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In Vitro Effect of Laser Beam on Antifungal Activity of Crude Petroleum Ether Extract Produced From Leaves of *Populus Spp.*

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ANTIMICROBIAL substances have been found in plants because medicinal plants are a rich supply of medications, traditional medicines, and ingredients for drug manufacture. This study aimed to examine how laser irradiation affected the crude petroleum ether extract made from populus spp. leaves. In this study, crude petroleum extract exposed to laser light was tested in vitro for its ability to inhibit the growth of several fungal species, including *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger, Penicillium brevicompactum*, and *Penicillium expansum*, which were isolated from isolate bank of the Veterinary-Microbiology Laboratory- Hospital in Wasit. Before and after being exposed to laser light, *Populus spp*. extracts had no antifungal effects on culture using well diffusion techniques. No statistically significant differences were found when comparing the biomass concentration of fungus exposed to light for 30 and 60 minutes with the control group. The GC-MS analysis revealed the existence of additional substances in the crude petroleum ether extracts, whether they had been subjected to laser light or not. Different substances, including Dimethyl methylphosphonate, Propanoic acid, 2-chloro-, 1-methy lbutyl ester 3-Chloropropionic acid, 3-chloropr op-2-enyl ester tetra-n-Propoxymethane, cis-9-Hexadecenoic acid cis-Vaccenic acid, Propyl tetradecyl carbonate - Carbonic acid, butyl decyl ester, Carbonic acid, heptadecyl propyl ester and Hexanedioic acid, bis(2-ethylhexyl) ester. The current study hypothesizes that short-term laser treatment of a crude petroleum ether extract made from Populus spp. leaves causes changes in the plant compounds that can be detected through GC-MS thanks to the radiation's increased energy consumption.

Keywords: *Populus spp*, Leaves, Petroleum ether extract, Antifungal activity, GC-MS analysis, Laser beam.

Introduction

The development of potent antimicrobial agents to treat and prevent infectious diseases has drawn scientific attention to two key areas: natural plant-based products, and synthetic chemistry [1]. Among other things, plants have played a special, integral role in the production of food, medicines, clothing, and shelter. Numerous studies have been conducted on natural substances to find new drugs [2]. For more than 5000 years, people have employed plants as a source of medications, antineoplastics, analgesics, and cardioprotective substances [3]. In recent years, especially in underdeveloped countries where between 70 and 90% of the population still uses conventional plant-based treatments, humans have been using natural antibiotics to prevent infections [4,5].

Drugs with large concentrations of natural products and their derivatives were approved by the Food and Drug Administration (FDA) [6,7]. The natural chemicals with antioxidant and antibacterial activities have been extracted from *Populus spp*. The leaf extract from Populus euphoretic exhibits antibacterial effects on some microbes [8]. Native Americans and people from Eurasia regularly treated ailments including arthritis and sexual disorders with *Populus tremuloides* [9]. Other researchers have noted the antibacterial and antioxidant effects of *Populus alba* extracts [10]. Additionally, it has been discovered that the

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aforementioned plant extract has the capacity to inhibit bacterial lysis, the energy production pathway, protein and nucleic acid synthesis, cytoplasmic membrane function, modification of membrane permeability, reduction of the ability to form biofilms, and bacterial resistance to particular conventional antibiotics [11]. Low-level laser therapy is a common resource that is employed nowadays as a noninvasive method. There have been numerous studies done to determine the real efficacy of low-level laser therapy in healing soft tissue injuries and the results have been encouraging [12]. Although low intensity laser therapy has been the subject of some research papers, especially those that focused on the visible and infrared light spectrums, little is known about the mechanisms of interaction with biological tissue and the photochemical and photothermal effects that this enchantment can also have on the tissues [12]. Despite several studies showing the bactericidal and/or bacteriostatic of laser effect, there still exists a conundrum. Studies have found that bacteria, especially Escherichia coli, grow more quickly when exposed to lasers; nevertheless, there are few studies demonstrating the fungicidal effects of lasers [13,14]. The discovery of laser medicine has enabled numerous unique therapeutic techniques that are efficient against hazardous pathogenic organisms [14]. Photo antimicrobial therapy has several benefits over conventional therapies, including safety, effectiveness, and convenience of use. It also has broad-spectrum activity against bacteria, fungus, viruses, and protozoa [15]. According to studies, using lasers has bactericidal and antibacterial effects [16]. Light may promote the growth of a wide variety of organisms, including fungi and the secondary metabolites they generate [17]. The objective of the current study is to assess and compare the efficacy of a semiconductor laser system in inhibiting the growth of several fungal species in vitro.

Material and Methods

This research was carried out at the University of Al-Hamdaniya Laser and Photonics Research Center. Numerous isolates that have already been collected from animals that were clinically infected are kept in the isolate bank of the Veterinary Microbiology Laboratory at the Wasit Veterinary Hospital. This study did not require ethical approval, as determined by the ethical approval committee.

-Plant sample preparation and extraction

This procedure was carried out according to the ref. [18]. *Populus spp*. growing in Thi-Qar Province, south of Iraq, was harvested for their healthy leaves (Figure 1). For the purpose of eliminating dust and debris, the collected leaves were washed twice. Tap water was used for the initial wash, and distilled water was applied for the subsequent wash. After drying out at room temperature, the leaves were ground into a fine powder. 350 g of the powder were submerged in 700 mL of petroleum ether for four days. After that, a layer of filter paper (0.5 g) was used to separate the powder from the petroleum ether. The powder was placed in a clean glass beaker (1000 ml), filled with 700 ml of aqueous methanol 80% (80% absolute methanol and 20% distilled water), and allowed to stand at room temperature until all of the petroleum had completely evaporated. The powder, whose methanol had completely evaporated, was soaked in petroleum ether before the next processes were carried out in the same order as with methanol and petroleum ether. The chloroform filtrate was then separated from the powder. Finally, a solid extract was produced by leaving the filter at 37 °C for 3 days.

Fig. 1. *Populus spp*. tree

Laser application

The semiconductor laser system was used to irradiate a quantity of the crude petroleum extract at 450 nm wavelength and 50 mW power after it had been dissolved in Dimethylsulfoxide (DMSO) to a solution containing 50 mg/ml. For one hour, a plastic tube containing the solution was exposed to vertical laser radiation at a distance of 10 cm (Figure 2).

Five isolates of fungus, including Aspergillus fumigatus, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium brevicompactum* and *Penicillium expansum*, were utilized to assess the inhibitory action of the laser exposed on the crude petroleum ether extracts of *Populus spp*. on the fungal growth. A semiconductor laser system (450 nm wavelength and 50 mW power laser) (China business) was used to study the effects of direct vertical exposure to intense light (at a distance of 10 cm) on the culture of fungus over the course of two irradiation durations (30 and 60 minutes). Each radioactive culture was subcultured for three days at 37°C on Sabouraud dextrose agar (Oxoid Company).

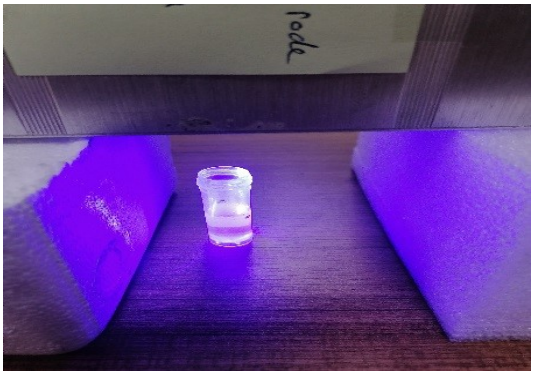

Fig. 2. Illustration of the use of laser radiation on the crude petroleum ether extract produced from leaves of *Populus spp.*

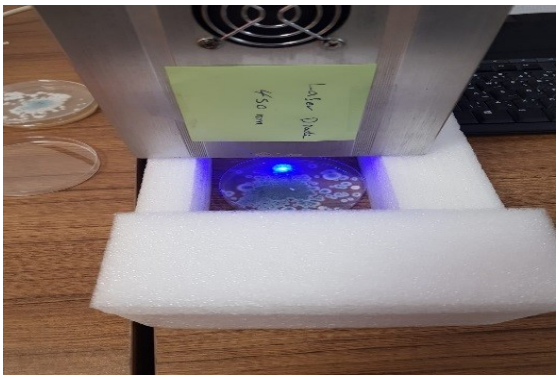

Fig. 3. Laser irradiated directly and vertically on culture of fungi by a semiconductor laser system

Antifungal activity test of the crude petroleum ether extract produced from leaves of populus spp.

Aspergillus fumigatus, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium brevicompactum*, and *Penicillium expansum* were used as test fungi in this experiment to compare the antifungal activity of populus leaf extracts exposed to and not exposed to laser light. Aseptically, portions of the crude petroleum extracts—either those not exposed to laser light or those exposed to laser light—were dissolved in DMSO to obtain concentrations of 50 mg/mL, and discs of the antifungal ketoconazole were used as a positive control. Additionally, Sabouraud dextrose agar (SDA) petri dishes were made, and each dish was inoculated with an active inoculum of the mold species *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Penicillium brevicompactum, and Penicillium expansum.* After a short while, three wells with a diameter of 6 mm were created in each dish, and 100 L of the aforementioned extract was added to each well. Dishes were incubated for three days at 37°C, after which the inhibitory zones were measured in millimeters with a ruler [19].

Dry weight of yeast

In this work, the concentration of fungus in suspension both before and after laser irradiation was measured. A single colony was chosen, placed into 40 mL of Sabouraued dextrose broth, and then incubated for two days at 37 °C. This was carried out three times to observe the possible growth in biomass activity. The culture was then centrifuged for 15 minutes at 2000 x g, and the supernatant fraction was decanted. After centrifuging the remaining material at 2000 x g for 15 minutes, it was transferred to a culture plate and allowed to dry

overnight at 35 C after being cleaned with ice-cold water. The dry yeast pellet on the culture plate was weighed and recorded as weigh 2 (W2), while the weight of the new culture plate was recorded as weigh 1 (W1). The yeast dry weight (DCW) was then estimated using the formula: W= W2-W1 [20, 21].

Gas chromatography–mass spectrometry Analysis

At the Ministry of Industry and Minerals Corporation for Research and Industrial Development, Iben Al-Betar Research Center, analysis of the crude petroleum ether extract made from *Populus spp*. leaves (non-exposed or exposed to the laser beam) was conducted. Gas chromatography was performed on the extracts using an Agelint 7820A GC Mass Spectrometer under the following conditions for the analytical column: GC inlet line temperature: 250 °C, aux heaters temperature: 300 °C, analytical column: Agelint HP-5ms ultra lneit (30 m length x 250 mm inner diameter x 0.25 mm film thickness), injection of 1 l, pressure: 11.933 psi He 99.99% gas, 250°C injector temperature, m/z 25–1000 scan range, splitless injection method, and the following oven program parameters: temperature: Ramp 1 60 C and hold for 3 minutes. Ramp 2: from 60°C to 180°C for 7°C/min; Ramp 3: from 180°C to 280°C for 8°C/min; and Ramp 4: from 280°C for 3 min.

Results

Antifungal Activity

The *Populus spp.* leaves used in this study's crude petroleum ether extract, whether they had been subjected to laser light or not, had none whatsoever.

The statistical study of fungus biomass concentrations under light irradiation settings (30 and 60 minutes) and the control group revealed no significant changes (Tabe 1 and Fig. 4).

TABLE 1. The impact of lasers on fungal biomass growth following exposure to laser irradiation lights for 30 and 60 minutes.

Type of fungi	Groups	Time (mean ±SE)		*LSD*
		30 minutes	60 minutes	
Aspergillus flavus	Culture without exposure to laser	0.15 ± 0^{Aa}	0.15 ± 0^{Aa}	0.050
	Culture exposure to laser	0.20 ± 0.01^{Aa}	0.50 ± 0.02^{Bb}	
Aspergillus fumigatus	Culture without exposure to laser	0.08 ± 0.003^{Aa}	0.08 ± 0^{Aa}	0.013
	Culture exposure to laser	0.30 ± 0.003^{Ba}	0.31 ± 0.006^{Ba}	
Aspergillus niger	Culture without exposure to laser	0.58 ± 0.01^{Aa}	0.57 ± 0.006^{Aa}	0.031
	Culture exposure to laser	0.58 ± 0.02^{Aa}	0.82 ± 0.006^{Bb}	
Penicillium brevicompactum	Culture without exposure to laser	0.20 ± 0^{Aa}	0.20 ± 0^{Aa}	0.034
	Culture exposure to laser	0.52 ± 0.02^{Ba}	0.52 ± 0.006^{Ba}	
Penicillium expansum	Culture without exposure to laser	0.62 ± 0.04^{Aa}	0.64 ± 0.01^{Aa}	0.080
	Culture exposure to laser	1.22 ± 0.02^{Ba}	1.52 ± 0.01^{Bb}	

[a] Similar capital letters indicate that there is no difference horizontally at $P<0.05$.

[b] similar small letters indicate that there is no difference vertically at $P<0.05$.

GC-MS analysis

The GC-MS analysis showed presence of other compounds (Tables: 2 and 3) the simple Different substances, including Dimethyl methylphosphonate, Propanoic acid, and petroleum ether extracts that had been subjected to laser light or not 1-methylbutyl ester, 2-chloro 3-Chlorpropionic acid, Tetra-n-Propoxymethane, cis-9-Hexadecenoic acid, cis-Vaccenic acid, and propyl tetradecyl carbonate are all components of the compound 3-chloropr op-2-enyl ester. Hexanedioic acid, bis(2-ethylhexyl) ester, butyl decyl ester, and heptadecyl propyl ester of carbonic acid.

Discussion

Even though they are contagious, several fungi illnesses in animals can self-cure [22]. Some experts suggested that employing unique medications as a preventative step to lessen the spread of contagious fungi infections, Recent study is focused on the use of medical plants that include active compounds but do not have any negative effects on patients in order to avoid the harmful effects of chemicals and some antimicrobial medications [22,23]. Numerous varieties of pathogenic fungus contain virulence traits that heighten their pathogenicity and provide a barrier to the therapeutic process [24]. Researchers are

looking for materials that can manufacture various pharmaceutical medications as well as stop the spread of diseases. Recently, 74% of pharmaceuticals produced from plants have been used in modern treatment [25]. Since the extract content depends on the affinity between the solvent and the plant, choosing the right solvent is thought to be a crucial step in producing a plant extract. When it comes to extracting a plant's essential oils, pure Petroleum ether might be used [26]. Unlike other materials, using water as a solvent, as in pumpkin leaves, led to significant results [27]. In other words, these chemicals have a high affinity for polar solvents like water and pure methanol because they are polar substances [28,29]. The *Populus spp*. plant's leaves were used to make the Petroleum ether extract, which was then studied for its antifungal properties both before and after exposure to the laser. These experiments formed the basis for the development of this technique.

The current investigation can explain why the petroleum ether extract that was not exposed to laser light did not produce inhibitory values against the fungi. The bioactive substances found in the plant extracts, including glycosides, tannins, saponins, and phenols, prevent the growth of microbes [30]. The addition of a second polar fluid will increase the polarity of the solvents. The bioactive substances discovered in plant matrices are medium-sized compounds because of their aromatic delocalized - electrons [30]. This event demonstrates the requirement for polar solvents to be used to extract the matrices because they are very polar and are one of the most amazing fermenting microorganisms [31-33].

In this investigation, the crude petroleum ether extract made from *Populus spp*. plant leaves was dissolved in DMSO to form a solution, and then irradiated with a semiconductor laser system at 450 nm wavelength and 50 mW power. Comparing the bioagent to samples that had not been exposed to laser irradiation, it was discovered that the bioagent had been laser-stimulated. No statistically significant difference was found between the control group and the biomass concentration of fungus exposed to light for 30 and 60 minutes. However, the bactericidal or bacteriostatic effect of laser radiation states that the radiation is absorbed through chromophores and that this may cause conformational modifications in some molecules, producing free radicals and reactive oxygen that, in turn, promote the disruption of bacteria's and fungi's membranes [35].

GC-MS has been used to identify several extract chemicals, such as Dimethyl methylphosphonate, Propanoic acid, 1-methylbutyl ester, 2-chloro 3-Chlorpropionic acid, Tetra-n-Propoxymethane, 3-Chloroprop-2-enyl EsterVaccenic acid, propyl tetradecyl carbonate, and 9-Hexadecenoic acid Hexanedioic acid, bis (2-ethylhexyl) ester, butyl decyl ester, and heptadecyl propyl ester of carbonic acid.

Bibliography

References in the text should be indicated by Arabic numerals in square brackets that run consecutively through the paper. Authors should ensure that all references are cited in the text and vice versa. The reference list should contain only literature references; other information (e.g. experimental details) should be placed either in the body of the text, or as a footnote. Each reference should contain only one literature citation. Authors are expected to check the original source reference for accuracy. Journal titles should be abbreviated according to American Chemical Society guidelines (The ACS Style Guide; Dodd, J. S., Ed.: American Chemical Society: Washington, DC, 1997). See examples for journal articles [1], books [2], multi-author books [3], proceedings [4] and personal communications [5], shown in References below.

Conclusions

The current study hypothesizes that the short-term laser treatment's effects on the crude Petroleum ether extract made from Populus spp. leaves are related to changes in the plant compounds that can be detected through GC-MS by way of increased energy consumption provided by the radiation. More research, especially in vivo research, is required to corroborate the findings of this study since, despite the outcomes of this work, the in vitro study did not emphasize favorable impacts. Although this study did not show that the wavelengths utilized affected the growth of fungi, there is a very important clinical significance since it may demonstrate the existence of bactericidal, bacteriostatic, or fungicidal effects of low-level laser therapy.

Acknowledgment

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Conflicts of interest

The authors declare no conflict of interest.

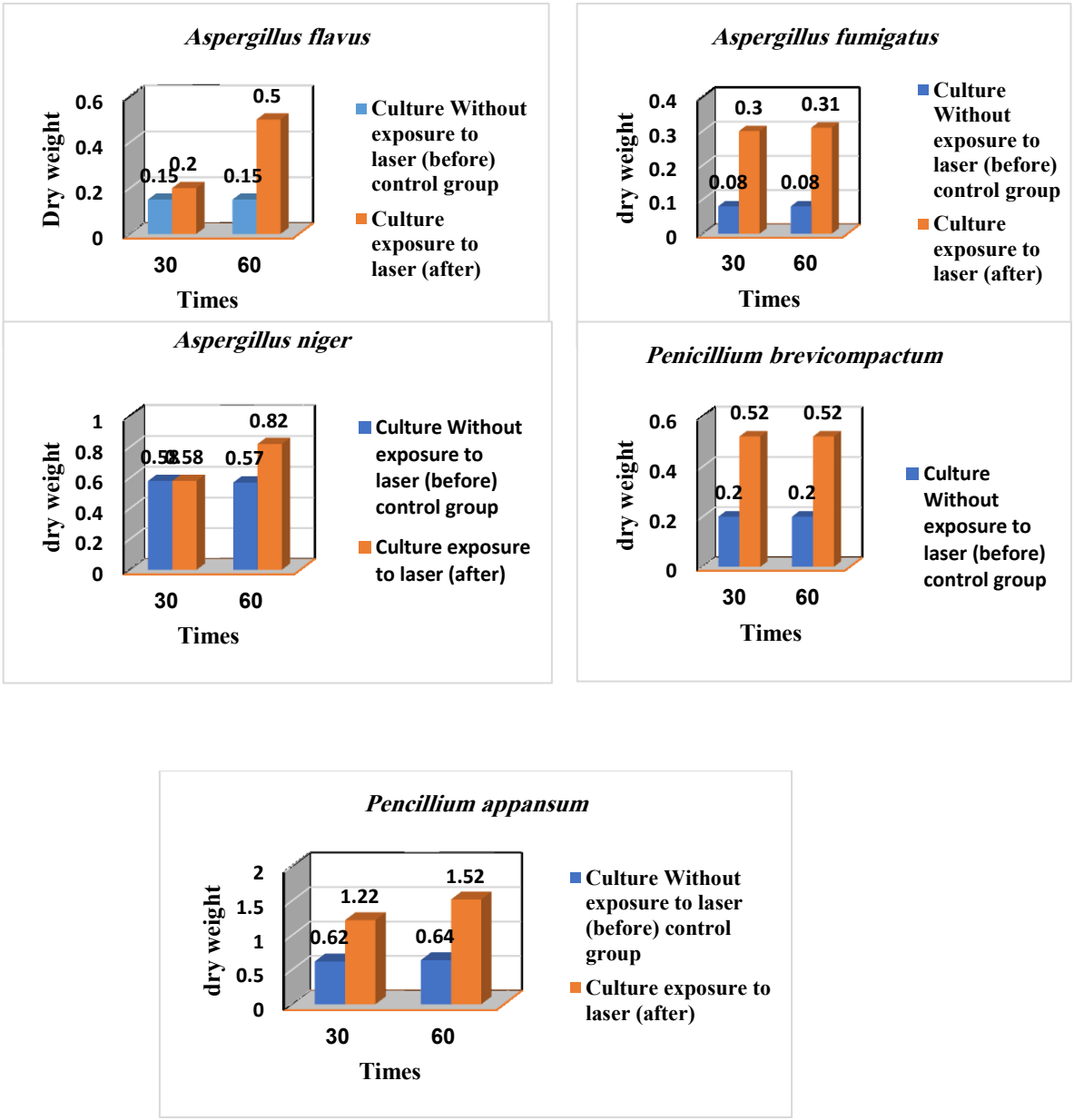

Fig. 4. Comparing the biomass content of fungus exposed to light for 30 and 60 minutes statistically with the control group.

TABLE 2. GC-MS analysis of the non-laser exposed crude petroleum ether extract

Compounds	Peak	Retentions time (RTs)
Dimethyl Sulfoxide	1	6.489
3 Dimethyl Sulfoxide	2	7.201
Dimethyl methylphosphonate	3	7.331
Dimethyl Sulfoxide	4	7.471
Pyrazolo[1,5-a]pyrimidine-3-carbon itrile, 2-methylthio-7-	5	7.870
(2-pyridyl)1H-Trindene, 2,3,4,5,6,7,8,9-octah ydro-1,1,4,4,9,9-		
hexamethyl-		
Ether, bis(p-tert-butylphenyl)		
Dimethyl Sulfoxide	6	8.032
Dimethyl Sulfoxide	7	9.057
Dimethyl Sulfoxide	8	12.088
Dimethyl Sulfoxide	9	12.790
Dimethyl Sulfoxide		
Pyrimidine-4,6(3H,5H)-dione, 2-but Ylthio-		
Dimethyl Sulfoxide	10	13.707
Dimethyl Sulfoxide	11	13.933
Dimethylsulfoxonium formylmethylid		
Propanoic acid, 2-chloro-, 1-methy lbutyl ester	12	14.073
3-Chloropropionic acid, 3-chloropr op-2-enyl ester		
tetra-n-Propoxymethane		
Dimethyl Sulfoxide	13	14.289
Dimethyl Sulfoxide		
Pyrimidine-4,6(3H,5H)-dione, 2-but ylthio		
Dimethyl Sulfoxide	14	15.821
Dimethyl Sulfoxide	15	16.102
Dimethyl Sulfoxide	16	17.051
Dimethyl Sulfoxide	17	18.357
Dimethyl Sulfoxide	18	18.583
Pyrimidine-4,6(3H,5H)-dione, 2-but ylthio-	19	19.338
Dimethyl Sulfoxide		
Hexanedioic acid, bis(2-ethylhexyl) ester	20	23.934
Hexanedioic acid, bis(2-ethylhexyl) ester		
Hexanedioic acid, bis(2-ethylhexyl) ester		

TABLE 3. GC-MS analysis of the laser exposed the crude petroleum ether extract

Compounds	Peak	Retentions time (RTs)
Dimethyl methylphosphonate **Dimethyl sulfone** **Dimethyl methylphosphonate**	1	7.622
Dimethyl Sulfoxide	2	7.838
Dimethyl Sulfoxide	3	8.107
Dimethyl Sulfoxide	4	8.927
Dimethyl Sulfoxide	5	9.370
Dimethyl Sulfoxide	6	12.067
Dimethyl Sulfoxide	7	13.717
Dimethyl Sulfoxide	8	13.955
Pyrimidine-4,6(3H,5H)-dione, 2-but ylthio- **Dimethyl Sulfoxide Pyrimidine-4,6(3H,5H)-dione, 2-but ylthio-**	9	14.084
Dimethyl Sulfoxide	10	14.257
Dimethyl Sulfoxide	11	16.134
Dimethyl Sulfoxide	12	18.367
Dimethyl Sulfoxide	13	18.605
Dimethyl Sulfoxide	14	19.489
cis-9-Hexadecenoic acid **cis-Vaccenic acid** **Oleic Acid**	15	20.654
Oleic Acid	16	20.924
Chloroacetic acid, pentadecyl este **Cyclohexane, 1-(1,5-dimethylhexyl) -4-(4-methylpentyl)-** **Propyl tetradecyl carbonate** **Carbonic acid, butyl decyl ester** **Carbonic acid, heptadecyl propyl ester**	17	21.151
Dimethyl Sulfoxide	18	21.453
Bromodifluoroacetylchloride **Dimethyl Sulfoxide** **Diisooctyl adipate**	19	23.967
Dimethyl Sulfoxide	20	30.041

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التأثير المختبري لشعاع الليزر على النشاط المضاد للفطريات لمستخلص الأثير النفطي الخام المنتج من أوراق *Populus spp*

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تعتبر النباتات الطبية مصدرًا غنيًا لإنتاج الأدوية والأدوية التقليدية وعوامل تخليق الأدوية ، لذلك تم اكتشاف المركبات المضادة للميكروبات من النباتات. هدفت هذه الدراسة إلى تحليل آثار استخدام اشعة الليزر على مستخلص إيثر البترول الخام الناتج من أوراق نبات *populus spp* ، وفي هذه الدراسة تم اختبار مستخلص البترول الخام المعرض بالليزر الذي تم اختباره في المختبر كعامل مثبط لنمو الأنواع الفطرية بما في ذلك *Penicillium* ،*Aspergillus niger* ،*Aspergillus flavus* ،*Aspergillus fumigatus.* *brevicompactum* و *Penicillium expansum* التي تم الحصول عليها من بنك العزلات البيطري - معمل الأحياء الدقيقة - مستشفى واسط. نتج عن ذلك أن مستخلصات *Populus spp* قبل وبعد التعرض لشعاع الليزر لم ينتج عنها أي آثار مضادة للفطريات للزروع الفطرية باستخدام طريقه الانتشار في الاكار

وأظهر التحليل الإحصائي لتركيز الكتلة الحيوية للفطريات تحت ظروف الاشعاع الضوئي ٣٠ و ٦٠ دقيقة ومقارنة بمجموعة التحكم أظهرت عدم وجود فروق معنوية ذات دلالة إحصائية. أظهر تحليل *GC-MS* وجود مركبات أخرى في كل من مستخلصات الإيثر البترولي الخام المعرض والغير معرض لليزر ، حيث تم الكشف عن مركبات مختلفة ، مثل ثنائي ميثيل فوسفونات ، وحمض البروبانونيك ، ٢-كلورو- ، ١-ميثي لبيوتيل. إستر ٣-حمض كلوروبروبيونيك ،٣-كلوروبر *cis-9-Hexadecenoic* ،*op-2-enyl ester tetra-n-Propoxymethane* *butyl decyl ester* ،*Propyl tetradecyl carbonate Carbonic acid* ،*acid cis-Vaccenic acid* *heptadecyl propyl ester and Hexanedic* ،*Carbonic acid* حمض ثنائي (٢-إيثيل هكسيل) استر. تفترض الدراسة الحالية أن تأثيرات العلاج بالليزر قصير المدى على مستخلص الأثير النفطي الخام المنتج من أوراق نبات *Populus spp* مرتبطة بالتعديلات المتولدة في مركبات النبات ، والتي يتم اكتشافها من خلال *GC-MS* عن طريق زيادة استهلاك الطاقة من الإشعاع.