



Phytonutrients and Antimicrobial Activities of Methanolic Extract from Hafr Al-Batin Truffles

Ghassab M. Al-Mazaideh^{1*} and Farhan K. Al-Swailmi²

¹*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hafr Al-Batin, Hafr Al-Batin, Saudi Arabia.*

²*Department of Pharmacy Practice, College of Pharmacy, University of Hafr Al-Batin, Hafr Al-Batin, Saudi Arabia.*

Authors' contributions

This work was carried out in collaboration between all authors. Author GMA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FKA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i24B31437

Editor(s):

(1) Dr. Asmaa Fathi Moustafa Hamouda, Jazan University, Saudi Arabia.

Reviewers:

(1) María del Carmen Cortés López, Instituto Mexicano del Seguro Social, México.

(2) Maulana Yusuf Alkandahri, Buana Perjuangan Karawang University, Indonesia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/67737>

Original Research Article

Received 08 February 2021

Accepted 14 April 2021

Published 17 April 2021

ABSTRACT

The desert truffle is a wild mushroom, also referred to as Kamah or Fagaa. Kamah is a rich source of polysaccharides that have medicinal, antitumoral, antibacterial, and immune-stimulant effects. Studies of hypogeous fungi, especially desert truffles, have recently entered traditional studies of epigeous higher *Basidiomycetes*. Based on the tasty desert truffle Kamah obtained from Hafr Al-Batin Governorate, Saudi Arabia, as a source of potential antimicrobial agents with both the aim of obtaining novel agents toward bacteria and Fungi of clinical significance. We specifically tested the antibacterial and antifungal efficacy of methanolic extracts of Kamah against the Gram-negative bacterial pathogens reference strains *E. coli* ATCC® 8739, *P. Aeruginosa* ATCC®9027, *S. aureus* ATCC®6538, *Enterococci* NCTC®775 and opportunistic fungus *C. albicans* ATCC®1231. The extract had MIC (minimum inhibitory concentrations) varying from 100 g/ml to 500 g/ml against the pathogens examined. The LC-QTOF-MS (liquid chromatography coupled to quadrupole time of flight mass spectrometry) phytoconstituents assay chromatogram indicated that the methanolic extracts of Kamah comprises 264 with retention periods varying from 1.04 to 18.86, which were

*Corresponding author: E-mail: gmazaideh@uhb.edu.sa;

categorized as unsaturated and saturated natural ingredients such as aromatic compounds, carboxylic acids, oxygenated hydrocarbons, fatty acids, amino acids, and vitamins). The main compounds were discovered to be 21 with peak areas larger than 2×10^{-5} and retention periods varying from 2.3 to 9.13. The main known substances with the maximum peaks were adenosine (11.724), phenylalanine (7.711), phenprobamate (7.711), and 5-hydroxytryptophan (5.711). Such preliminary findings, we assume, are encouraging in terms of obtaining a beneficial antibiotic substitute to battle antibiotic-resistant pathogens especially eye infections.

Keywords: Kamah; Hafr Al-Batin; antibacterial; antifungal; LC-QTOF-MS.

1. INTRODUCTION

Many of available drugs have been derived directly or indirectly from medicinal plants. These bioactive compounds are mostly isolated from different parts of plants such as leaves, stems and roots. Their bioactivity properties like antimicrobial and antioxidant in vitro testing have noticed in many publications in the last decade [1-5]. The antioxidant and antimicrobial activity of bioactive compounds are mainly related to their ability to chelate metals, redox properties, and reactivity as quenching species of singlet oxygen [6]. Due to these properties, medicinal plants have been used for many years to treat health disorders and prevent diseases. These plants have always been a very good source of many drugs.

Truffles are an exceedingly unusual black mushroom, widely classified as a delicacy and they are one of the most precious of the scents. They are another often known for its fragrance but have some bioactive compounds in it as well. An *ascomyctic* fungus is a member of the *Tuberales* order of the Phylum of the *Peziza* Subclass. While the *basidiomycetes* develop underground, true truffles are only included in the genus *Tuber*. It is possible that the term truffle came from the word tuber in Latin, which has a related meaning of swelling or lump, which means "potato tuber." Truffles are hypogeous, ascomycetous, microaerobic fungi that type ectomycorrhizae (ECM) with the roots of the both gymnosperms and angiosperms [7-9].

Humans have used edible and medicinal mushrooms for thousands of years. Many of the previously known bioactive molecules have been found in different types of mushrooms, including antitumor compounds. Polysaccharides are the most frequently encountered and most strong polysaccharide-derived antitumor and immunomodulators [10]. In the far East, such as China, where much of the medical understanding and usage of polysaccharides arose. Ganoderma

lucidum (Reishi), Inonotus obliquus (Chaga), and many other mushrooms have been gathered and used for centuries in all three of these countries [11].

In Saudi Arabia several places cultivate truffle and have also been an important food for everyday use and income for a long time. The citizens of Saudi Arabia historically live quite well under current natural conditions in the processing and production of desert truffles [12]. The desert truffle, also known as Kama'a or Fagaa, is a family mushroom. It resembles a truffle in form but has a softer consistency, and it sprouts in the desert sand after a thunder and lightning storm. They are found in the Mediterranean region's dry climate, especially in Hafr Al-Batin, Saudi Arabia, during the springtime, and at truffle festivals [13]. Those aspects of mycological research still form the basis of current medical study, and practice, particularly in the field of stomach, prostatic, and respiratory diseases. Medicinal mushrooms provide a limitless supply of nutritionally, antitumorally, antibacterial and immunostimulatory polysaccharides [14]. The study of hypogeous fungi, in particular so-called desert truffle, has been accompanied recently by traditional studies of epigeous higher Basidiomycetes.

Truffle extracts have been investigated extensively for their therapeutic properties. The antibacterial effects of truffle extract, for example, have been well established in the treatment of a variety of diseases, including trachoma [15,16]. Schillaci et al. (2017) investigated the antibacterial activities of the edible desert truffle mushrooms *Tirmania pinoyi*, *Terfezia claveryi*, and *Picoa juniperi* against the strains *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) [17]. Paul et al. [18] discovered that *Terfezia* truffles had antifungal efficacy against plant pathogenic fungi. In other research, *Tirmania pinoyi* extract was shown to have antimicrobial properties against *B. subtilis* [18].

S. aureus is a significant eye pathogen that can infect the tear duct, eyelid, conjunctiva, cornea, anterior and posterior chambers, and vitreous chamber [19]. The infections that affect the cornea (keratitis) or the inner chambers of the eye (endophthalmitis) are the most dangerous because they can result in a loss of vision or even blindness. Antimicrobial factors are expressed constitutively in each of these ocular sites, and these defences are augmented by a protective host response to the organism. Predisposing factors, such as the use of contact lenses prior to the development of bacterial keratitis or, in the case of endophthalmitis, the trauma caused by cataract surgery or intravitreal injection, are common causes of infection. The bacterial surface's structural carbohydrates trigger an inflammatory response that reduces bacterial load but also contributes to tissue damage. Several bacterial secreted proteins, such as alpha-toxin, beta-toxin, gamma-toxin, Panton-Valentine leukocidin, and other two-component leukocidins, mediate tissue damage and contribute to the inflammatory response's induction. Quantitative animal models of keratitis and endophthalmitis have revealed new information about *S. aureus* virulence and host factors involved in infection control.

The aim of this analysis was to identify phytonutrients and describe antimicrobial activity of methanolic extract from Hafr Al-Batin truffles LC-QTOF-MS (liquid chromatography coupled to quadrupole time of flight mass spectrometry) method which was used for this research as a phytoconstituents endeavor. This additional aim was to research the biological importance of these metabolites and metabolism of truffles was also completed to establish how they may be used as a therapeutic antibacterial agent. There's no recent data on Hafr Al-Batin truffles, so this paper was a breakthrough in identifying desert truffle phytoconstituents and their antibacterial function.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Kamah

Kamah brought from Hafr Al-Batin Governorate, Saudi Arabia during springtime 2021 and they were finding in Hafr Al-Batin truffle festival.

2.1.1 Preparation of methanolic extract

Twenty-five grams of fresh Kamah were soaked in 250 ml of 96% methanol and it was put in the shaker device at 150 rpm, in dark place for five

days at room temperature and stored in a refrigerator for one day. The extract was then filtered using a Buchner funnel under vacuum. The filtrate was centrifuged at 3000 rpm for 10 minutes, and then extract concentrated in the rotary evaporator. The crude was left in open vials in the fume hood for two days at room temperature and stored thereafter at 4°C in a glass container until further use [20].

2.1.2 Microorganism and growth conditions

The extraction was tested for growth of bacterial strains and fungus in vitro using disc diffusion method and in agar well diffusion method. This study work was applied two Gram negative bacteria: *Escherichia Coli* (*E. Coli*, ATCC® 8739) and *Pseudomonas Aeruginosa* (*P. Aeruginosa*, ATCC®9027), two Gram positive bacteria: *Staphylococcus aureus* (*S. aureus*, ATCC®6538) and *Enterococci* (NCTC®775). In addition, *C. albicans* was tested as opportunistic fungal pathogen of humans (*C. albicans*, ATCC®1231). Test crude was added to a blank disk where on the following concentration 100 µl, 125 µl, 250 µl, 500 µl, 1000 µl, 1500 µl and 2000 µl. The disk left to absorb the sample for 30 seconds then placed in plate with a culture of CRM (*Calicchia resuspension medium*) [21]. The media that was used for microorganism: *E. Coli* with brilliance *E. Coli* - coliform selective medium, *P. Aeruginosa* with baird parker agar base, *S. aureus* with slantez and bartley medium, *Enterococcus* with *Pseudomonas* cetrimide agar, and *C. albicans* with sabaraud dextrose agar. The samples then placed in incubator as indicated temperature 37°C except for *C. albicans* at 25°C. The MIC (Minimum inhibitory concentration) were misused after 72hrs and after 5 days for *C. albicans*. The sample was tested directly from crude extract prepared for LC/MS/MS analysis.

2.2 Plant Constituents' Experimental Procedure

Stock solutions were prepared by dissolving of the appropriate amount of substance in Dimethyl sulfoxide-DMSO (analytical grade), then diluted with Acetonitrile then Centrifuged of each sample at 4000 rpm for 2.0 min to be used for identification of exact MS and retention time. All the other reagents, Acetonitrile, methanol, water, and formic acid used were LC/MS grade.

2.2.1 Instrumentation and ms parameters

Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker

Daltonik Elute UPLC system (Bremen, Germany) was used for screening compounds of interest. Standards were used for identification of m/z with high resolution Bruker TOF MS and exact retention time of each analyte after chromatographic separation. This instrument was operated using the Ion Source Apollo II ion Funnel electrospray source. The capillary voltage was 2500 V, the nebulizer gas was 2.0 bar, the dry gas (nitrogen) flow was 8 l/min, and the dry temperature was 200°C. The mass accuracy was < 1 ppm; the mass resolution was 50000 FSR (Full Sensitivity Resolution) and the TOF repetition rate was up to 20 kHz using Elute UHPLC coupled to a Bruker impact II QTOFMS. Chromatographic separation was performed using Bruker Solo C18 2 µm UHPLC column (100 mm x 2.1 mm x 2.0 µm) at a flow rate of 0.51 mL / min and a column temperature of 40°C. Solvents: (A) water with 0.05% formic acid and (B) acetonitrile. The LC gradient was 0 - 27 min linear gradient from 5% - 80% B; 27 - 29 min for 95% B; 29.1 min for 5% B, total analysis time was 35 min with injection volume of 3 µl.

3. RESULTS

3.1 Microbiological Analysis

Table 1 summarizes the MIC (µg/ml) of Kamah extract on the different bacterial strains and one fungus. The MIC values showed antimicrobial effect of methanolic extract of Kamah against four bacterial strains including *E. Coli*, *P. Aeruginosa*, *S. aureus* and *Enterococcus*. In addition to *C. albicans* as one of the fungal pathogens of humans.

Table 1 summarizes the MIC (minimum inhibitory concentration) results of Kamah methanolic extract on the different bacterial strains. The MIC values showed that negative Gram bacteria (*E. coli* and *P. Aeruginosa*) were inhibited at 100 µl and 125 µl, respectively. The positive Gram bacteria (*S. aureus* and *Enterococcus*) were

inhibited at 100µl and 125 µl, respectively. Fungi (*C. albicans*) was inhibited at 500 µl.

3.2 LC-QTOF-MS Analysis

Fig. 1 shows the extracted constituents of methanolic extract of Kamah which they were investigated using LC-QTOF-MS/M. Each constituent in the methanolic extract was quantified and identified by comparing mass fragmentation patterns with standards such as Wiley 9 library spectral data and NIST (Fig. 1).

Table 2 contains the data of identified 21 major compounds (more than 2×10^{-5} peak area) from methanolic extract of Kamah which includes retention time (RT, min), Area, molecular formula, measured m/z values (precursor mass) and calculated mass error (ppm), and the hit identities from the search of three compound databases, namely PubChem, KEGG Compound and ChemSpider. The raw data of all analytes (264 natural compounds) detected during the LC-QTOF-MS/MS analysis.

4. DISCUSSION

Drug discovery and phytomedicine have recently become hot subjects around the globe in order to discover and cultivate novel antioxidant and antibacterial compounds. Plants are being increasingly important in these fields due to their abundance of secondary metabolites such as alkaloids, polyphenols, terpenoids, hormones, glycosides, as well as other natural ingredients. These constituents demonstrated that herbal medicine has a significant role in clinical studies in infection disorders and in improving health treatment [22]. The capacity of bacteria to intercalate on DNA is related to their action against alkaloids and their derivatives, such as strongly aromatic planar quaternary [23,24]. Several natural phytochemical constituents can be found in Kamah's methanolic extract (Table 2). Antimicrobial and antioxidant activity are believed to be due to the presence of these substances.

Table 1. MIC (µg/ml) values of Kamah methanolic extract

Microorganism	Media	MIC mg/ml
E. Coli	Brilliance E. Coli /coliform selective medium	100µl
P. Aeruginosa	Baird Parker agar base	125µl
S. aureus	Slantez and bartley medium	100µl
Enterococcus	Pseudomonas cetrimide agar	125µl
C. albicans	Sabaraud dextrose agar	500µl

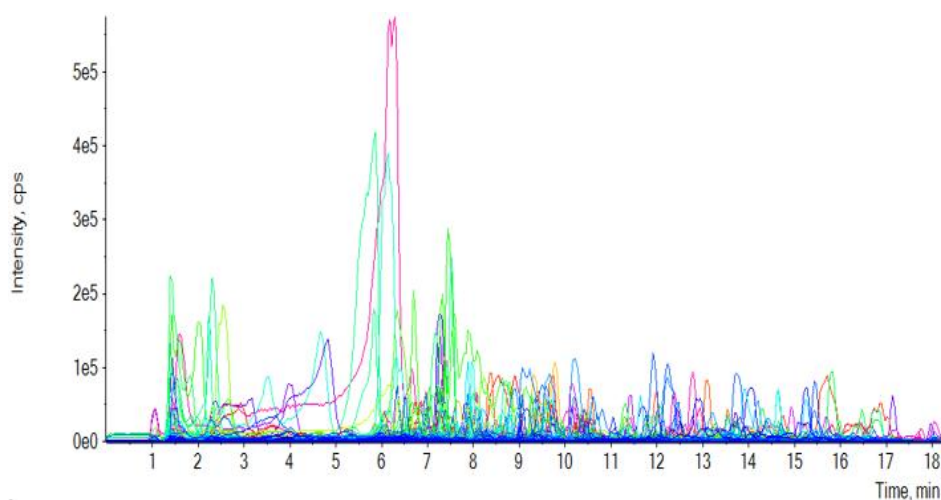


Fig. 1. Full peak chromatogram of methanolic extract of Kamah using LC-QTOF-MS/M

Table 2. LC-QTOF-MS phytoconstituents analysis of major compounds Kamah methanolic extract

No.	Component name	RT (min)	Area $\cdot 10^{-5}$	Molecular formula	Precursor mass	Mass error (ppm)
1.	Adenosine	5.74	11.724	C ₁₀ H ₁₃ N ₅ O ₄	268.104	-0.4
2.	Phenylalanine	6.12	7.711	C ₉ H ₁₁ NO ₂	166.086	0.8
3.	Phenprobamate	6.12	7.711	C ₉ H ₁₁ NO ₂	166.086	0.8
4.	5-hydroxytryptophan	4.75	5.627	C ₁₁ H ₁₂ N ₂ O ₃	221.092	1.9
5.	Vitexin	9.13	3.570	C ₂₁ H ₂₀ O ₁₀	433.113	2.6
6.	Isovitexin	9.13	3.570	C ₂₁ H ₂₀ O ₁₀	433.113	2.6
7.	Genistin	9.13	3.570	C ₂₁ H ₂₀ O ₁₀	433.113	2.6
8.	Emodin-8-glucoside	9.13	3.570	C ₂₁ H ₂₀ O ₁₀	433.113	2.6
9.	Afzelin	9.13	3.570	C ₂₁ H ₂₀ O ₁₀	433.113	2.6
10.	Sophoricoside	9.13	3.570	C ₂₁ H ₂₀ O ₁₀	433.113	2.6
11.	Apigenin-7-glucoside	9.13	3.570	C ₂₁ H ₂₀ O ₁₀	433.113	2.6
12.	Adenine	2.31	3.464	C ₅ H ₅ N ₅	136.062	0.6
13.	Salidroside	7.31	3.199	C ₁₄ H ₂₀ O ₇	301.155	-1.4
14.	Salidroide	7.31	3.199	C ₁₄ H ₂₀ O ₇	301.155	-1.4
15.	Nicotinamide	2.54	3.179	C ₆ H ₆ N ₂ O	123.055	-0.5
16.	Stachydrine hydrochloride	1.51	2.897	C ₇ H ₁₃ NO ₂	144.102	1.3
17.	<i>p</i> -Coumaric acid	3.99	2.333	C ₉ H ₈ O ₃	165.055	1.9
18.	Isoleucine	2.82	2.041	C ₆ H ₁₃ NO ₂	132.102	1.3
19.	Leucine	2.82	2.041	C ₆ H ₁₃ NO ₂	132.102	1.3
20.	Schaftoside	8.54	2.031	C ₂₆ H ₂₈ O ₁₄	565.155	1.9
21.	Isoschaftoside	8.54	2.031	C ₂₆ H ₂₈ O ₁₄	565.155	1.9

Polysaccharides used in medicinal mushrooms have dietary, antitumoral, antibacterial, and immune-stimulating properties. The antimicrobial potential of aqueous extracts of the edible Desert Truffles from Saudi Arabia mushrooms *Tirmania pinoyi*, *Terfezia clavaryi*, and *Picoa juniperi* was investigated [17]. The antibacterial function of acid-soluble protein extracts from these three organisms in vitro against the Gram-

positive human pathogenic strain *S. aureus* and the Gram-negative strain *P. aeruginosa* demonstrated minimum inhibitory concentrations of 50% against the pathogens studied.

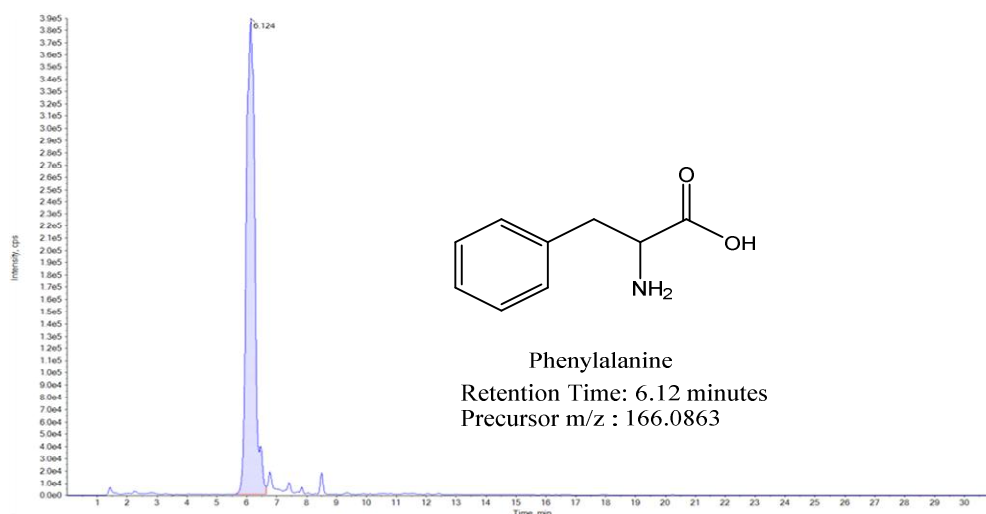
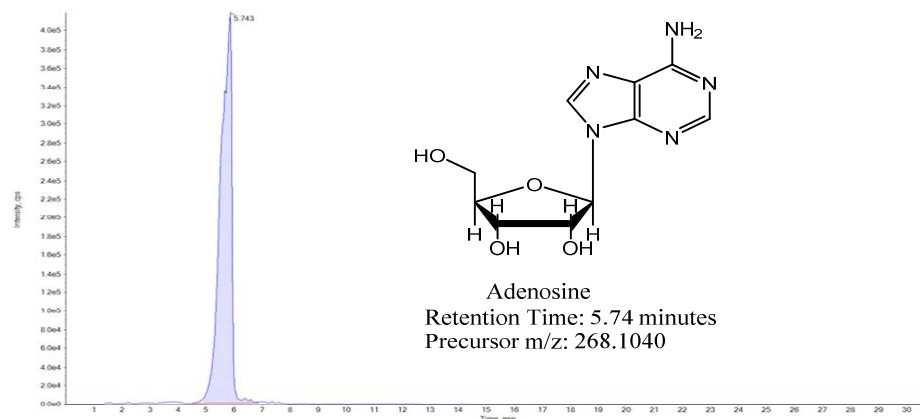
In the third stage of the disease, truffle juice was shown to be very effective against trachoma. Truffle juice was also discovered to have an important inhibitory effect on trachomas fibrosis.

Organic antibiotics, on the other hand, or cortisone-based products, have a host of side effects. As a consequence, we firmly advocate that truffle juice be used in combination with normal eye infections care at all times.

Methanolic extract of Kamah has antimicrobial effects on the growth of *E. coli*, *P. Aeruginosa* as Gram-negative bacteria, *S. aureus* and *Enterococcus* as Gram-positive bacteria and *C. albicans* as fungus. This is the first report on Methanolic extract of Kamah as antimicrobial effects with CRM. Methanolic extract showed potent antibacterial activities against *E. coli*, *P. Aeruginosa*, *S. aureus* and *Enterococcus* with MICs from 100 μ l to 25 μ l (Table 1). Weak antifungal effect with *C. albicans* was reported with MIC 500 μ l. Based on the findings of this study, it can be concluded that Methanolic extract of Kamah is a potent source of antimicrobial agents against Gram-positive and

Gram-negative bacterial strains and a weak antifungal agent. So that, it could be used as natural antibacterial agent.

LC-QTOF-MS phytoconstituents analysis chromatogram showed that the methanolic extract of Kamah contains 264 with retention times between 1.04 and 18.86 and they classified as saturated and unsaturated natural products (oxygenated hydrocarbons, carboxylic acids, fatty acids, amino acids, aromatic compounds and vitamins). Major compounds were found to be 21 with peak area more than 2×10^{-5} with retention times between 2.3 and 9.13 (Table 2). The highest major identified compounds peaks with retention time were adenosine (11.724), phenylalanine (7.711), phenprobamate (7.711), 5-hydroxytryptophan (5.627). Fig. 2 shows the structure of the main compounds detected with peak area more than 2×10^{-5} .



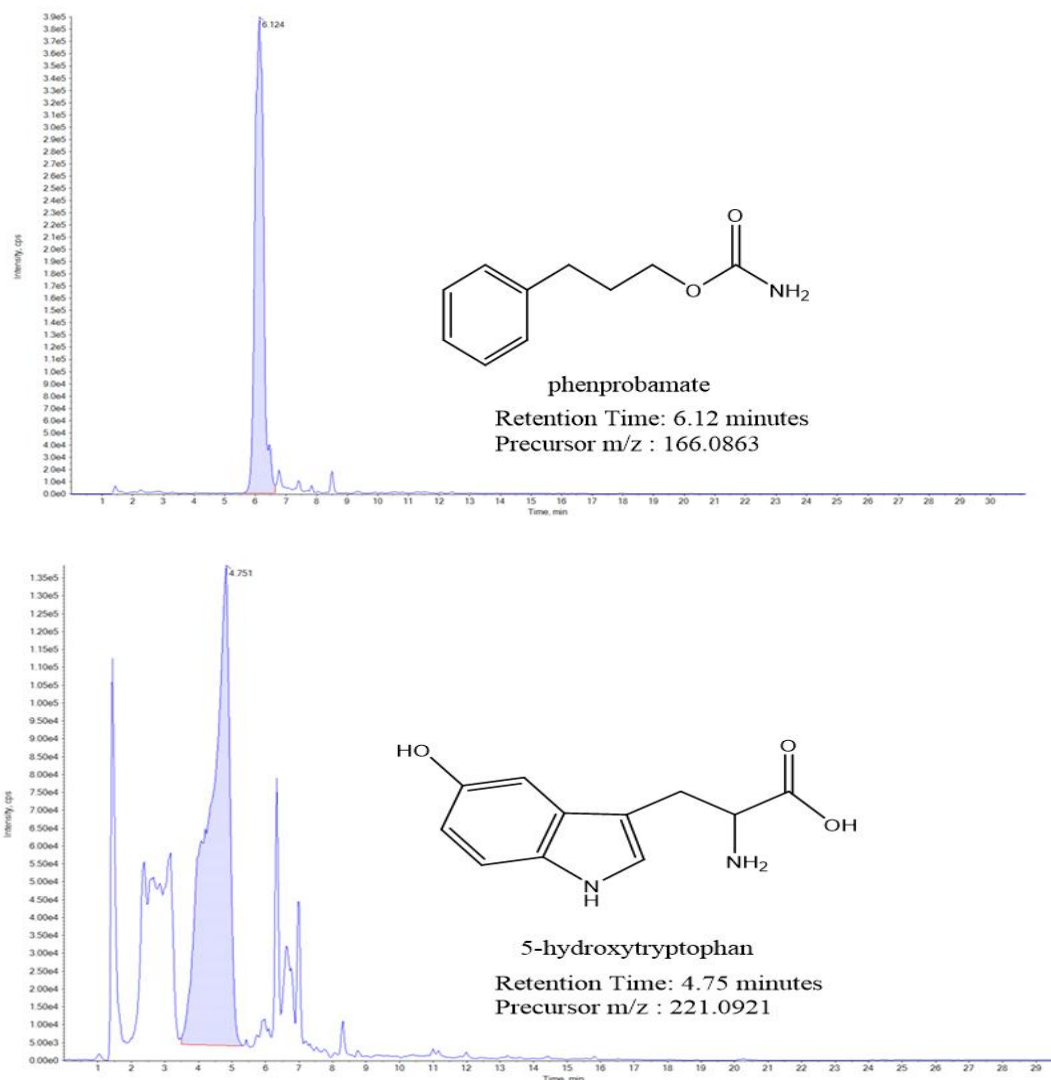


Fig. 2. Structures and Chromatograms of the main compounds (Adenosine, Phenylalanine, Phenprobamate and 5-hydroxytryptophan) detected Kamah methanolic extract

The properties of the described compounds are significant in Kamah because of their antibacterial and antioxidant action, as well as their ability to inhibit lipid oxidation [25]. As a result, Kamah is regarded as one of the most intriguing functional foods. As scavengers of many oxygen molecules, raw kamah have a high antioxidant activity. This result encourages the use of natural Kamah extracts instead of synthetic antioxidants. Despite their intriguing chemical structure and antioxidant function, desert truffles have been shown to have promising antibiotic activity [26]. All of these findings point render desert truffles a fascinating crop to produce and development of a valuable

antibiotic substitute to combat antibiotic-resistant pathogens.

5. CONCLUSION

The methanolic extract of Kamah is an active source of antioxidant and antimicrobial agents against Gram-positive and Gram-negative bacterial species, according to the results of this experimental work. As a consequence, it has the ability to be used as a natural antibacterial and antioxidant. Obviously, the extract's antifungal properties were not shown. This may be attributed to the fact that most of the active

ingredients are present in very low concentrations. Our findings may be used to direct prospective in vivo research on the medicinal use of Kamah and its ingredients in treating cancer. As a consequence, it may be used as a natural antibacterial and antioxidant agent in the treatment of a number of diseases, including eye infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research, University of Hafr Al Batin for funding this work through the research group project No. G-113-2020.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shalayel MHF, Nour S, Al-Mazaideh GM, Mahmoud SY, Farrag ES. Antifungal efficacy of clove flower buds (*Syzygium aromaticum*) oil and aqueous extract against dermatophytes clinical isolates. *Med Sci.* 2021;25(109):744-750.
2. Aldal'in HK, Al-Mazaideh GM, AL-Nadaf AH, Al-Rimawi F, Afaneh AT, Marashdeh A, Abu-Lafi S, Alakhras F, Ayyal-Salman H, Jamhour RMAQ. Phytochemical constituents of *securigera securidaca* seed extract using. *Trop J Nat Prod Res.* 2020;4(9):540-544. DOI: org/10.26538/tjnpr/v4i9.8
3. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. *Food Bioprod Proc.* 2011;89(3):217-33.
4. Martins S, Amorim ELC, Peixoto Sobrinho TJS, Saraiva AM, Pisciotano MNC, Aguilar CN, Teixeira JA, Mussatto SI. Antibacterial activity of crude methanolic extract and fractions obtained from *Larrea tridentata* leaves. *Ind Crop Prod.* 2013;41:306-11.
5. Wedian F, Al-Qudah MA, Al-Mazaideh GM. Corrosion inhibition of copper by *Capparis spinosa* L. Extract in Strong Acidic Medium: Experimental and Density Functional Theory. *Int J Electrochem. Sci.* 2017;12:4664-4676.
6. Sumayya AR, Srinivasan S, Amatullah N. Screening and biochemical quantification of phytochemicals in fenugreek (*Trigonella foenum-graecum*). *Res J Pharm., Biol Chem Sci.* 2012;3(1):165-169.
7. Patel S. Food, health and agricultural importance of truffles: A review of current scientific literature. *Curr Trends Biotechnol Pharm.* 2012;6:15-27.
8. Zambonelli A, Iotti M, Hall I. Current status of truffle cultivation: Recent results and future perspectives. *Micol Ital.* 2015;44:31-40. DOI: 10.6092/issn.2465-311X/5593
9. Payen T, Murat C, Bonito G. Truffle phylogenomics: New insights into truffle evolution and truffle lifecycle. *Adv Botan Res.* Elsevier: Amsterdam, The Netherlands. 2014;70:211-234.
10. Tzianabos AO. Polysaccharide immunomodulators as therapeutic agents: Structural aspects and biological function. *Clin Microbiol Rev.* 2000;13:523-533.
11. Hobbs C. Medicinal value of *Lentinus edodes* (Berk) Sing (Agaricomycetidae). A literature reviews. *Int J Med Mushrooms.* 2000;2:287-302.
12. Hashem A, Alqarawi AA, Shah MA, Wirth S, Egamberdieva D, Tabassum B, Abd_Allah EF. Desert truffles in Saudi Arabia: Diversity, ecology, and conservation. In: Sharma S, Varma A. (eds) *Microbial Resource Conservation.* Soil Biol. Springer, Cham. 2018;54. Available:https://doi.org/10.1007/978-3-319-96971-8_13
13. Manzelat SM. Mushrooms in Saudi Arabia (local and imported). *Int J Curr Res Biosci. Plant Biol.* 2019;6(8):8-12. DOI:https://doi.org/10.20546/ijcrbp.2019.608.002
14. Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl. Microbiol. Biotechnol.* 2002;60(3):258-74. DOI: 10.1007/s00253-002-1076-7 Epub 2002 Sep 10. PMID: 12436306.
15. Al-Qarawi AA, Mridha MAU. Status and need of research on desert truffles in Saudi Arabia. *J Pure Appl Microbiol.* 2012;6:1051-1062.

16. El-Enshasy H, Elsayed EA, Aziz R, Wadaan MA. Mushrooms and truffles: Historical biofactories for complementary medicine in Africa and in the Middle East. *Evid-Based Compl Alter Med*. 2013;2013:1-10. Available:<https://doi.org/10.1155/2013/620451>
17. Schillaci D, Cusimano MG, Cascioferro SM, Stefano VD, Arizza V, Chiaramonte M, Inguglia L, Bawadekji A, Davino S, Gargano ML, Venturella G. Antibacterial activity of desert truffles from Saudi Arabia Against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J Med Mushrooms*. 2017;19(2):121-125. DOI: 10.1615/IntJMedMushrooms.v19.i2.30
18. Paul NC, Kim WK, Woo SK, Park MS, Yu SH. Diversity of endophytic fungi associated with *Taraxacum coreanum* and their antifungal activity. *Mycobiology*. 2006;34:185-190.
19. O'Callaghan RJ. The pathogenesis of *Staphylococcus aureus* eye infections. *Pathogens*. 2018;7(1):9. DOI: 10.3390/pathogens7010009 PMID: 29320451; PMCID: PMC5874735.
20. Tofighi Z, Molazem M, Doostdar B, Taban P, Shahverdi A, Samadi N, Yassaa N. Antimicrobial activities of three medicinal plants and investigation of flavonoids of *tripleurospermum disciforme*. *Iran J Pharm Res*. 2015;14(1):225-231.
21. Calicchia ML, Wang CIE, Nomura T, Yotsuzuka F, Osato DW. Selective Enumeration of *Bifidobacterium bifidum*, *Enterococcus faecium*, and streptomycin-resistant *Lactobacillus acidophilus* from a mixed probiotic product. *J Food Prot*. 1993;56(11):954-957. DOI: 10.4315/0362-028X-56.11.954 PMID: 31113077.
22. Chung YC, Chang C, Chao WW, Lin CF, Chou ST. Antioxidative activity and safety of the 50 ethanolic extracts from red beam fermented by *Bacillus subtilis* MR-K1. *J. Agricult Food Chem*. 2002;50(8):2454-2458.
23. Maiti M, Kumar GS. Biophysical aspects and biological implications of the interaction of benzophenanthridine alkaloids with DNA. *Biophys Rev*. 2009;1(3):119-129. DOI:10.1007/s12551-009-0014-7
24. Jaradat NA, Abualhasan M, Iyad Ali I. Comparison of anti-oxidant activities and exhaustive extraction yields between wild and cultivated *Cyclamen persicum*, *Malva sylvestris* and *Urtica pilulifera* leaves. *J Appl Pharm Sci*. 2015;5(4):101-107.
25. Murcia MA, Martinez-Tome M, Vera A, Morte A, Gutierrez A, Honrubia M, Jimenez AM. Effect of industrial processing on desert truffles *Terfezia clavaryi* Chatin and *Picoa juniperi* Vittadini: Proximate composition and fatty acids. *J Sci Food Agric*. 2003;83:535-541. DOI: 10.1002/jsfa.1397
26. Janakat SM, Al-Fakhiri SM, Sallal AK. Evaluation of antibacterial activity of aqueous and methanolic extracts of the truffle *Terfezia clavaryi* against *Pseudomonas aeruginosa*. *Saudi Med J*. 2005;26(6):952-957. PMID: 15983681.

© 2021 Al-Mazaideh and Al-Swailmi; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/67737>