DocID: https://connectjournals.com/03896.2020.20.6121

Biochem. Cell. Arch. Vol. 20, No. 2, pp. 6121-6128, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

ANALYTICAL AND BIOLOGICAL ACTIVITY STUDY OF ONE OF THE ISOLATED EXTRACTS OF TRUFFLES ETHYL 6-METHYL-2-OXO-4-(2-THENYL)-1,2,3,4-TETRAHYDROPERIMIDINE-5-CARBOXYLATE (EMOTTC) AGAINST THREE OF *CANDIDA* SPECIES

Dawood CH. Al-Bahadily[1], Rana D. S. Alkamil[1], Qutaiba A. Qasim[1], Falah Hassan Shari[2] and H. N. K. AL-Salman[3*]

[1]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Basrah, Iraq.
[2]Department of Pharmacology and Toxicology, College of Pharmacy, University of Basrah, Iraq.
[3]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Iraq.
*e-mail: hsennaserh@yahoo.com

(Received 10 June 2020, Revised 4 September 2020, Accepted 13 September 2020)

ABSTRACT : Six compounds were extracted from the organic layer extract for truffles using a cellulite apparatus and a mixture of organic and inorganic solvents. The water layer extracts were neglected due to the lack of compounds benefiting the purpose of the laboratory experiment. Using solvents like, chloroform, ethyl acetate, methanol, n-Hexane and separating suppression the compound was isolated from (*EMOTTC*) and obtained On 200 g of this compound where the rate of extraction was higher when using methanol and less in chloroform than the biological efficacy of the compound (*EMOTTC*) was studied. Mass spectroscopy was performed for all compounds extracted from the organic layer and especially determined the chemical composition of the isolated compound *EMOTTC*. Various internationally recognized chemical statements were made for the purpose of proving the nature of the chemical composition of each compound separately. Biological efficacy was achieved For EMOTTC against three *Candida* sp. By taking the highest *EMOTTC* concentration and lowest concentration, a study of fungal ovarian inhibition using potato agar-dextrose (PDA) showed excellent bioavailability against *Candida* sp and demonstrated the ability to assess inhibition and its biological effect against the three types of *Candida* sp. The presence of the *EMOTTC* biologically active compound in the compound It has proven medicinal medicinal importance in treating some infections caused by fungal candidiasis. However, for the purpose of obtaining the advantages of biological activity and the toxicity of this compound, this requires extensive study.

Key words : *Candida* sp., truffle extracts, *EMOTTC* biological activity.

INTRODUCTION

Truffles (in the Arabian Peninsula) or thunder (in Sudan), and truffles (in Libya, Tunisia, Algeria, Morocco, and Mauritania), is the name of a family of fungi called Terfeziaceae (Latin: Terfeziaceae) which is a seasonal wild fungus that grows in the desert after a rain of depth of 5 to 15 centimeters underground and used as food. Truffle weight usually ranges from 30 to 300 grams. It is considered one of the tastiest and most valuable desert fungi. Chemical contents can be known through truffle analysis. The analyzes have proven to contain 9% protein, 13% starchy materials, 1% fat, and contain minerals similar to those in the human body such as phosphorous, sodium, calcium, and potassium. It also contains vitamin B2, B2 that is rich in vitamin A. It also contains a quantity of nitrogen in addition to carbon, oxygen, and hydrogen, which makes the composition similar to the composition of meat. And the taste of cooked ones like the taste of lamb kidneys. It contains the amino acids necessary to build the cells of the human body (Dillon *et al*, 2019; Lockhart *et al*, 2017; Garcia *et al*, 2018).

Truffle extracts contain many biologically active organic compounds against Candida fungi that cause various infections in human tissues. The most important organic compounds extracted is (*EMOTTC*), which is a truffle extract that has a high killing property against different types of inflammatory Candida fungi that cause various inflammatory infections in the human body systems (Caldara *et al*, 2018; de Oliveira *et al*, 2018). Candida is a genus of yeasts and is the most common cause of fungal infections worldwide. Many species are harmless commensals or endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease, known as an opportunistic infection. Candida is located on most of the mucosal surfaces and

mainly the gastrointestinal tract, along with the skin. *Candida albicans* is the most commonly isolated species and can cause infections (candidiasis or thrush) in humans and other animals. In winemaking, some species of Candida can potentially spoil wines (Shi *et al*, 2018; CAML *et al*, 2018; Marcos *et al*, 2016; Liedke *et al*, 2017; Denning *et al*, 2015). Many species are found in gut flora, including *C. albicans* in mammalian hosts, whereas others live as endosymbionts in insect hosts. Systemic infections of the bloodstream and major organs (candidemia or invasive candidiasis), particularly in patients with an impaired immune system (immunocompromised), affect over 90,000 people a year in the US. The genome of several *Candida* species has been sequenced.Antibiotics promote yeast (fungal) infections, including gastrointestinal (GI) *Candida* overgrowth and penetration of the GI mucosa. While women are more susceptible to genital yeast infections, men can also be infected. Certain factors, such as prolonged antibiotic use, increase the risk for both men and women. People with diabetes or the immunocompromised, such as those infected with HIV, are more susceptible to yeast infections. *Candida antarctica* and *Candida rugosa* are a source of industrially important lipases, while *Candida krusei* is prominently used to ferment cacao during chocolate production. *Candida rugosa* is also used as an enzyme supplement to support fat digestion with its broad specificity for lipid hydrolysis (Shirazi *et al*, 2015; Lopez-Ribot *et al*, 2004; Bistoni *et al*, 2004; Richards *et al*, 2004; Ibrahim *et al*, 2005).

Carboxyl esters containing sulfur and its derivatives are long-known compounds with antibacterial agents that have biological activity and significant influence on a wide range of bacteria. This is due to the presence of the aromatic ring, which has spread widely due to its various uses as anti-bacterial, anti-fungal and anti-fungal infections (Spellberg *et al*, 2005; Alaa *et al*, 2018).

EMOTTC is a sulfur beta-lactam compound, and it is an effective substance against a number of biological activities due to its selection. *EMOTTC,* as a powder whose other chemical name is ethyl 6-methyl-2-oxo-4-(thiophen-2 -yl) -1,2,3,4-tetrahydropyrimidine-5-carboxylate and 6-Methyl-2-oxo-4-thiophen-2-yl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester and ethyl 4-methyl-2-oxo-6-(2-thienyl) -1,3,6-trihydropyrimidine-5-carboxylate and has the molecular formula $C_{12}H_{14}N_2O_3S$, molecular weight 266.32 g / mol, has a melting point of 100-105 °C Its chemical is now classified as an antibacterial and antifungal. These compounds are classified as a biochemical for their high

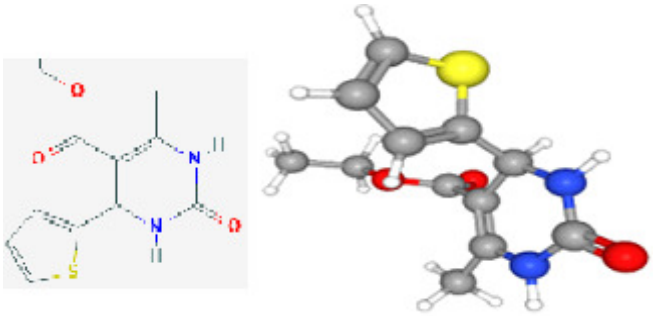

Fig. 1 :

toxicity (Al-salman *et al*, 2017; Al-salman *et al*, 2018; Hussein *et al*, 2020; Rana *et al*, 2020).

The antifungal activity is usually increased by increasing the number of amine groups in the solution when the NH_2 and -S- groups enter the chemical composition of *EMOTTC*. A number of researchers indicated that the presence of amine groups is more effective in eliminating various fungi. The NH_2 and -S groups are particularly bound to the outer surface molecules of the fungus, which disrupt the fungus membrane causing its melting and thus the death of the fungal cell. On this basis, the amine groups attracted high antifungal activity as a new class of antibiotics (Mohammed *et al*, 2018; Al-Sowdani *et al*, 2015; Mohamed *et al*, 2018; Dawood *et al*, 2019).

In other laboratory experiments conducted by researchers using compounds containing groups of hydroxy, nitrogen, carboxyl and sulfur on a wide range of fungi, it was observed to have a inhibitory effect on the formation of white tablets when incubated for 3 hours before fungal growth. There are some strains that have a pronounced effect regardless of time to add the compound (Al-Hashimia *et al*, 2018).

In order to obtain the inhibitory effect against the various fungi, the structural properties of the carboxylic ester compounds must be improved by adding a chain of alkyls of different lengths that increase the efficiency of the compound against the fungi by attaching the beta-lactam group to another group containing sulfur, as it is considered the increase of the double electrons that are An important nitrogen source is an important factor for achieving optimal value is to reduce the fungi activity as known. The high density of electrons causes a significant loss of fungi activity, in addition to the presence of ammonia in the structure of the beta-lactam sulfur compounds, which increases the permeability of the fungal cell membrane and thus the cell is subject to degradation, the permeability of the cell components inside and its death (Buffie *et al*, 2015; Mika *et al*, 2016; Komarova *et al*, 2017; Martinez *et al*, 2015).

MATERIALS AND METHODS

The experiment was carried out using reagents and solvents that were highly titrated and the deionized water (18.2 Mega ohms.) was used for the experiment at 25°C (Hu *et al*, 2015).

Chemicals used in the experiment

- $C_6H_{14}$for HPLC graduate, BDH Comp.
- $C_4H_8O_2$ for HPLC graduate, BDH Comp.
- C_2H_5OH and CH_3OH, BDH Comp.
- $CHCl_3$ for HPLC graduate.
- potato dextrose agar (PDA)(Cultivated medium), BDH Biological.

Collect of the experiment materials

Ripe Truffles were collected and washed from the soil with water. Then the villi and peels were separated from the rest of the fruits. After the impurities were isolated, the purified fruits were cut and placed on a large-size filter paper at 25°C, dried, ground, and used.

Prepare Truffle extracts

It was adopted on the internationally recognized method where 200 g of purified powder was dissolved well from impurities and dissolved in one liter of deionized water where it was thoroughly mixed for 24 hours at room temperature for the purpose of increasing the recovery rate after which the filtration solvents in the rotary evaporator were separated and from Then it was dried from the remaining water by an oven at a temperature of 40 degrees Celsius after that put the dry powder in airtight containers (PVC) and kept at a temperature of 18 degrees Celsius until use (Ghareeb *et al*, 2015).

Extraction methods

The process of extracting the truffles purified was carried out with two basic steps, the first step was by suppressing the separation and mixture of solvents like methanol: chloroform: deionized water (10: 40: 40) where six compounds were extracted from the organic layer and the aqueous layer was neglected due to the absence of biological important compounds, namely (EMOTTC), 1-Methoxybenzene, -4- (4-iodobenzylidenamino), (MBIBI), Octaethylene glycol, (OEG), Nortazettine (NTN) 5. Alpha.-pregnan-3.beta. -diol (aP, bD), 5- beta-Pregnane-3-alpha, 11-alpha, 20-beta-trio, (bP, bT). After the organic and aqueous layers were isolated from each other, the compound was isolated (EMOTTC) from the organic layer, to be a compound that will be biologically studied against different types of fungi of the asses. The specific method for isolating the EMOTTC purifier from the organic layer with different solvent methanol, chloroform, ethyl acetate and hexane separately (Barka *et al*, 2016).

A 10 g of ripe and dried truffle powder was dissolved with 50 ml of deionized water and then the pH value was determined, after which chemical and physical quality tests were carried out and the percentage of aggregates and biologically active components were determine.

RESULTS

Using a UV-Vis spectrometer that contains a double visible UV ray and different wavelengths, *EMOTTC* was detected at a maximum wavelength of 390 nm using a nitrous detector. The organic compound *MBIBI* was detected at a maximum wavelength of 310 nm with the same detector as the *OEG* organic compound. It was detected at a maximum wavelength of 254 nm and measured by *NTN* at a maximum wavelength of 330 nm. The compound *a-P, b-D* was measured at a maximum wavelength of 290 nm and a measure of the compound *b-P, b-T* at a wavelength of maximum 300 nm. All maximum wavelengths that were determined for all active compounds after scanning were measured by the same UV-visible spectrum apparatus that had a double beam package. All compounds are shown in Fig. 2.

EMOTTC isolation and purification

After the organic and aqueous layers were isolated from each other, the aqueous layers were neglected because they did not contain compounds benefiting our work and then the *EMOTTC* was isolated and purified from the organic layer, a compound that will be biologically studied against different types of bacteria. A special accurate method was used to isolate and purify *EMOTTC* from the organic layer containing this compound using different solvents like CH_3OH,C_2H_5OH, $CHCl_3$, $C_4H_8O_2$, and n-Hexane. Each solvent is used in a separate way to precipitate, analyze and estimate the compound (Valle *et al*, 2015).

The chemical disclosure results

The results of the chemical disclosures indicate the presence of six active biological compounds in the organic layer extracted from the truffle plant. Are (EMOTTC), 1-Methoxybenzene, -4- (4-iodobenzylidenamino), (MBIBI), Octaethylene glycol, (OEG), Nortazettine (NTN) 5. Alpha.-pregnan-3.beta.-diol (a-P, b-D), 5- beta-Pregnane-3-alpha, 11-alpha, 20-beta-trio, (b-P, b-T). The results obtained were confirmed by GC-Mass technology and UV diagnostic results.

Determine the *EMOTTC* compound

Using a standard solution of *EMOTTC*, direct

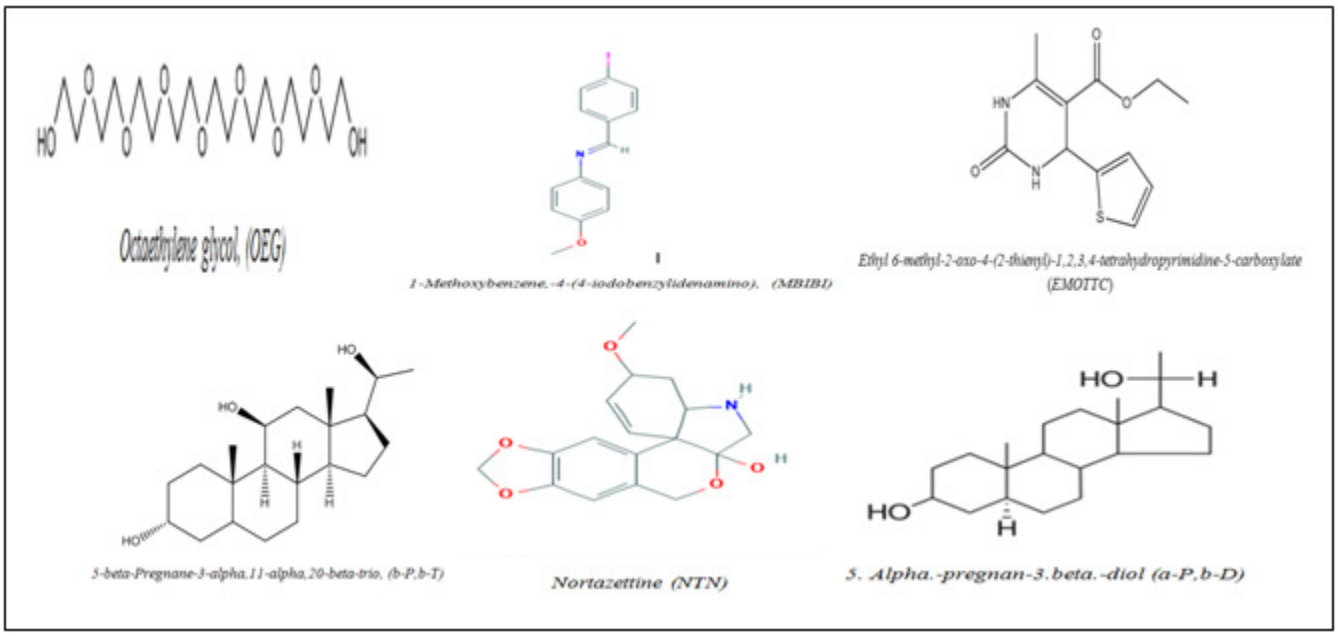

Fig. 2 : Compounds extracted in the organic layer.

measurements of plant extracts were performed in volume (2.0 ml) for different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml. *EMOTTC* absorption was measured along a 230 nm wavelength. The maximum wavelength after wiping is determined using the same UV-protected deionized device as blank. The values of the linear regression equation were used for Quercetin as well as the values of R^2, the *EMOTTC* concentration was calculated. The standard scheme was obtained for *EMOTTC* the extract was calculated after purification. SD means (n = 3).

The organic compound *EMOTTC* is expressed in mg/g unit of dry extract equivalent. The chemical composition of currently is not accurately classified in our databases, nor has information about this compound including synonyms and identifier numbers been specified, and we do not have complete information about this chemical.

Determination of the chemical composition of organic compounds in the extract by GC-Mass analysis

Table 2 and Fig. 3 show the analyzes using the GC-Mass technique where a molecular ion is formed for all compounds in the extract and it was found that the molecular ion equals the weight of the molecular formula of the compound minus one. Charts confirm Weigh all composite particles that give a good signal to isolate and separately identify all chemical compounds.

Statistical analysis of the data

Statistical analysis of the data tested for ANOVAis expressed as mean ± SD. (t) It was used and used to analyze the statistical function. P values <0.05 were found to be of excellent statistical significance.

Biologically active compounds extraction

Table 3 and Fig. 2 illustrate the biologically active compounds obtained from the organic layer in the *Truffles e*xtract. The bioactive compound *EMOTTC* was obtained against tree types of Fungal Candida.

The percentage obtained from extracts

Approximately 50g (20%) dry mass of all extract components was obtained from extracting 500-600 g of Truffles after 16 hours of continuous hot extraction in Soxhlet extract using ethanol solvent. The Copshan division method was used for raw alcohol extracts. Table 3 shows the different extraction ratios when using different solvents such as n-C_6H_{14}, $CHCl_3$, $C_4H_8O_2$, C_2H_5OH and CH_3OH.

The *EMOTTC* crude

The weight ratio of *EMOTTC* is calculated using the following linear regression equation obtained from the standard *EMOTTC*.

$y = 0.031x+0.020$, $R^2 = 0.9989$

Where, y is absorbance and x is the amount of *EMOTTC* that calculated by the microgram unit.

The crude extract of *EMOTTC* in methanol, chloroform, ethyl acetate, and n-Hexane was 89 ± 0.51, 71.18±0.99, 95.12 ± 0.50 and 80.32 ± 0.101, respectively, and the statistical significance was calculated for all values of the obtained results ($P = 0.001$). Ethyl acetate ($C_4H_8O_2$) extract was found to contain the highest amount of *EMOTTC* compound followed by methanol, hexane and chloroform. However, little quantity of

Fig. 3 : Analyzes GC-Mass technique for all compound extract.

Table 1 : Significance of organic compounds extracted from truffles in organic and aqueous layers.

Phase	*EMOTTC*	*MBIBI*	*OEG*	TBAC	*NTN*	*a-P, b-D*	*b-P, b-T*
Organic layer	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Aquatic layer	Negative	Negative	Negative	Negative	Negative	Negative	Negative

EMOTTC compounds was detected in chloroform extract. A significant difference in the aquatic layer contents for different extractions was observed by the ANOVA parameter test.

Yeasts isolates used in the study

The effect of the organic compound (*EMOTTC*) extracted from truffles on the growth of *Candida* sp.: *Candida albicans* (ATTC 10231, *Candida glabrata* (ATTC 90030) and *Candida krusei* (ATTC 6258) has been studied. These fungi coexist within the human body and cause various diseases in addition to reduce immunity.

Studying the effect of the compound (*EMOTTC*) on experimental yeastisolates and determining the lowest inhibiting and lethal concentration of fungi. The effectiveness of the compound (*EMOTTC*) in the radial growth of fungal pathogens within the human body has been studied. The following EMOTTC concentrations are included (30, 20, 10, 5, 2.5, 1.25, 0.65) µg / ml where (2, 1, 0.5, 0.1, 0.05, 0.01, 0.005) ml of the compound (*EMOTTC*) were taken by a small pipette and added to the prepared, sterile and cooled PDA medium to 45°C in the sterile conditions and with the following sizes: (0 99.90, 99.95, 99.99, 99.995, 99.999, 1.0) ml of PDA and after mixing well the well pour into sterile Petri dishes with a diameter of 9 cm, after hardening the center containing the essential oil to stand each dish with a piece of a fungal colony of 0.5 cm diameter. 5-7 days at room temperature and when the comparison treatment with the diameter (nutrient-free center) reached a greater degree towards the dish, then the inhibition of the fungi growth rate was calculated as in the following equation:

Percentage of inhibition = [(Fungi growth rate in the control sample – Fungi growth rate in treatment) / (Fungi growth rate in the control sample) × 100

The results of the statistical analysis shown in Fig. 3 indicate that the highest rate of inhibition of radial growth of fungi was recorded at a concentration of 30 ml 60.1%, 49.5% and 45.8% respectively in *Candida albicans*, *Candida glabrata* and *Candida krusel*, respectively, while the lowest 0.05 ml concentration inhibition ratio with 1.2%, 2.5% and 3.3% for *Candida albicans*, *Candida*

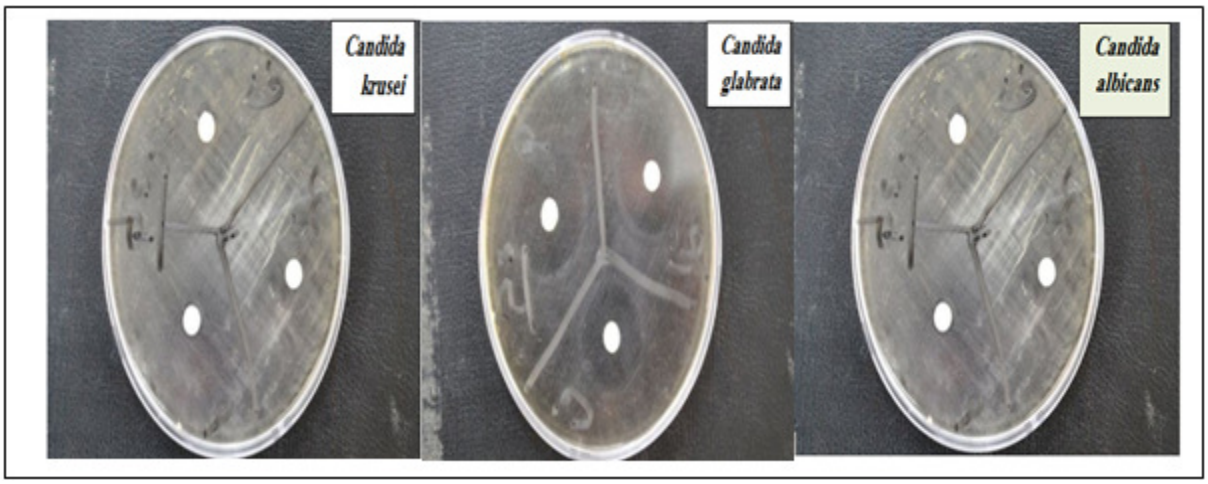

Fig. 4 : Test results for three types of yeast fungi.

Table 2 : Parameters of the analytical method of mass spectrum in the determination of truffle extracts.

Column	HP-5MS, 5% phenyl methyl Sillox (1629.5) m×0.250 µm I.D. × 0.25 µm, SS., Inlet He
EMV mode	Gain factor (1.10)
Resulting EM voltage	1220
Low mass	28.1
High mass	441
Threshold	150
Minimum quality for all compounds	(92-98%)
Flow rate	1.2 ml/min
Run time	30 min
Hold up time	6.890 min
Solvent delay	3.00 min
Average velocity	27.810 cm/s
Temperature	Initial 65°C to maximum 370°C
Pressure	10.23 Psi

Table 3 : The amount and % yields of organic extracts from Truffles.

The solvents of Extracts	Amount (gm)	Yields (w/w) %
CH_3OH	20	40
$CHCl_3$	8	16
$C_4H_8O_2$	16.5	33
C_6H_{14}	5.5	11

glabrata and *Candida krusel*, respectively.

Statistical analysis is performed by SPSS using ANOVA analysis to describe the tests between subject factors and groups of fungi. The results were: a high significant difference ($p < 0.002$) between the *EMOTTC* concentrations used; a high significant difference ($p < 0.002$) for *EMOTTC* interference and a high significant difference ($p < 0.002$) between the fungi used.

CONCLUSION

1. A number of global methods were tested, but one method was successfully conducted, through which six organic compounds were identified in the organic layer and the aqueous layer was neglected. The EMOTTC isolated compound was identified from the extracted compounds by the gas mass spectrometer. All the compounds extracted by the GC-MS spectroscope and the use of the molecular ion system for all the compounds were studied separately, as it was found that the mass of the molecular ion of each of them is equal to the atomic masses, as shown in the graphs that confirmed the current six organic compounds. All results indicate good purification and isolation of the six extracted compounds, especially EMOTTC.

2. The active multi-group compound such as *EMOTTC* extracted from *truffles* was used for the experimental study to inhibit the lethal effect of the toxins producing fungi as it was found that it contains the highest amount of *EMOTTC* extracted. The concentration of 2.0 ml (30 µg/ml) of *EMOTTC* resulted in a very large inhibition (56.4%) for the radial growth of *Candida albicans* experimentally observed compared to other

Candida glabrata species (43.6%), which may be due to its sensitivity to *EMOTTC*. In contrast, *Candida krusel* genus showed resistance to *EMOTTC* with significant inhibition (44.6%). Volumes (0.1 ml, 0.01 ml, and 0.001 ml) of the *EMOTTC* compound used in *Candida kruselfungi* in markedly significant value inhibition effects of 15.9%, 13.0%, and 6.5% were respectfully higher than those of both other fungi *Candida albicans*(15.2%, 9.7%, 4.5%) and *Candida glabratafungi* (11.2%, 9.1%, 5.7%) These results correspond to the indicated high efficiency of the *EMOTTC* compound of truffles in reducing fungi 29 in general with *Candida inhibition albicans*, *Candida glabrata*, and *Candida krusel* as shown on the net Chemical slides of compounds. The extract contains many biologically active organic compounds that can be used for future work.

ACKNOWLEDGMENTS

All authors would like to thank all Professors in the lab. of the College of Pharmacy at Basra University for their cooperation in finding the best results in this manuscript. The authors also thank and appreciate the work supervisor, Dr. Hussein Nasser Khalaf Al Salman for improving this manuscript.

REFERENCES

Alaa G Al-Hashimia, Sinan J Abdul-Abbas, NajlaHussen Saper Al Garory, Shaker A N AL-Jadaan and Al-Salman H N K (2018) Isolation and diagnosis of phytochemicals extracted from pomegranate husks and study of the biological effect of extract (O-Veratramide) as an antimicrobial activity. *Int. J. Green Pharmacy* **12** (4), 310-321.

Al-Hashimia A G, Al-Salman H N and Al-Jadaan N A (2018) GC-mass analysis and estimation of pomegranate husks extracts and the biological efficacy of compound tri-butyl acetyl citrate as one of the extract against food fungi. *Orient. J. Chem.* **34**, 2089-2097.

Al-Salman H N and Jasim E Q (2018) Analytical methods for diagnosis a mixture of narcotic substances in seized materials. *Int. J. Green Pharm.* **12**, 216-226.

Al-Salman H N (2017) Analysis methods and qualitative diagnosis chromatographic for mixture of narcotic substances in seized materials. *Eur. J. Sci. Res.* **147**, 403-411.

Al-Sowdani K H and Al-Salman H N (2015) Determination of extracted methamphetamine from hashish narcotic plant by home-made ion chromatography system. *Int. J. Adv. Res.* **3**, 723-730.

Barka V E A, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C and Meier-Kolthoff J P (2016) et al. Taxonomy, physiology, and natural products of Actinobacteria. *Microbiol. Mol. Biol. Rev.* **80**, 1-43.

Bistoni F, Cenci E and Menacci A (2004) Mucosal and systemic T helper cell function after intragastric colonization of adult mice with *Candida albicans*. *J. Infect. Dis.* **168**, 1449-1457.

Buffie C G, Bucci V, Stein R R, McKenney P T, Ling L and Gobourne A (2015) Precision microbiome reconstitution restores bile acid mediated resistance to clostridium difficile. *Nature* **517**, 205-208.

CA M L, Vincent B M, Solis N V, Lancaster A K, Sullivan L B and Hartland C L (2018) Inhibiting mitochondrial phosphate transport as an unexploited antifungal strategy. *Nat. Chem. Biol*. **14**(2), 135–141.

Caldara M and Marmiroli N (2018) Tricyclic antidepressants inhibit Candida albicans growth and biofilm formation. *Int. J. Antimicrob. Agents* **52**(4), 500–505.

Dawood C H Al-Bahadily, Falah Hassan Shari, Mazin A A Najm and Al-Salman H N K (2019) Antimicrobial Activity of the Compound 2-Piperidinone, N-(4-Bromo-n-butyl)- Extracted from Pomegranate Peels. *Asian J. Pharmaceutics* **13** (1), 46-53.

de Oliveira Santos G C, Vasconcelos C C, Lopes A J O, de Sousa Cartágenes M, Filho A K D B and do Nascimento F R F (2018) Candida infections and therapeutic strategies: mechanisms of action for traditional and alternative agents. *Front. Microbiol.* **9**, 1351.

Denning D W and Bromley M J (2015) Infectious disease.How to bolster the antifungal pipeline. *Science* **347**(6229), 1414–1416.

Dillon Piccione, Susanna Mirabelli, Nicholas Minto and Tejas Bouklas (2019) Difficult but Not Impossible: in Search of an Anti-Candida Vaccine. *Topical Collection on Tropical Mycosis* **6**, 42–49.

Garcia-Carnero L C, Perez-Garcia L A, Martinez-Alvarez J A, Reyes-Martinez J E and Mora-Montes H M (2018) Current trends to control fungal pathogens: exploiting our knowledge in the host-pathogen interaction. *Infect. Drug. Resist*. **11**, 903–913.

Ghareeb M and Akhlaghi F (2015) Alternative matrices for therapeutic drug monitoring of immunosuppressive agents using LC-MS/MS. *Bioanalysis* **7**, 1037-1058.

Hu M, Liu X, Dong F, Xu J, Li S, Xu H and Zheng Y (2015) *Food Chem*. **175**, 395-400.

Hussein N AL-Salman, Eman T Ali, Omar A Almukhtar and Majid S Jabir (2020) 2- benzhydrylsulfinyl-N-hydroxyacetamide extracted from fig: A good therapeutic agent against *Staphylococcus aureus*. *AIP Conference Proceedings* 25 March: 020223-5

Ibrahim A S, Spellberg B J, Avenissian V, Fu Y, Filler S G and Edwards Jr J E (2005) Vaccination with recombinant N-terminal domain of Als1p improves survival during murine disseminated candidiasis by enhancing cell-mediated, not humoral, immunity. *Infect. Immun*. **73**, 999–1005.

Komarova A S, Chermenskaya T D and Chelovechkova V V (2017) *J. Anal. Chem.* **72**, 1076-1081.

Liedke S C, Miranda D Z, Gomes K X, Goncalves J L S, Frases S and Nosanchuk J D (2017) Characterization of the antifungal functions of a WGA-Fc (IgG2a) fusion protein binding to cell wall chitin oligomers. *Sci. Rep*. **7**(1), 12187.

Lockhart S R, Berkow E L, Chow N and Welsh R M (2017) *Candida auris* for the clinical microbiology laboratory: not your grandfather's *Candida* species. *Clin. Microbiol. Newsl*. **39**(13), 99–103.

Lopez-Ribot J L, Casanova J L and Murgui A (2004) Antibody response to Candida albicans cell wall antigens. *FEMS Immunol. Med. Microbiol.* **41**, 187–196.

Marcos C M, de Oliveira H C, de Melo W C M A, da Silva J F, Assato P A and Scorzoni L (2016) Anti-immune strategies of pathogenic fungi. *Front. Cell Infect. Microbiol*. **6**, 142.

Martinez G, Morales A, Maestro A, Cermenj S, Oliva J and Barba A J (2015) *AOAC Int.* **98**, 6, 1745-1751.

Mika A and Stepnowski P (2016) Current methods of the analysis of immunosuppressive agents in clinical materials: A review. *J. Pharm. Biomed Anal.* **127**, 207-231.

Mohamed Senouci Bereksi, Hafida Hassaïne, Chahrazed Bekhechi and Djamel Eddine Abdelouahid (2018) Evaluation of Antibacterial Activity of some Medicinal Plants Extracts Commonly Used in Algerian Traditional Medicine against some Pathogenic Bacteria. *Pharmacogn J*. **10**(3), 507-512.

Mohammed A M, Ihmood K J and Abdullmajeed K A (2018) Synthesis characterization and biological activity evaluation of some new azo derivatives from 2- amino benzothiazole and their derivatives. *Kirkuk Univ. J. Sci. Stud.* **13**, 212-227.

Rana D S ALkamil, Dawood CH Al-Bahadily, RasoolChaloob, Falah Hassan Shari and AL-Salman H N K (2020) Estimation of Truffles Extracts and Study the Biological Efficacy of Ethyl 6-methyl-2-oxo-4-(2-thienyl)-1,2,3,4- tetrahydropyrimidine-5- carboxylate (EMOTTC) as One of the Extracts against Ophthalmic Bacteria. *Sys. Rev. Pharm.* **11**(6), 878- 887.

Richards M J, Edwards J R and Culver D H (2004) Nosocomial infections in pediatric intensive care units in the United States. *Pediatrics* **103**, 1-7.

Shi H, Dong S, Zhang X, Chen X, Gao X and Wang L (2018) Phage vaccines displaying YGKDVKDLFDYAQE epitope induce protection against systemic candidiasis in mouse model. *Vaccine* **36**(38), 5717–5724.

Shirazi F and Kontoyiannis D P (2015) Micafungin triggers caspase-dependent apoptosis in *Candida albicans* and *Candida parapsilosis* biofilms, including caspofungin non-susceptible isolates. *Virulence* **6**(4), 385–394.

Spellberg B, Ibrahim A S, Edwards J E Jr and Filler S G (2005) Mice with disseminated candidiasis die of progressive sepsis. *J. Infect. Dis*. **192**, 336–343.

Valle D L, Anrae J I, Puzon J J, Cabrera E C and Rivera W L (2015) Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. *Asian Pac. J. Trop. Biomed.* **5**, 532-540.