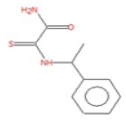
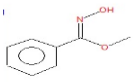
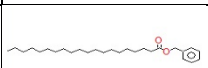
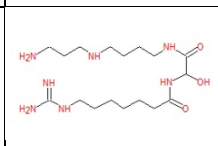
	Dermatophytes (Fungal type)	**Mean of Inhibition zone (mm) Concentration (mg mL^{-1})**				
		Comparison 1 (0.00) mg mL^{-1}	**Clotrimazole 2 mg mL^{-1} Comparison 2**	**2.5 mg mL^{-1}**	**5 mg mL^{-1}**	**10 mg mL^{-1}**
1	*T. mentagrophytes*	9.00	0.00	0.00	0.00	0.00
2	*T. rubrum*	9.00	0.00	0.00	0.00	0.00
3	*M. gypseum*	9.00	0.00	1.5	1.00	1.00
4	*M. canis*	9.00	0.00	1.85	1.25	1.00

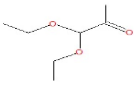
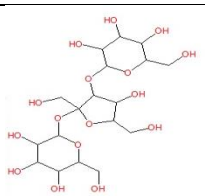
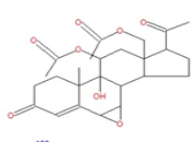
Assessment of biochemical compounds of *J. rigidus*

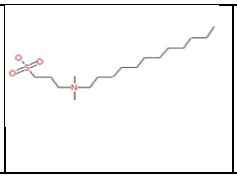
"The GC-MS analysis of methanol extract of *J. rigidus* leaves and stems are depicted with the presence of 20 components in Table 2. The separated compounds has different biological activities, as anxiolytic antimicrobial, anti-inflammatory spasmolytic, antiproliferative, anti-algal effects and antioxidant.

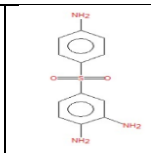
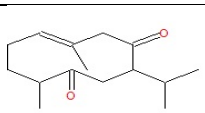
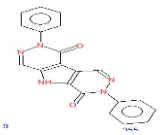
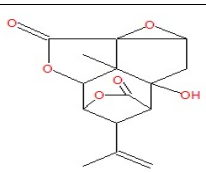
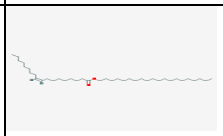
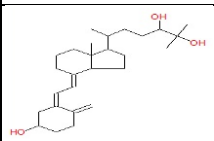
Table 2. Major phytochemical composites in methanolic extract of *J. rigidus* stems and leaves.

No.	Chemical name	Exact Mass	Chemical structure	MS Fragmentat-ions	formula	Pharmacology
1.	Methyl N-(N-benzyloxycarbonyl-beta-l-aspartyl)-beta-d-glucosaminide	442.158745		51,65,79,91 ,108,139, 182, 20,266	$C_{19}H_{26}N_2O_{10}$	Antioxidant antimicrobial
2.	Glucopyranoside, beta-D-fructofuranosyl, alpha-D	342.116212		60,73,85,10 3,131,163,1 91,215,256	$C_{12}H_{22}O_{11}$	Skin conditioning
3.	Benzaldehyde.3,4dimethoxy(4-5 dihydro-5-methyl	293.083412		51,77, 105, 121, 136, 188, 253, 281, 322	$C_{13}H_{15}N_3O_3S$	

4.	Chloro(2-methyloxiran-2-yl)acetic acid, t-butyl ester	206.070972		57,77, 91, 119, 149, 165, 207, 253,284	$C_9H_{15}ClO_3$	
5.	Methyl (13E,16E)-13,16-octadecadienoate #	294.25588		55, 74, 95, 109, 149, 177,205, 242	$C_{19}H_{34}O_2$	
6.	Mercapto-1,2-propanediol-	108.024501		59,77, 90, 108	$C_3H_8O_2S$	Antibiotic Anticancer activity
7.	2(1-phenyl-ethylamino)-2-thioxo-acetamide	208.067		51,77, 91, 105, 120, 148, 163, 175, 191, 208	$C_{10}H_{12}N_2OS$	Antioxidant antimicrobial
8..	methyl N-hydroxybenzenecarboxymidate	151.16		55, 73, 105, 133, 151	$C_8H_9NO_2$	Fungicides
9.	benzyl n-eicosanoate	402.349		57, 71, 91, 108, 126, 147, 207, 281, 311	$C_{27}H_{46}O_2$	antibacterial, antifungal
10.	(-)-Gusperimus	387.295		59, 72, 86, 100,128,187,212, 237, 265	$C_{17}H_{37}N_7O_3$	Antibiotics, Antineoplastic Treatment of nephritis Hypoglycemic Agents

11.	Pyruvaldehyde, 1-(diethyl acetal)	146.18		59,75,87,101	$C_7H_{14}O_3$	
12.	α-D-Glucopyranoside,O-α-Dglucopyranosyl-(1.fwdarw.3)-ß-Dfructo	504.4		60,73,85,97,113,126,145,192	$C_{18}H_{32}O_{16}$	Anti-inflammatory Antistress Antiosteoporotic Antidiabetic cardioprotective
13	(15-acetyl-12-acetyloxy-11-hydroxy-10-methyl-7-oxo-3-oxapentacyclo[9.7.0.0^{2,4}.0^{5,10}.0^{1} ^{4,18}]octadec-5-en-14-yl)methyl acetate	460.5		55,79,152,235,279,385,400,460	$C_{25}H_{32}O_8$	

14	1-Dodecanaminium, N,N-dimethyl-N-(3-sulfopropyl)-, hydroxide, inner salt	336.6		58,69,84,97,122,152,180,213	$C_{17}H_{38}NO_3S^+$	

15	3,4,4'-Triaminodiphenylsulfone	263.32		57,83,125,181,220,248	$C_{12}H_{13}N_3O_2S$	
16	3-Isopropyl-6,10-dimethyl-6-cyclodecene-1,4-dione #	236.35		55,69,82,109,137,180,236	$C_{15}H_{24}O_2$	Anti-viral antibacterial Antifungal Antitumor ( )
17	5,7-Dihydro-2,7-diphenyl-1H-pyrrolo[2,3-d:4,5-d']dipyridazine-1,6(2H)-dione	355.3		51,77,93,119,149,165,187,224,238,267,327,355	$C_{20}H_{13}N_5O_2$	Anti-angiogenic Effect Antitumor( )
18	(1aR(1aalpha,2abeta,3beta,6beta,6abeta,8aS*,8bbeta,9R*))-Hexahydro-2a-hydroxy-8b-methyl-9-(1-methylethenyl)-3,6-methano-8H-1,5,7-trioxa cyclopenta(ij)cycloprop(a)azulene-4,8(3H)-dione	292.28		59,95,149,165,193,210,233,252,292	$C_{15}H_{16}O_6$	Treating neuromuscular or neurologic disease treatment of cancer
19	O-eicosanyl (9Z)--1 octadecenoate	563.0		57,69,97,180,222,264,283,325,407,480	$C_{38}H_{74}O_2$	
20	9,10-Secocholesta-5,7,10(19)-triene-3,21,25-triol, (3.beta.,5Z,7E)-	416.6		55,118,136,158,207,253,383,416	$C_{27}H_{44}O_3$	

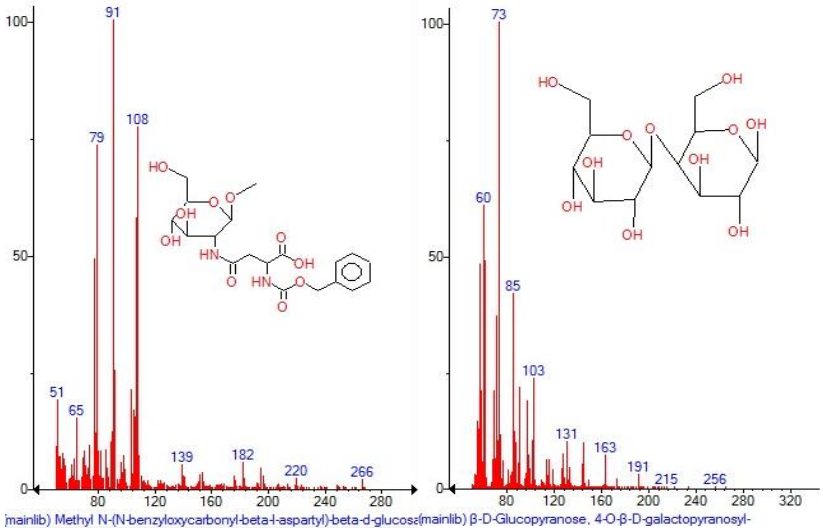


Fig. 1

Fig. 2

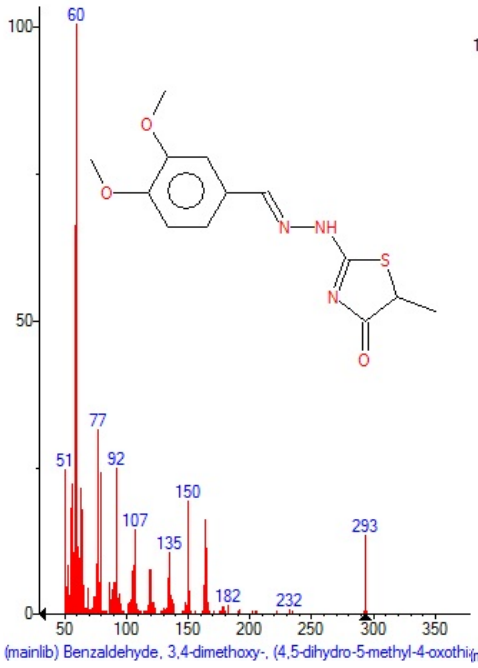


Fig. 3

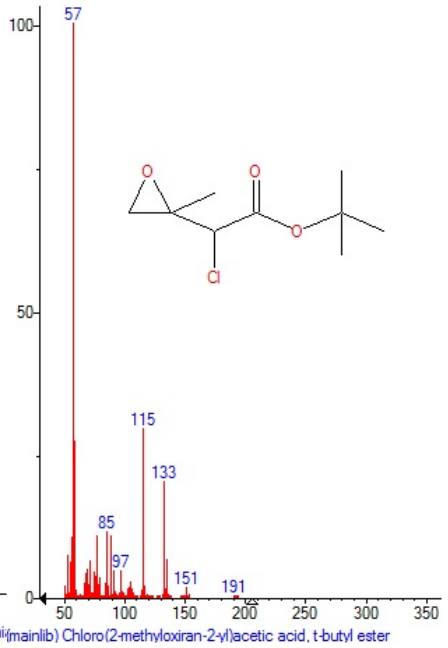


Fig. 4

Figs. 1-4. GC-MS chromatogram of the methanolic extract of *Juncus rigidus* leaves and stem.

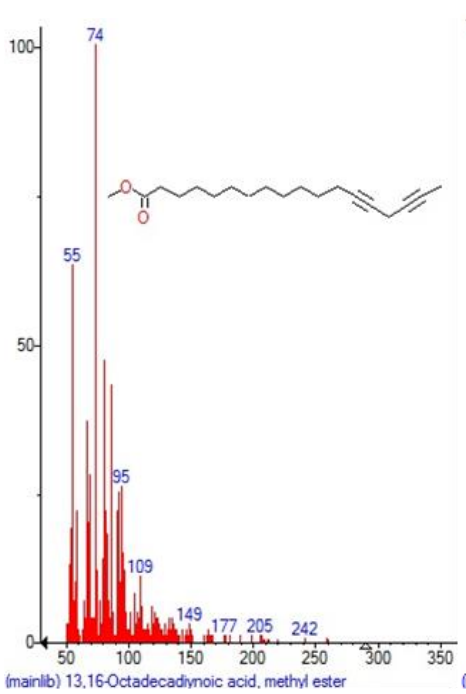


Fig. 5

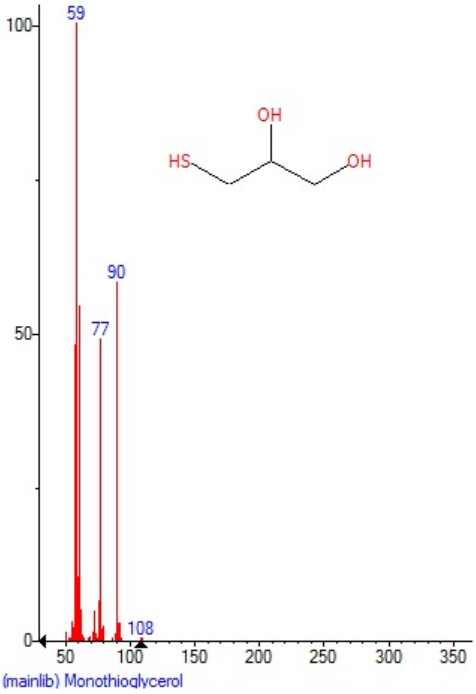


Fig. 6

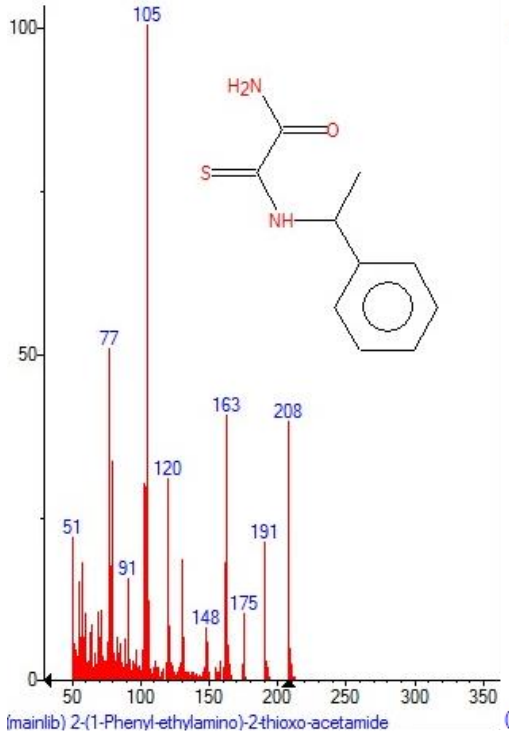


Fig. 7

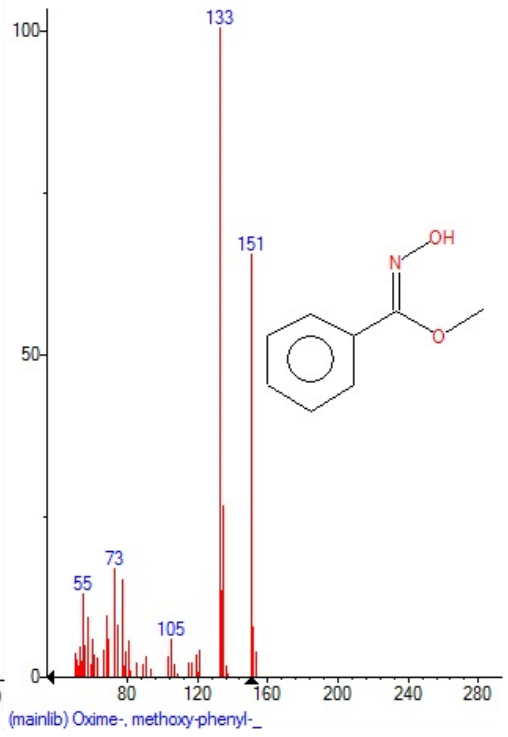


Fig. 8

Figs. 5-8. GC-MS chromatogram of the methanolic extract of *Juncus rigidus* leaves and stem.

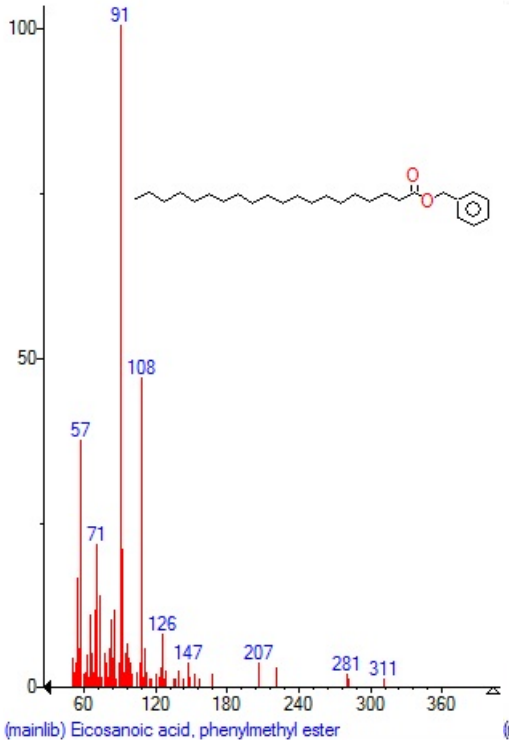


Fig. 9

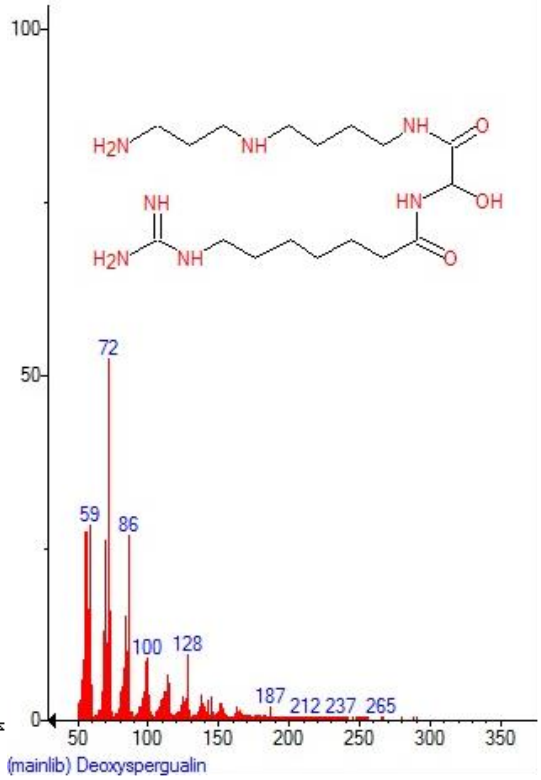


Fig. 10

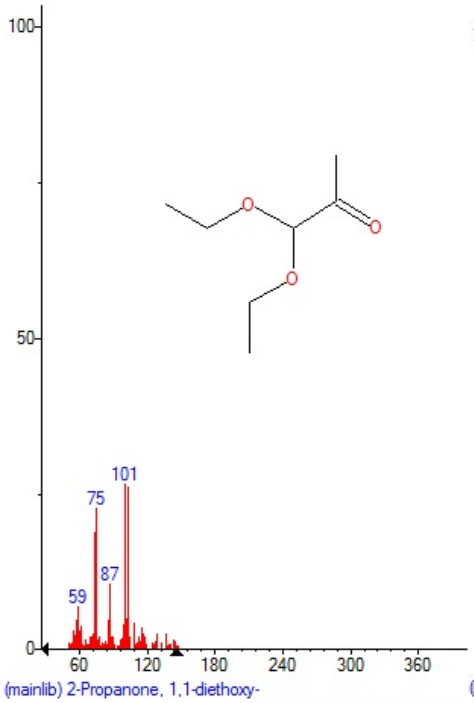


Fig. 11

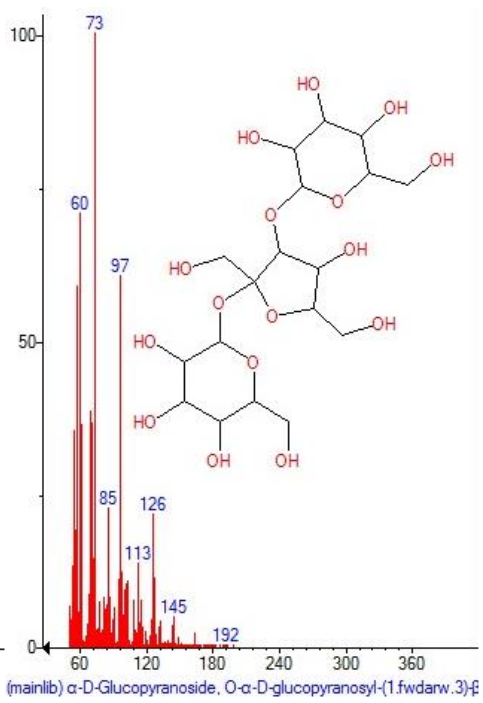


Fig. 12

Figs. 9-12. GC-MS chromatogram of the methanolic extract of *Juncus rigidus* leaves and stem.

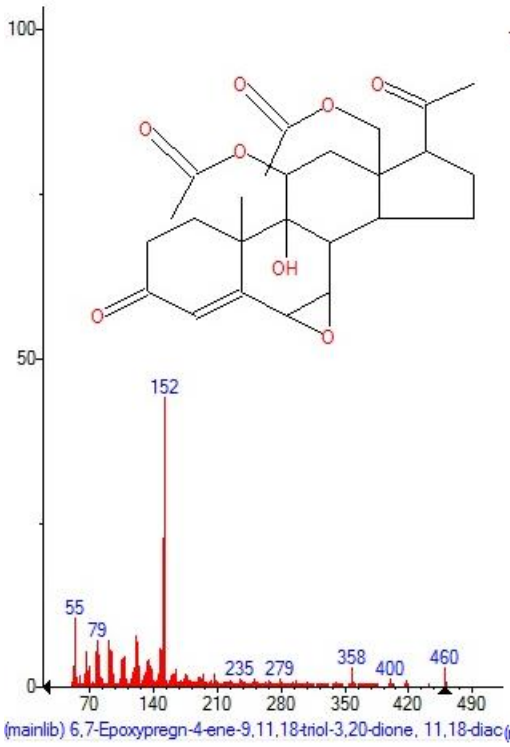


Fig. 13

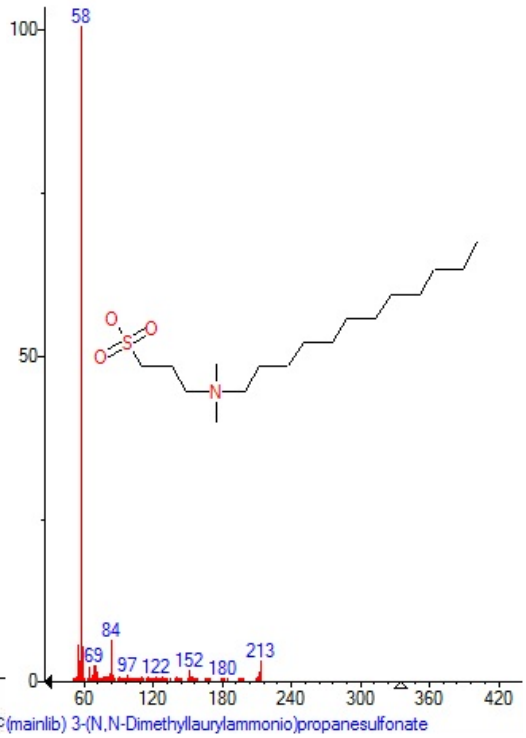


Fig. 14

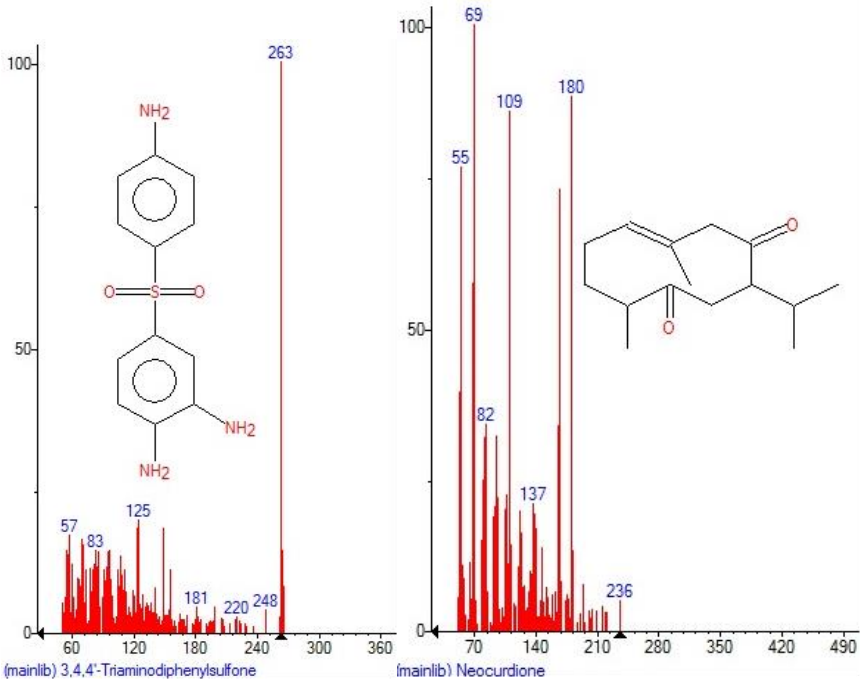


Fig. 15 Fig. 16

Figs. 13-16. GC-MS chromatogram of the methanolic extract of *Juncus rigidus* leaves and stem.

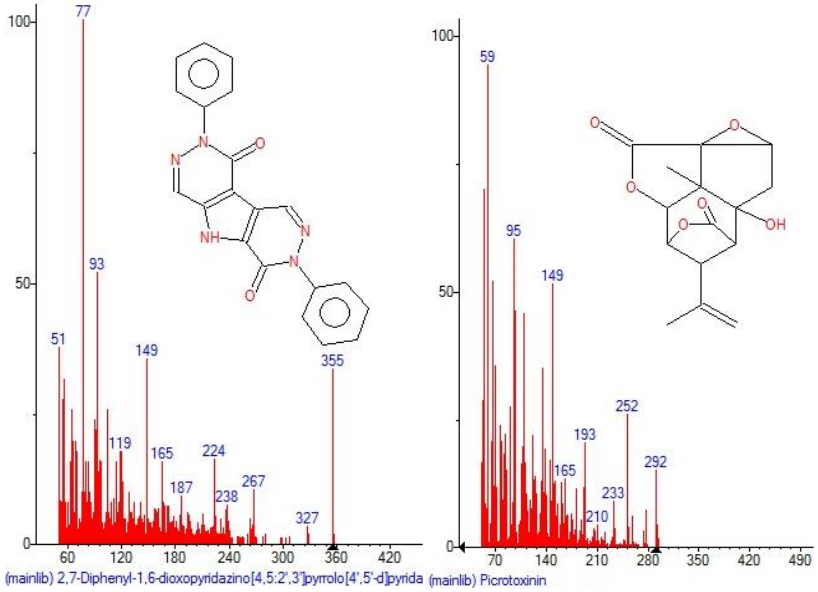


Fig. 17 Fig. 18

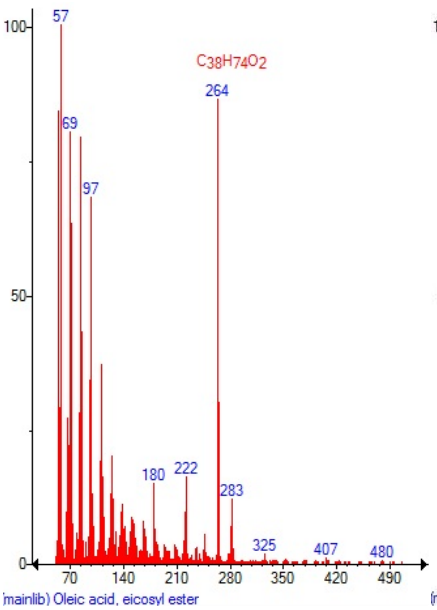


Fig. 19

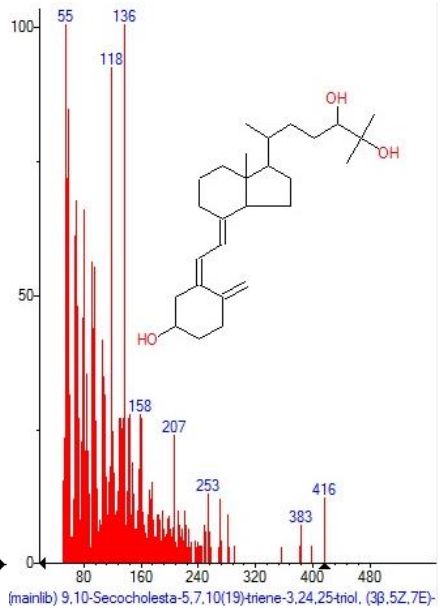


Fig. 20

Figs. 17-20. GC-MS chromatogram of the methanolic extract of *Juncus rigidus* leaves and stem.

REFERENCES

El-Shamy, AI, Abdel-Razek, AF & Nassar, MI 2012, Phytochemical review of *Juncus* L. genus (Fam. Juncaceae). *Arabian Journal of Chemistry*, pp: 1-11. https://doi.org/10.1016/j.arabjc.2012.07.007.

Al-Shurait, EA & Al-Ali, RM 2022, Optimal conditions for anthocyanins extracting from some food wastes. *Caspian Journal of Environmental Sciences*, 20: 503-512.

Abu Serag, NA, Al Gara,awi, NI & Ali, AM 2019, Analysis of bioactive phytochemical compound of (*Cyperus aucheri* Jaub.) By using gas chromatography-mass spectrometry. IOP Conference Series: Earth and Environmental Science, 388: 012063.

Adem, RS, Ayangbenro, AS & Gopane, RO 2020, Phytochemical screening and antimicrobial activity of *Olea europaea* subsp. *africana* against pathogenic microorganisms, *Scientific African*, 10: e00548.

AlAmery, SF & AlGaraawi NI 2020, Phytochemical profile and antifungal activity of stems and leaves methanol extract from the *Juncus maritimus* linn. Juncaceae family against some dermatophytes fungi. AIP Conference Proceedings 2290, 020034-1–020034-17.

Allaith, SA, Alfekaik, DF &Alssirag, MA 2019, Identification of *Pistacia vera* and *Prunus amygdalus* Batsch seed oils using GC-MS as useful methodology for chemical classification., IOP Conference Series: Earth and Environmental Science 388: 012061.

Champion, R, Burton, J, Burns, D & Breathnach, S 1998, Text book of dermatology. 6th ed. Blackwell Science Ltd, P. 1277-1376.

El Kady, IA, Mohamed, SS & Mostafa, EM 1993, Antibacterial and antidermatophyte activities of some essential oils from spices. *Qatar University Scientific Journal*, 13: 63-69.

Gahukar RT 2012, Evaluation of plant-derived products against pests and diseases of medicinal plants: A review. *Crop Protection*, 42: 202-20

El Khateeb, AY, Elsherbiny, AE, Tadros, LK, Ali, SM & Hamed, HB 2013, Phytochemical analysis and antifungal activity of fruit leaves extracts on the mycelial growth of fungal plant pathogens. *Journal of Plant Pathology & Microbiology*, 4: 1000199

Hamza, SM, Al Saadi, SA, Al Abbawy, DH 2020, A study of physical and anatomical characteristics of the heavy metal accumulation of *Juncus rigidus*. *Bulletin of the Iraq Natural History Museum*, 16: 63-81.

Kúsz, N, Stefkó, D, Barta, A, Kincses, D, Szemerédi, N, Spengler, G, Hohmann, J & Vasas, A 2021, Juncaceae Species as Promising Sources of Phenanthrenes: Antiproliferative Compounds from *Juncus maritimus* Lam. *Molecules*, 26: 999.

Lee, CL, Chang, FR, Yen, MH, Yu, D, Liu, YN, Bastow, KF, Morris Natschke, SL, Wu, YC & Lee KH 2019, Cytotoxic phenanthrenequinones and 9,10-dihydrophenanthrenes from *Calanthe arisanensis*. *Journal of Natural Products*, 72: 210–213.

Lin, Y, Wang, F, Yang, LJ, Chun, Z, Bao, JK & Zhang, GL 2013, Anti-inflammatory phenanthrene derivatives from stems of *Dendrobium denneanum*. *Phytochemistry*, 95: 242-251.

Ma, W, Zhang, Y, Ding, YY, Liu, F & Li, N 2016, Cytotoxic and anti-inflammatory activities of phenanthrenes from the medullae of *Juncus effusus* L. *Archives of Pharmacology Research*, 39: 154-160.

Naser AL-Isawi, HI 2022, Effects of applying cold and hot aqueous extracts of ginger to control onion rot disease caused by *Aspergillus niger*. *Caspian Journal of Environmental Sciences*, 20: 611-616.

Porusia, M & Septiyana, D 2021, Larvicidal activity of *Melaleuca leucadendra* leaves extract against *Aedes aegypti*. *Caspian Journal of Environmental Sciences*, 19: 277-285.

Saeed, A, Haider, A, Saqib, S, Khan, SA & Faryald K 2019, In-vitro antifungal efficacy of tissue conditioner-chitosan composites as potential treatment therapy for denture stomatitis. *International Journal of Biological Macromolecules*, 125: 761-766.

Sahli, R, Rivière, C, Siah, A, Smaoui, A, Samaillie, J, Hennebelle, T, Roumy, S, Ksouri, R, Halama, R & Sahpaz, S 2018, Biocontrol activity of effusol from the extremophile plant, *Juncus maritimus*, against the wheat pathogen *Zymoseptoria tritici*. *Environmental Science and Pollution Research*, 25: 29775–29783.

Salih, AH Salih, RY & Ahmed, H 2022, Bioactivity of *Cymbopogon Citratus* aqueous extract against measles virus and some bacterial isolates. *Caspian Journal of Environmental Sciences*, 20: 585-592.

Shuang, sh, LvYu, Fu, Jun, Ch, Yuan, J & Sui qing, Ch 2020, Six phenanthrenes from the roots of Cymbidium faberi Rolfe. and their biological activities, *International Journal of Molecular Sciences*, 20: 4422.

Szepietowski, JC & Schwart, RA 2005, Tineabarbae. Umdnj-New Jersey Medical School.

Thuerig, B, Ramseyer, J, Hamburger, M, Oberhänsli, T, Potterat, O, Jakob Schärer, H & Tamm, M 2016, Efficacy of a *Juncus effusus* extract on grapevine and apple plants against *Plasmopara viticola* and *Venturia inaequalis* and identification of the major active constituent. *Pest Management Science*, 72: 1718-1726.

Townsend, CC & Guest, E 1985, Flora of Iraq, Monocotyledones, excluding Gramineae. Vol. 8, Ministry of Agriculture & Agrarian Reform Republic of Iraq, pp. 310-355

Wahid, AZ & Jafar, FN 2005, Test of life effectiveness of *Carthamus tinctorius* extract toward germ and fungi. *Al-Basrah Research Journal*, 31: 39-47.

Yoshihito, N, Yumoto, H, Kumar, K, Matsuo, T, Hirota, K, Kan Nagao, YM, Tomotake, Y, Jimbo, R & Ichikawa, T 2018, Antifungal and mechanical properties of tissue conditioner containing plant-derived component: an in vitro study: tissue conditioner with plant-derived component. *Journal of Prosthodontics*, 27: 665-669.

Zhang, Y, Zhang, Q, Xin, W, Liu, N & Zhang, H 2019, Nudol, a phenanthrene derivative from *Dendrobium nobile*, induces cell cycle arrest and apoptosis and inhibits migration in osteosarcoma cells. *Drug Design, Development and Therapy*, 13: 2591-2601.

Bibliographic information of this paper for citing:

AlGaraawi, N, I, AlAmery, S, F, Alwahed Alsirrag, M, A 2022 First record of phytochemical and the antifungal activity of methanol extracts from vegetative parts of *Juncus rigidus* plants in Iraq. Caspian Journal of Environmental Sciences, 20: 1011-1021.