HSPI The Open Access Publisher

J P S P JOURNAL OF PLANT SCIENCE AND PHYTOPATHOLOGY
ISSN 2575-0135

Research Article

Phytochemical Compounds and the Antifungal Activity of *Centaurium pulchellum* Ethanol Extracts in Iraq

Noor Jawad Khadhum, Neepal Imtair Al-Garaawi* and Antethar Jabbar Al-Edani

Department of Biology, College of Education for Pure Science, Kerbala University, Kerbala, Iraq

Abstract

The current study included a variety of phytochemical substances that were extracted from *Centaurium pulchellum* and showed a wide range of medicinal properties from the plant's reproductive and vegetative parts against the pathogenic fungus Aspergillus flavus. The vegetative and reproductive components of *Centaurium pulchellum* were subjected to (GC-MS) analysis for phytochemical study. The data indicated that fungal activity was the highest. Four extract concentrations of 5, 10, 15, and 20 mg/ml were utilized in the investigation, and the diameter of the colonies measured at each concentration was 90.00, 36.00, 28.00, 18.00, and 0.00 mm, respectively.

Nine bioactive phytochemical compounds were found in *Centaurium pulchellum*'s vegetative and reproductive portions, according to GC-MS analysis of the chemicals. Another study reported phytochemical substances that: 1-H-Imidazole-2-carboxaldehyde, 1-methyl-;Acetaminophen; n-Hexadecanoic acid; Mercaptoacetic acid, 2TMS derivative; 1.2,3-Dimethyl-5-(trifluoromethyl)-1,4-benzenediol #; Mercaptoethanol, 2TMS derivative-; Bis-(3,5,5-trimethylhexyl) phthalate Tetrakis(trimethylsilyl) orthosilicate #;- 1.1-Isopropoxy-3,3,3-trimethyl-1-[(trimethylsilyl)oxy] disiloxanyl tris(trimethylsilyl) orthosilicate #.

More Information

***Address for correspondence:**
Neepal Imtair Al-Garaawi, Department of Biology, College of Education for Pure Science, Kerbala University, Kerbala, Iraq,
Email: neepal.i@uokerbala.edu.iq

Submitted: June 12, 2024
Approved: June 24, 2024
Published: June 25, 2024

How to cite this article: Khadhum NJ, Al-Garaawi NI, Al-Edani AJ. Phytochemical Compounds and the Antifungal Activity of *Centaurium pulchellum* Ethanol Extracts in Iraq. J Plant Sci Phytopathol. 2024; 8: 079-083.

DOI: 10.29328/journal.jpsp.1001137

Keywords: *Pulchellum centaurium*; Phytochemical; Gas chromatography-mass spectrometry; Antifungal

Introduction

Centaurium pulchellum is a small flowering plant belonging to the genus *Centaurium*, which belongs to the Gentianaceae family, It is commonly known as the lesser centaury and is called (a dandelion weed) [1]. The plant under consideration is a perennial species known for its highly acerbic flavor, erect glabrous, stem 30 cm - 50 cm in height, 4-angled, leaves 2-4 pairs below the lowermost inflorescence branch, cyme branch spreading at a wide angle, calyx lobes 5, linear-subulate, 5 mm, corolla tube 10mm long, limb 5 rarely 4, lobes narrowly ovate-oblong, 4.5 mm, stamens inserted in throat, stigma distinctly 2- branched, capsule about as long as calyx [2,3]. It thrives in damp soil environments, rocky mountain clefts, and in proximity to pristine water sources, originating from the Mediterranean territories, it is indigenous to a vast expanse extending across most of Eurasia to the northerly regions, encompassing Scandinavia, and reaching as far as the Himalayas and China) [4-6].

This plant is a rich source of many active compounds that can be used in many fields, isolated as the secoiridoids and the xanthones found in the roots and stem of *C. pulchellum* [7-9]. Also, the plant extract acts as an antibacterial agent on brine shrimp, due to the two isocoumarin derivatives, one (erythricin) and the other (erythrocentaurin) [9]. Šiler, et al. [10,11] studied the main secondary metabolites: secoiridoid glycosides, monoterpenoid, and phenolics, and further investigated for antioxidant and antimicrobial(antibacterial and antifungal) activity for five centaury species one of them were *C. pulchellum*. The extract of centaur flowers contains (82 compounds) and six distinct groups have been identified: glycosides, iridoid glycosides, phenolic acid, aglycones, and their derivatives [12]. In the roots, the chemical constituent Ethyl N-docosanoylanthranilate, demonstrates efficacy in combating *Aspergillus flavus* fungal strains [13].

Aspergillus flavus represents a fungal species within the *Aspergillus* section *Flavi* and has served as a paradigmatic system for elucidating fungal development and toxin synthesis. The impact of *A. flavus* on human health is multifaceted, encompassing the generation of highly carcinogenic mycotoxins known as aflatoxins and the induction of aspergillosis in immunocompromised individuals. Furthermore, the infection of crops by *A. flavus* leads to economic repercussions due to diminished yields and *aflatoxin*

contamination. *A. flavus*, classified as a saprophytic fungus, propagates in the environment predominantly through the production of asexual spores (conidia), which confer extended viability in adverse environmental conditions. Conidia exhibit a structural composition comprising the rodlet layer, cell wall, and melanin, originating from a specialized asexual structure termed the conidiophore [14].

Materials and methods

Research subject and sample

"The fungi under study were identified in the agricultural college laboratory at Kerbala University. They were isolated from plants infected by Aspergillus in Kerbala fields."

Microscopic assessment

Samples were analyzed by the following procedure: The region was sterilized in 70% alcohol to obtain rid of saprophytes fungi and microbes, then a sample from the affected plant and put on a clean slide with a drop of 0% KOH and then covered was put on and heated on a flame then observed by a microscope looked after spores of Aspergillus flavus or hypha. The observed distinguished as in: [15-17].

Plant extract extraction

The dried aerial parts were pulverized and extracted in a Soxhlet. The polar compounds were extracted with 1.5 liters of methanol, then filtered and evaporated. After that, the compounds were weighed, and their yield percentage, expressed as a percentage of their dry weight, was calculated. The yield of the dark green extract from methanol was 57.5 grams (0.095%), the plant extracts were placed in the dark and refrigerated at a temperature of 4 °C. Wahid & Jafar method [18].

Alcoholic extract from *C. pulchellum* plants is cultivated and used to study the growth of pathogenic fungi

Steps from El-Kady, et al. [19] were applied viz., "four concentrations (5, 10, 15, and 20 mg/ml) of the methanol extracted extracts of *C. pulchellum* were combined with (PDA) grown media (three tests for each concentration)." then hardening a medium and creating a hole in the middle of those dish with a cork borer that was pierced 5 mm. Placing a disk in the center of dishes that had a diameter of 5 mm, these dishes put fungal inoculum and allowed to grow for ten days. The plates were placed in the incubator at 25 °C, and after ten days, the developing colony's diameter was recorded. Results were noted, so the ratio of inhibition was measured. Then compute the inhibition ratio by [20]:

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

Obtaining and preparing plant

The *Centaurium pulchellum* plant was collected from several parts of Iraq (Zakho, the mountain zones of Iraq, Mosul a hills zone, Baghdad, Karbala in the middle of Iraq a lowlands zone, Basra in the south of Iraq) in the period April-May 2023. Following that, the plants were cleaned and allowed to air dry. After being powdered, 40 g of plants were soaked in 200 ml of ethanol and filtered.

Participants extract identification using mass spectrometry and gas chromatography (GC/MS)

GC-MS analysis was used to identify *C. pulchellum* phytochemically in 'a (Scion Instruments Company. Model: SCiON 436) GC-MS Column: SCION-5MS {ID = 0.25 mm, length = 15 m, df = 0.25 µm} Carrier gas: Helium device controlled by a computer at 60 eV. Using a tiny syringe, approximately 1 µL of the ethanol extract was put into the GC-MS column, and the scanning process took 45 minutes. Column oven: initial temperature is 50 °C increase by 10 °C / min to 230 °C, Sample Preparation: 100 µL of the sample is diluted with 5 ml of N-Hexane (HPLC-Grade) before injection. Gas flow ratio: 1 ml/min Pressure: 10 psi m/z Range: 1 – 2000, split ratio 1:50. *(This method is innovative in Al-Zahraa Center for Medical and Pharmaceutical Research Sciences (ZCMPRS))*[21].

Statistical analyses

Performed using STATGRAPHICS Centurion XV software, version 15.1.02 (StatPoint, Inc. 1982-2006, USA). The data analyzed of variance (ANOVA), and comparisons between the mean values of each of the concentrations investigated were made by the Least Significant Difference (LSD) test calculated at the 95% confidence level.

Results and discussion

Antifungal activity

Aspergillus flavus was chosen for the current investigation to evaluate the ethanol extract's efficacy of *C. pulchellum* parts, the extract showed a high antifungal activity against *Aspergillus flavus.* Used 4 extract concentrations (5,10,15,20 mg/ml) showed results (36.00, 28.00, 18.00, 0.00 mm) diameter of colonies in *Aspergillus flavus* the results showed in Table 1. These results are in agreement with Šiler, et al. [10,11] and Todorovic, et al. 2022 [12] when they studied the activity of *C. pulchellum* against the *Aspergillus flavus* .while Janković, et al. Miana & Al-Hazimi [22,23] obtained some secondary metabolites that act as Antifungal and They discovered that fungi are inhibited by the ethanolic extract of *C. pulchellum* parts.

Evaluation of the biochemical substances of *centaurium pulchellum*

Nine components are present in the GC-MS ethanolic extract of the vegetative and reproductive portions of *C. pulchellum*, as shown in Table 2. These substances have a variety of biological actions; a high level of phenols the examination of the acetaminophen compound revealed that 82% of the compounds were analgesic, antibiotic, antipyretic, and anti-inflammatory, the resulting in agreement with Šiler, et al. [10,11] study they mentioned that the most of compounds belong to phenols than the Organosilanes compounds which have antifungal activity (Table 2) (Figures 1-10) lists the remaining chemicals' antifungal and antioxidant properties.

Table 1: Ethanol extracts from *C. pulchellum* possess antifungal effects.

Fungal type	Mean of Inhibition zone (mm)					
	First, compare using distilled water (0.00 mg /ml)	Comparison 2 with Clotrimazole (2 mg/ml)	Concentration (5 mg/ml)	Concentration (10 mg/ml)	Concentration (15 mg/ml)	Concentration (20 mg/ml)
Aspergillus flavus	90	0	0.36	0.28	0.18	0

Table 2: Major phytochemical composites in ethanolic extract of *Centaurium pulchellum.*

Peak number	Retention time (min)	Area	%Total	M.wt	Chemical type	Name	biological activity
1	9.218	753171	0.310	110	aromatic heterocycle	1H-Imidazole-2-carboxaldehyde, 1-methyl-	Antifungal, Antibacterial activity [24]
2	14.436	2.011e+8	82.667	151	phenols	Acetaminophen	Antifungal antimicrobial analgesic, antibiotic, antipyretic, anti-inflammatory, antioxidant, antispasmodic properties, antitoxic, cytotoxic, Šiler, et al. [11]
3	17.125	2.945e+6	1.210	256	Fatty acid	n-Hexadecanoic acid	anti-oxidant, nematicide, hypocholesterolemic, pesticidal, hemolytic, 5-Alpha reductase inhibitor, antiadrogentic [25]
4	18.236	7.455e+6	3.064	236	Carboxylic acid	Mercaptoacetic acid, 2TMS derivative	Antifungal, Antibacterial, Antiinflammatory, [26]
5	18.929	2.745e+6	1.128	206	phenols	1.2,3-Dimethyl-5-(trifluoromethyl)-1,4-benzenediol #	Antifungal, Šiler, et al. [11]
6	20.024	2.834e+6	1.165	222	Carboxylic acid	Mercaptoethanol, 2TMS derivative-	Antifungal, Antibacterial, [26]
7	20.556	3.498e+6	1.437	418	aromatic dicarboxylic acid	Bis-(3,5,5-trimethylhexyl) phthalate	Antifungal, Antibacterial, Antiinflammatory, antipromastigotes activity and antiamastigotes [26-28]
8	21.716	1.017e+7	4.180	384	Organosilanes	Tetrakis(trimethylsilyl) orthosilicate #	Anti-microbial [24]
9	22.086	7.277e+6	6.20	576	Organosilanes	1.1-Isopropoxy-3,3,3-trimethyl-1	Anti-microbial [24]

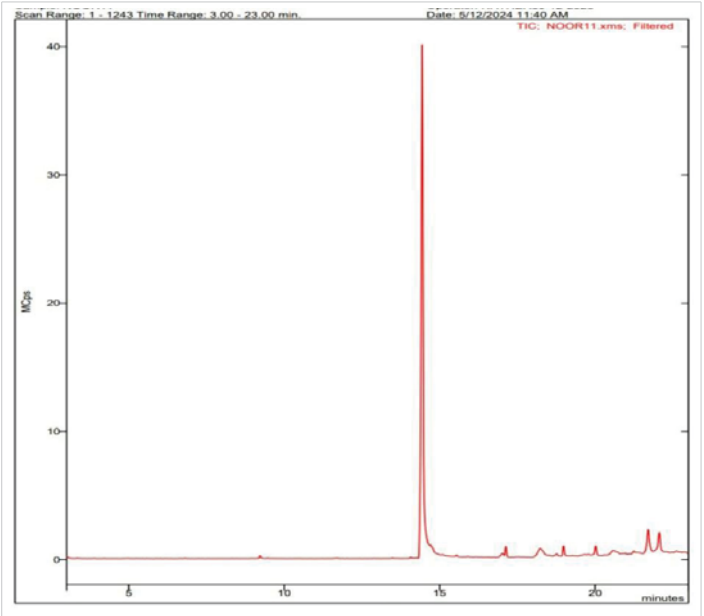

Figure 1: GC-MS chromatogram of methanolic extract of *C. pulchellum.*

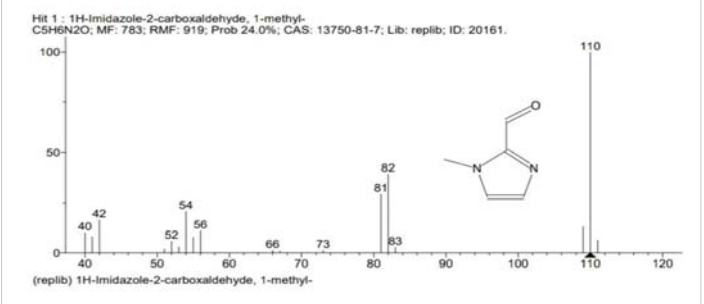

Figure 2

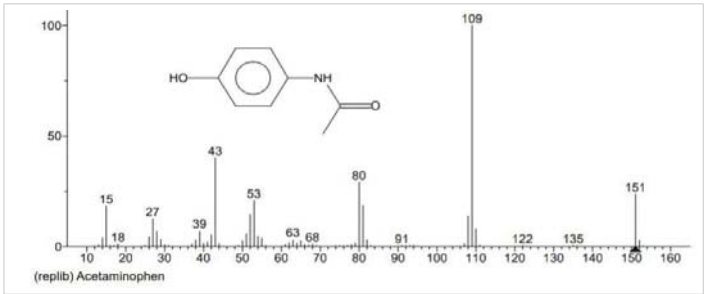

Figure 3

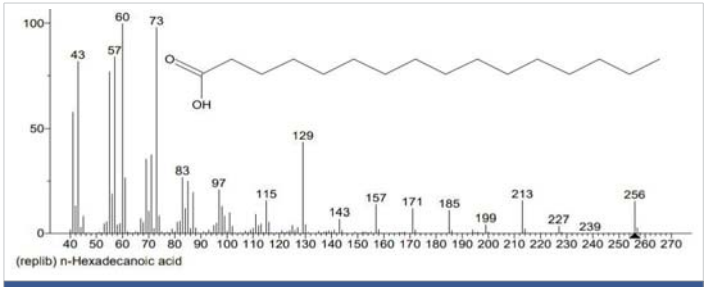

Figure 4

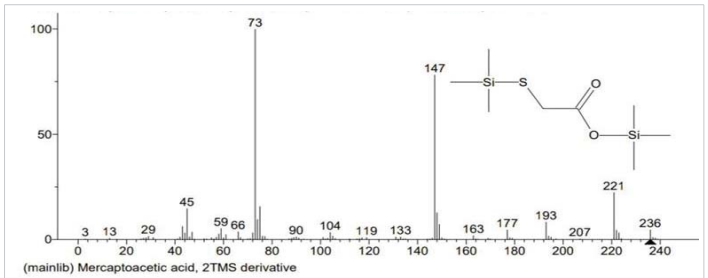

Figure 5

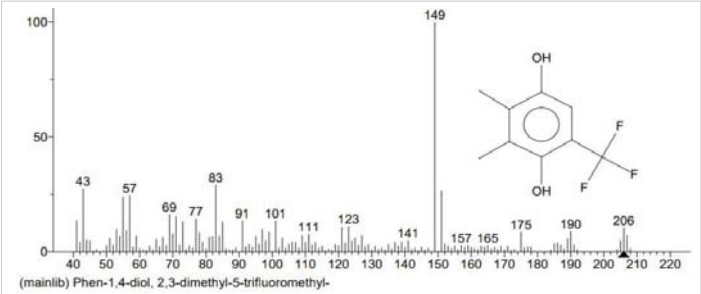

Figure 6

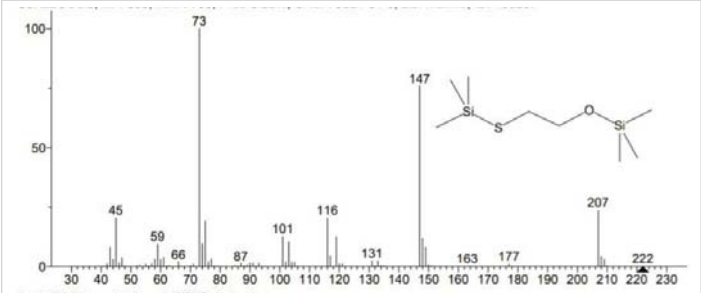

Figure 7

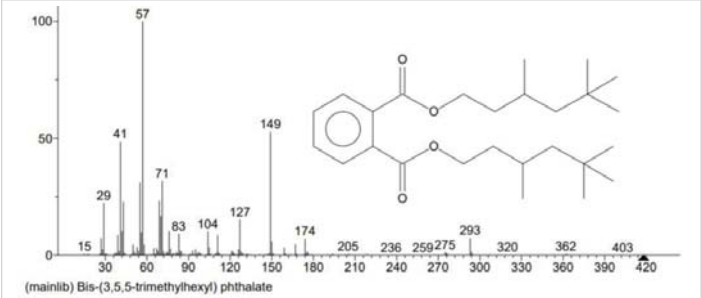

Figure 8

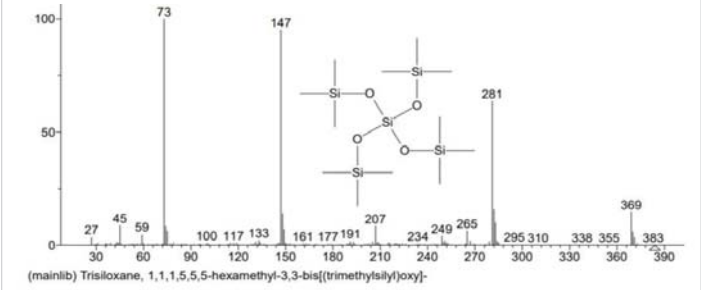

Figure 9

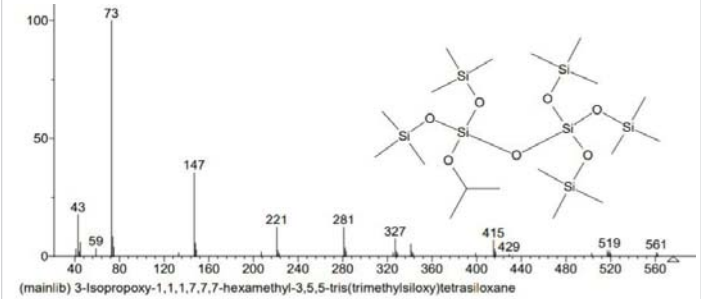

Figure 10

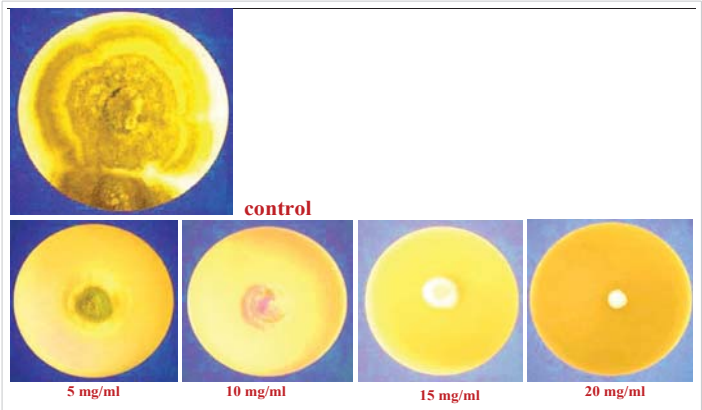

Plate 1: *Aspergillus flavus* growth cultivated in different concentrations of methanol extract of *C. pulchellum*.

Conclusion

In the current study, nine compounds from aerial parts of *Centaurium pulchellum* the plant collected from different places in Iraq, were identified by (GC-MS) analysis. this plant shows bioactive compounds useful for many herbal formulations especially antifungal activity against *Aspergillus flavus* because of the high levels of phenols and Organosilanes so it is important as a plant phytopharmaceutical. However, some studies are needed to undertake its chemotaxonomy properties, bioactivity, and profile of toxicity.

References

1. Ressources U. internationale pour la conservation de la nature et de ses. A guide to medicinal plants in North Africa. IUCN; 2005.
2. Ghazanfar S, Edmondson JR. Flora of Iraq.Lythyraceae to Camanulaceae Ministry of Agriculture Republic of Iraq.vol.5.part 2.p28. 2013.
3. Al-Allaq SA, AL-Mossawei AH, Alzhra LA. Species of Centaurium Hill. In Iraq and its geographical distribution. Baghdad Science Journal. 2013; 10(1):22-31.
4. Mifsud S. *Centaurium pulchellum* (Lesser Centaury): MaltaWildPlants. Com-the online Flora of the Maltese Islands. 2002.
5. Saeed JF. Morphological Study of the Centaurium pulchelum and C. erythraea (Gentianaceae) in Kurdistan region of Iraq. Education. 2013; 7(1):01-06.
6. Ubsdell RAE. Studies on variation and evolution in Centaurium erythraea Rafn and C. littorale (D. Turner) Gilmour in the British Isles. 3. Breeding systems, floral biology, and general discussion. Watsonia. 1979; 12:225-232.
7. Krstić D, Janković T, Šavikin-Fodulović K, Menković N, Grubišić D. Secoiridoids and xanthones in the shoots and roots of *Centaurium pulchellum* cultured in vitro. In Vitro Cellular & Developmental Biology-Plant. 2003;3 9:203-207.
8. van der Sluis WG. Chemotaxonomical investigations of the genera Blackstonia and Centaurium (Gentianaceae). Plant Systematics and Evolution. 1985; 149:253-286.
9. Bibi H, Ali I, Sadozai SK, Atta-Ur-Rahman. Phytochemical studies and antibacterial activity of *Centaurium pulchellum* Druce. Nat Prod Res. 2006 Aug ;20(10):896-901. doi: 10.1080/14786410500162047. PMID: 16854716.
10. Siler B, Misić D, Nestorović J, Banjanac T, Glamoclija J, Soković M, Cirić A. Antibacterial and antifungal screening of *Centaurium pulchellum* crude extracts and main secoiridoid compounds. Nat Prod Commun. 2010 Oct;5(10):1525-30. PMID: 21121240.

11. Siler B, Zivković S, Banjanac T, Cvetković J, Nestorović Živković J, Cirić A, Soković M, Mišić D. Centauries as underestimated food additives: antioxidant and antimicrobial potential. Food Chem. 2014 Mar 15;147:367-76. doi: 10.1016/j.foodchem.2013.10.007. Epub 2013 Oct 11. PMID: 24206732.

12. Todorović M, Milutinović M, Božunović J, Aničić N, Petrović L, Skorić M, Mišić D. Comparative metabolomics of two Centaurium species displaying variable flower coloration phenotypes. In 4th International Conference on Plant Biology [and] 23rd SPPS Meeting; 2022 Oct 6-8; Belgrade, Serbia. Belgrade: Institute for Biological Research" Siniša Stanković"–National Institute of Republic of Serbia, University of Belgrade. 2022; 145.

13. Tan RX, Kong LD, Wei HX. Secoiridoid glycosides and an antifungal anthranilate derivative from Gentiana tibetica. Phytochemistry. 1998 Apr;47(7):1223-6. doi: 10.1016/s0031-9422(97)00698-5. PMID: 9611826.

14. Cho HJ, Son SH, Chen W, Son YE, Lee I, Yu JH, Park HS. Regulation of Conidiogenesis in Aspergillus flavus. Cells. 2022 Sep 7;11(18):2796. doi: 10.3390/cells11182796. PMID: 36139369; PMCID: PMC9497164.

15. Szepietowski JC, Schwart RA. Tineabarbae. Umdnj-New Jersey medical school. 2005.

16. Ayman Y El-Khateeb, Elsherbiny A Elsherbiny , Louis K Tadros , Safaa M Ali and Hassan B Hamed.(2013) . Phytochemical Analysis and Antifungal Activity of Fruit Leaves Extracts on the Mycelial Growth of Fungal Plant Pathogens. J Plant Pathol Microb. Vol 4 , Isse 9 • 1000199

17. Champion R, Burton J, Burns D, Breathnach S. Textbook of dermatology. 6th ed. Oxford: Blackwell Science Ltd; 1998. p. 1277-1376.

18. Wahid AZ, Jafar FN. Test of life effectiveness of Carthamus tinctorius extract toward germ and fungi. AlBasrah Res J. 2005;31(3B):39-47.

19. El-Kady IA, Mohamed SS, Mostafa EM. Antibacterial and anti-dermatophyte activities of some essential oils from spices. Qatar Univ Sci J. 1993;13(1):63-69.

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

21. Al-Zahraa Center for Medical and Pharmaceutical Research Sciences (ZCMPRS) Email: zcmprs@alzahraa.edu.iq.

22. Janković T, Krstić D, Savikin-Fodulović K, Menković N, Grubisić D. Xanthones and secoiridoids from hairy root cultures of Centaurium erythraea and C. pulchellum. Planta Med. 2002 Oct;68(10):944-6. doi: 10.1055/s-2002-34927. PMID: 12391565.

23. Miana GA, Al-Hazimi HMG. Xanthones of *Centaurium pulchellum*. Phytochemistry. **1984; 23:**1637-1638.

24. Militello M, Settanni L, Aleo A, Mammina C, Moschetti G, Giammanco GM, Blàzquez MA, Carrubba A. Chemical composition and antibacterial potential of Artemisia arborescens L. essential oil. Curr Microbiol. 2011 Apr;62(4):1274-81. doi: 10.1007/s00284-010-9855-3. Epub 2010 Dec 25. PMID: 21188586.

25. Kumaradevan G, Damodaran R, Mani P, Dineshkumar G, Jayaseelan T. Phytochemical screening and GC-MS analysis of bioactive components of ethanol leaves extract of Clerodendrum phlomidis (L). Am J Biol Pharm Res. 2015;2:142-148.

26. Li M, Zhou L, Yang D, Li T, Li W. Biochemical composition and antioxidant capacity of extracts from Podophyllum hexandrum rhizome. BMC Complement Altern Med. 2012 Dec 22;12:263. doi: 10.1186/1472-6882-12-263. PMID: 23259745; PMCID: PMC3542176.

27. AlAmery SF, AlGaraawi NI. Phytochemical profile and antifungal activity of stems and leaves methanol extract from the Juncus maritimus Linn. Juncaceae family against some dermatophytes fungi. AIP Conf Proc. 2020;2290:020034-1–020034-17.

28. Al-Garaawi NI, et al. Analysis of bioactive phytochemical compound of Cyperus alternifolius L. by using gas chromatography-mass spectrometry. IOP Conf Ser Mater Sci Eng. 2019;571(1):1-19.