Düzce University Journal of Science & Technology

Research Article

In Vitro Antimicrobial Activity Screening of *Leucoagaricus leucothites* and Determination of the Ethanol Extract Composition By Gas Chromatography/Mass Spectrometry

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DOI: 10.29130/dubited.642707

ABSTRACT

Macrofungi are good food resources, which have not only medicinal properties but are nutritive as well. For centuries they have been used for preventing several diseases including cancer, hypercholesterolemia and hypertension. They are also known to present antimicrobial activity, thus the aim of the present study is to put forward antimicrobial activity of ethanol extract of *Leucoagaricus leucothites* (Vittad.) Wasser 1977, a macro fungi and in addition to determine the chemical composition by Gas Chromatography-Mass Spectrometry. Nineteen bacteria strains and one yeast strain are used in antimicrobial screening. According to the data obtained from the study *L. leucothites* presented both antibacterial and antifungal activity against the bacteria and yeast strains used at different concentrations with different levels. Although there are some previous studies, it can be proposed that this study is the first detailed screening report regarding the antibacterial and antifungal potential of *L. leucothites* and the compounds found in *L. leucothites* ethanol extract.

Keywords: *Leucoagaricus leucothites, Mushroom, Antimicrobial activity, Chemical composition, Disk diffusion method, GC-MS*

Leucoagaricus leucothites'in *In Vitro* Antimikrobiyal Aktivite Taraması ve Etanol Ekstrakt Kompozisyonunun Gaz Kromatografisi/Kütle Spektrometresi ile Belirlenmesi

ÖZET

Makro mantarlar sadece tıbbi özelliklere sahip olmayan, aynı zamanda da besleyici olan iyi besin kaynaklarıdır. Yüzyıllar boyunca kanser, hiperkolesterolemi ve hipertansiyon gibi çeşitli hastalıkları önlemek için kullanılmışlardır. Ayrıca makro mantarların antimikrobiyal aktivite gösterdikleri de bilinmektedir, bu nedenle bu çalışmanın ana amacı, bir makro mantar olan *Leucoagaricus leucothites* (Vittad.) Wasser 1977'nin etanol ekstraktının antimikrobiyal aktivitesini ortaya koymak ve ayrıca Gaz Kromatografisi-Kütle Spektrometresi ile kimyasal bileşimin belirlenmesini sağlamaktır. Antimikrobiyal taramada on dokuz bakteri, bir adet maya suşu kullanılmıştır. Bu çalışmadan elde edilen verilere göre *L. leucothites* farklı konsantrasyonlarda kullanılan tüm mikroorganizmalara karşı farklı seviyelerde hem antibakteriyel hem de antifungal aktivite ortaya koymuştur. Her ne kadar daha önce yapılmış birkaç çalışma bulunsa da; bu çalışmanın, *L. leucothites*'in antibakteriyel ve

Received: 04/11/2019, Revised: 28/01/2020, Accepted: 04/02/2020

antifungal potansiyeli ve *L. leucothites* etanol ekstraktında bulunan bileşikler ile ilgili ilk ayrıntılı tarama raporu olduğu öne sürülebilir.

Anahtar Kelimeler: *Leucoagaricus leucothites, Mantar, Antimikrobiyal aktivite, Kimyasal bileşim, Disk difüzyon yöntemi, GC-MS*

I. INTRODUCTION

Mushrooms are accepted as good medicinal and nutritive food resources, which are respectable sources of several vitamins, including vitamin B and D, and some essential minerals, including selenium [1-7]. Furthermore, they are known to include several pharmaceutical compounds, therefore they have a very common use against numerous health problems for centuries, such as antimicrobial agents against more than a few infectious diseases, anti-hypertensives, anti-arrhythmic agents, medications for asthma, anti-neoplastic drugs, analgesics and anti-inflammatory drugs [8-11].

Antimicrobial agents are compounds, those are in use in order not only to prevent but also to treat diseases caused by microorganisms. On the other hand it is quite common that microorganism may change their responses against antimicrobial agents, which can mostly the main reason of antibiotic resistance [12]. World Health Organization (WHO) [12] clearly proposed that microorganisms are developing a tremendous resistance against common antimicrobials all over the world. Thus, success rates in treating infections caused by these microorganisms will decrease, as these antimicrobials appear to be ineffective day by day. Therefore, researchers all over the world have been working harder in order to determine novel antimicrobial compounds [13,14].

Right after the discovery of penicillin by Fleming, researchers increased their attention on the antimicrobial potentials of fungi, which can be used to discover such novel antimicrobial compound candidates [15]. So far, quite a lot of antimicrobial agents were obtained from fungi, which have antimicrobial, antiviral, antidiabetic, anti-inflamatory, anti-fibrotic, liver protective and immune modulatory activities [16-21].

In this study the antimicrobial activity of *Leucoagaricus leucothites* (Vittad.) Wasser 1977 is investigated against nineteen bacteria strains and one yeast strain with a common method known as the disk diffusion method and the compounds found in the *L. leucothites* ethanol extract were determined by gas chromatography/mass spectrometry.

II. EXPERIMENT

A. MACROFUNGI

The macrofungi (*L. leucothites*), which were used for their antimicrobial activity, were obtained from Belgrad Forest, İstanbul, TURKEY. A sample of this macrofungi was stored as a reference.

B. EXTRACTION OF ACTIVE COMPOUNDS

Air dried macrofungi were ground into fine powder by a lab scale blender. Active compounds present in macrofungi were extracted by ethanol (Merck, Germany) through shaking at room temperature for 3 days. Right after the shaking process, the mixture was filtered (Whatman No. 1), and then the ethanol was removed at 30°C through a rotary evaporator (Heidolph Hei-Vap Value HL/HB-G1) [22]. The residue was used to prepare the stock solution.

C. MICROORGANISMS

Bacillus subtilis DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus durans* (food isolate), *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* (food isolate), *Escherichia coli* ATCC 25922, *Escherichia coli* (food isolate), *Klebsiella pneumoniae* (food isolate), *Listeria innocua* (food isolate), *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescence* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis* (food isolate), *Salmonella kentucky* (food isolate), *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (clinical isolate) and *Staphylococcus epidermidis* DSMZ 20044.

D. INOCULA

The conditions for incubation were 24 hours & 37 ˚C, and 48 hours & 27 ˚C for bacteria and *C. albicans* respectively [23]. Inoculum for each microorganism was prepared in sterile saline solution (0.9% w/v) and 0.5 McFarland standard was used to adjust the turbidity of all inocula [24-26].

E. ANTIMICROBIAL ACTIVITY TEST

Disk diffusion test was done according to Andrews [27]. 50, 70 and 180 µL of extract stock was used, so that 16.25, 22.75 and 58.50 mg extract were loaded on 6 mm diameter sterile paper disks [28,29]. The remaining ethanol on paper disks was removed according to the protocols defined previously [14,29]. The inhibition zones were determined in millimeters after incubation at suitable time and temperature combination defined previously [22,23].

F. GC-MS ANALYSIS

The composition of *L. leucothites* ethanol extract was determined by GC-MS analysis defined in previous studies [30-32].

G. POSITIVE AND NEGATIVE CONTROLS

Ethanol loaded antibiotic disks and empty disks were tested as negative controls and ciprofloxacin as positive control.

H. STATISTICS

All test were applied as triplicates. One-way analysis of variance (ANOVA), which is a parametric method was performed (P = 0.05). Pearson correlation coefficient was determined for any possible correlation between the intensity of antimicrobial activity and concentration. R Studio, version 3.3.2 was used for statistical analysis [33].

III. RESULTS & DISCUSSION

Table 1 shows the inhibition zones in millimeters, which are defined with standard errors as the mean values of triplicates.

Empty sterile disks and solvent, which were used as negative controls; presented no activity. Moreover, statistical analysis demonstrated no significant difference between the effects of replicates ($p>0.05$). In contrast, a strong positive correlation (Pearson correlation coefficient = 0.6806) was detected between the effects of *L. leucothites* extracts and extract volume used.

Table 1 presents that 50 µL ethanol extract of *L. leucothites* had antibacterial activity against *S. infantis*, *S. enteritidis* ATCC 13076, *P. fluorescens* P1, *P. aeruginosa* DSMZ 50071, *L.*

monocytogenes ATCC 7644, *L. innocua*, *E. coli* and *E. aerogenes* ATCC 13048 with inhibition zones of 7 mm. 70 µL ethanol extract of *L. leucothites* was presented antibacterial activity against *S. kentucky*, *S. infantis*, *S. enteritidis* ATCC 13076, *P. aeruginosa* DSMZ 50071, *L. innocua*, *K. pneumoniae*, *E. coli* and *B. subtilis* DSMZ 1971 with inhibition zones of either 7 or 10 mm. 180 µL ethanol extract of *L. leucothites* was presented antibacterial and antifungal activity against all strains with inhibition zones ranging between 7 and 11 mm.

The highest activities observed were for 180 µL ethanol extract, which contains 58.50 mg extract, against *E. coli* with 11 mm of inhibition zone and *S. infantis* with 10 mm of inhibition zones.

Results showed that ciprofloxacin have higher activities when they are compared to the activity of *L. leucothites* extract. Increasing the tested amount could possibly increase the activity and in addition purifying the active compound and using against microorganisms would definitely present better activities. On the other hand, the positive control ciprofloxacin presented no activity against *C. albicans* and *E. coli* (food isolate), but *L. leucothites* extract showed for both *C. albicans* and *E. coli* (food isolate).

The GC-MS analysis of *L. leucothites* ethanol extract with its major components and their composition percentages are given in Table 2.

According to Figure 1 and Table 2 the ethanol extract of *L. leucothites* was found to be mostly composed of 9,12-Octadecadienoic acid, which is about 38% of all the extract. On the other hand, the second major compound found in the extract was Ergosta-5,8,22-trien-3-ol, (3.beta.,22E) with about 11%. Other compounds found in the extract from higher percentage to lower were palmitic acid, ethyl oleate, ethyl linoleate, 9,12-octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester, hexadecanamide, docosane, 5,6-Dihydroergosterol, (22E)-Ergosta-5,7,9(11),22-tetraen-3.beta.-ol and 3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester.

Previous studies have presented that some of the major compounds found in the *L. leucothites* ethanol extract have well-known antimicrobial activity. For example, 9,12-octadecadienoic acid, which was observed as the highest compound found in the extract composition, was previously reported to have antimicrobial activity [34,35]. In addition, palmitic acid, which forms the 8.31% of the extract, was also previously proven to present antimicrobial activity [36].

Some studies in the literature have also shown that the ethyl and methyl esters of n-9, n-7 and n-6 fatty acids have strong antimicrobial activity. Although the mechanism of their antimicrobial activity are unknown yet, it is known that these fatty acids form the cell membrane, thus they and their ethyl and methyl esters could possibly target the membranes. As a result, microorganisms can be killed by penetrating and disrupting the normal functions of the membranes [36,37]. Thus, ethyl oleate and ethyl linoleate, which are found in the composition of the extract with 7.10% and 6.31% respectively, could possibly affected antimicrobial activity of the extract too.

Table 1. *Disk diffusion test result for L. leucothites (Inhibition zones in mm).*

Microorganisms	**50** *(µL)*	**70** *(µL)*	**180** *(µL)*	**Ciprofloxacin**
B. subtilis	-	7,00 ± 0,00	9,00 ± 0,00	36
C. albicans	-	-	9,00 ± 0,00	-
E. faecalis	-	-	8,00 ± 0,00	19
E. faecium	-	-	7,00 ± 0,00	29
E. aerogenes	7,00 ± 0,00	-	8,00 ± 0,00	30
E. durans	-	-	8,00 ± 0,00	24
E. coli ATCC 25922	-	-	9,00 ± 0,00	-
E. coli	7,00 ± 0,00	10,00 ± 0,00	11,00 ± 0,33	-
K. pneumoniae	-	7,00 ± 0,00	7,00 ± 0,00	30
L. innocua	7,00 ± 0,00	7,00 ± 0,00	9,00 ± 0,00	18

Table 1 (continuation). *Disk diffusion test result for L. leucothites (Inhibition zones in mm).*

L. monocytogenes	7,00 ± 0,00	-	9,00 ± 0,00	20
P. aeruginosa	7,00 ± 0,00	7,00 ± 0,00	9,00 ± 0,00	28
P. fluorescens	7,00 ± 0,00	-	9,00 ± 0,00	19
S. enteritidis	7,00 ± 0,00	7,00 ± 0,00	9,00 ± 0,00	36
S. infantis	7,00 ± 0,00	7,00 ± 0,00	10,00 ± 0,33	24
S. kentucky	-	7,00 ± 0,00	9,00 ± 0,00	34
S. typhimurium	-	-	9,00 ± 0,00	35
S. aureus ATCC 25923	-	-	8,00 ± 0,00	22
S. aureus	-	-	8,00 ± 0,00	22
S. epidermidis	-	-	8,00 ± 0,00	34

Table 2. *Biochemical composition of L. leucothites.*

No	**Retention Time**	**Compound**	**Formula**	**Molecular Weight** *(g.mol^{-1})*	**Area** *(%)*
1	48,231	Palmitic acid	$C_{16}H_{32}O_2$	256,424	8,31
2	54,640	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280,445	38,22
3	55,181	Ethyl linoleate	$C_{20}H_{36}O_2$	308,499	6,31
4	55,372	Ethyl Oleate	$C_{20}H_{38}O_2$	310,514	7,10
5	55,875	Hexadecanamide	$C_{16}H_{33}NO$	255,439	3,04
6	56,350	Docosane	$C_{22}H_{46}$	310,601	2,97
7	64,040	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	213,316	1,11
8	70,253	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354,524	3,52
9	85,650	(22E)-Ergosta-5,7,9(11),22-tetraen-3.beta.-ol	$C_{28}H_{42}O$	394,630	2,02
10	87,160	Ergosta-5,8,22-trien-3-ol, (3.beta.,22E)-	$C_{28}H_{44}O$	396,648	11,33
11	87,642	5,6-Dihydroergosterol	$C_{28}H_{46}O$	398,664	2,27

The GC-MS chromatogram of *L. leucothites* ethanol extract is given in Figure 1.

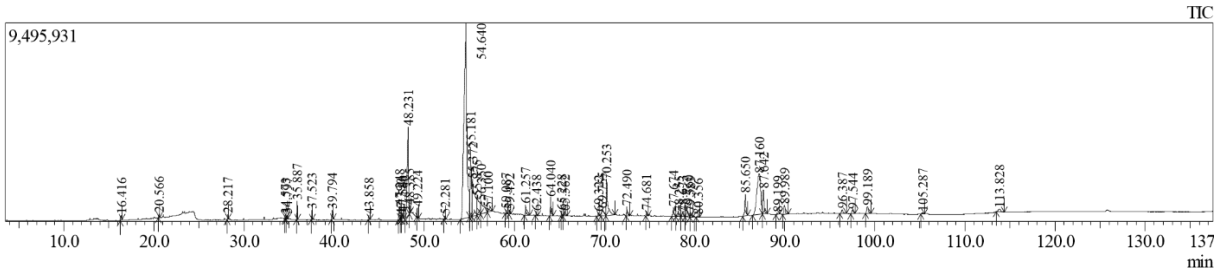

Figure 1. *The GC-MS chromatogram of L. leucothites ethanol extract*

9,12-octadecadienoic acid, palmitic acid, ethyl oleate and ethyl linoleate, which forms in total about 60% of the *L. leucothites* ethanol extract have antimicrobial activity, so observing an antimicrobial activity for *L. leucothites* ethanol extract is logical.

Aslim and Ozturk have previously conducted a research about the antimicrobial activity of *L. leucothites* ethanol extract by agar well diffusion method on *Bacillus cereus* RSKK 867, *E. coli* O157:H7, *L. monocytogenes* ATCC 7644, *P. aeruginosa* ATCC 27853, *Proteus vulgaris* RSKK 96026, *S. aureus* ATCC 25923, *S. enteritidis* 171, *Yersinia enterocolitica* ATCC 1501 and *Shigella sonnei* RSKK 8177 and they observed similar results, which is obtained in our study. Although there are some similar microorganisms used both in the previous study and our study, since the methods

used to determine the antimicrobial activity were different, comparing the results won't be possible [38].

Sevindik et al tested both the antibacterial and antifungal activity of *L. leucothites* ethanol extract against *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 by agar dilution method and they also found that the extract was active against the microorganisms used [39].

IV. CONCLUSION

As a result, the results of the experiments showed that *L. leucothites* have antimicrobial activity, thus it could possibly have pharmaceutical uses. On the other hand, additional experiments are required to understand the activity mechanisms of the active substances in details.

On the other hand, there have been no reports about the GC-MS analysis of *L. leucothites* ethanol extract insofar as the recent literature is taken into account and our results are the first results showing the GC-MS analysis of *L. leucothites* ethanol extract.

V. REFERENCES

[1] F. Watanabe, Y. Yabuta, T. Bito, and F. Teng, "Vitamin B12-Containing plant food sources for vegetarians," *Nutrients*, vol. 6, no. 5, pp. 1861-1873, 2014.

[2] G. Cardwell, J. Bornman, A. James, and L. Black, "A review of mushrooms as a potential source of dietary vitamin D," *Nutrients*, vol. 10, no. 10, pp. 1498, 2018.

[3] J. Falandysz, "Selenium in Edible Mushrooms," *J. Environ. Sci. Heal. C*, vol. 26, no. 3, pp. 256-299, 2008.

[4] M. Bonatti, P. Karnopp, H. M. Soares, and S. A. Furlan, "Evaluation of *Pleurotus ostreatus* and *Pleurotus sajor-caju* nutritional characteristics when cultivated in different lignocellulosic wastes," *Food Chem.*, vol. 88, no. 3, pp. 425-428, 2004.

[5] D. Agrahar-Murugkar and G. Subbulakshmi, "Nutritional value of edible wild mushrooms collected from the Khasi Hills of Meghalaya," *Food Chem.*, vol. 89, no. 4, pp. 599-603, 2005.

[6] L. M. Cheung and P. C. Cheung, "Mushroom extracts with antioxidant activity against lipid peroxidation," *Food Chem.*, vol. 89, no. 3, pp. 403-409, 2005.

[7] A. Imtiaj and T. S. Lee, "Screening of antibacterial and antifungal activities from Korean wild mushrooms," *World J Agric. Sci.*, vol. 3, no. 3, pp. 316-321, 2007.

[8] J. Clardy and C. Walsh, "Lessons from natural molecules," *Nature*, vol. 432, no. 7019, pp. 829-837, 2004.

[9] D. Webster, P. Taschereau, R.J. Belland, C. Sand, and R. P. Rennie, "Antifungal activity of medicinal plant extracts; preliminary screening studies," *J. Ethnopharmacol.*, vol. 115, no. 1, pp. 140-146, 2008.

[10] K. Canli, E. M. Altuner, I. Akata, Y. Turkmen, and U. Uzek, "In vitro antimicrobial Screening of *Lycoperdon lividum* and determination of the ethanol extract composition by gas chromatography/mass spectrometry," *Bangladesh J. Pharmacol.*, vol. 11, no. 2, pp. 389-394, 2016.

[11] K. Canli, I. Akata, and E. M. Altuner, "In vitro antimicrobial activity screening of *Xylaria hypoxylon*," *Afr. J. Tradit. Complement. Altern. Med.*, vol. 13, no. 4, pp. 42-46, 2016.

[12] WHO. (2019, Aug 15). *Antibiotic resistance* [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance.

[13] B. Paudel, H. D. Bhattarai, J. S. Lee, S. G. Hong, H. W. Shin, and J. H. Yim, "Antibacterial potential of antarctic lichens against human pathogenic gram-positive bacteria," *Phytother. Res.*, vol. 22, no. 9, pp. 1269-1271, 2008.

[14] E. M. Altuner, K. Canli, and I. Akata, "Antimicrobial screening of *Calliergonella cuspidata*, *Dicranum polysetum* and *Hypnum cupressiforme*," *J. Pure Appl. Microbio.*, vol. 8, no. 1, pp. 539-545, 2014.

[15] N. Bala, E. A. Aitken, N. Fechner, A. Cusack, and K. J. Steadman, "Evaluation of antibacterial activity of Australian basidiomycetous macrofungi using a high-throughput 96-well Plate assay," *Pharm. Biol.*, vol. 49, no. 5, pp. 492-500, 2011.

[16] B. Dülger, F. Şen, and F. Gücin, "Antimicrobial activity of the macrofungi *Russula delica* Fr.," *Turkish J. Biol.*, vol. 23, no. 1, pp. 127-134, 1999.

[17] N. Gunde-Cimerman, "Medicinal value of the genus Pleurotus (Fr.) P. Karst.(Agaricales Sl, Basidiomycetes)," *Int. J. Med. Mushrooms*, vol. 1, no. 1, pp. 69-80, 1999.

[18] V. E. C. Ooi, "Medicinally important Fungi," in *Science and Cultivation of Edible Fungi*, V. Griensven, Ed., Rotterdam: Balkema, 2010, pp. 41-51.

[19] S. P. Wasser and A. L. Weis, "Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives," *Int. J. Med. Mushrooms*, vol. 1, no. 1, pp. 31-62, 1999.

[20] S. P. Wasser and A. L. Weis, "Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective," *Crit. Rev. Immunol.*, vol. 19, no. 1, pp. 65-96, 1999.

[21] B. Dulger, T. B. Suerdem, D. Yesilyurt, N. Hacioglu, and A. Camdeviren, "Evaluation of antimicrobial activity of the macrofungus *Phellinus torulosus*," *J. Biol. Sci.*, vol. 5, no. 4, pp. 436-439, 2005.

[22] E. M. Altuner and K. Canli, "In vitro antimicrobial screening of *Hypnum andoi* AJE Sm," *Kastamonu Üniv. Orman Fak. Derg.*, vol. 12, no. 1, pp. 97-101, 2012.

[23] E. M. Altuner, "Bazı karayosunu türlerinin antimikrobiyal aktivitesinin belirlenmesi", Doktora tezi, Ankara Üniversitesi, Ankara, Türkiye, 2008.

[24] K. A. Hammer, C. F. Carson, and T. V. Riley, "Antimicrobial activity of essential oils and other plant extracts," *J. Appl. Microbiol.*, vol. 86, no. 6, pp. 985-990, 1999.

[25] E. M. Altuner, I. Akata, and K. Canli, "In vitro antimicrobial activity screening of *Bovista nigrescens* Pers," *Kastamonu Üniv. Orman Fak. Derg.*, vol. 12, no. 1, pp. 90-96, 2012.

[26] K. Canli, A. Yetgin, I. Akata, and E. M. Altuner, "In vitro antimicrobial screening of *Aquilaria agallocha* roots," *Afr. J. Tradit. Complement. Altern. Med.*, vol. 13, no. 5, pp. 178-181, 2016.

[27] J. M. Andrews, "BSAC standardized sisc susceptibility testing method (Version 6)," *J. Antimicrob. Chemother.*, vol. 60, no. 1, pp. 20-41, 2007.

[28] A. M. Mahasneh and A. A. El-Oqlah, "Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Jordan," *J. Ethnopharmacol.*, vol. 64, no. 3, pp. 271-276, 1999.

[29] S. Silici and A. N. Koc, "Comparative study of in vitro methods to analyse the antifungal activity of propolis against yeasts isolated from patients with superficial mycoses," *Lett. Appl. Microbiol.*, vol. 43, no. 3, pp. 318-324, 2006.

[30] M. A.Hossain and A. Rahman, "Chemical composition of bioactive compounds by gc-ms screening and anti-fungal aroperties of the crude extracts of cabbage samples," *Asian J. Biotechnol.*, vol. 3, pp. 68-76, 2011.

[31] K. Canli, A. Yetgin, I. Akata, and E. M. Altuner, "Antimicrobial activity and chemical composition screening of *Anacyclus pyrethrum* Root," *Indian J. Pharm. Educ.*, vol. 51, pp. 244-248, 2017.

[32] K. Canli, A. Yetgin, I. Akata, and E. M. Altuner, "Antimicrobial activity and chemical composition screening of *Epilobium montanum* Root," *Indian J. Pharm. Educ.*, vol. 51, pp. 239-243, 2017.

[33] Core R Team. (2019, Aug 15). *R: A language and environment for statistical computing* [Online]. Available: https://www.R-project.org/.

[34] C. S. Kalaivani, S. S. Sathish, N. Janakiraman, and M. Johnson, "Gc-ms studies on *Andrographis paniculata* (Burm. f.) Wall. Ex Nees-a medicinally important plant," *Indian Int. J. Med. Arom. Plants*, vol. 2, no.1, pp. 69-74, 2012.

[35] M. M. Rahman, S. H. Ahmad, M. T. M. Mohamed, and M. Z. Ab Rahman, "Antimicrobial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava*, and *Andrographis paniculata*," *Sci. World J.*, 2014, Art no. 635240.

[36] C. B. Huang, B. George, and J. L. Ebersole, "Antimicrobial activity of n-6, n-7 and n-9 Fatty acids and their esters for oral microorganisms," *Arch. Oral. Biol.*, vol. 55, no. 8, pp. 555-560, 2010.

[37] C. S. Lunde, S. R. Hartouni, J. W. Janc, M. Mammen, P. P. Humphrey, and B. M. Benton, "Telavancin disrupts the dunctional integrity of the bacterial membrane through targeted interaction with the cell wall precursor lipid II," *Antimicrob. Agents Chemother.*, vol. 53, no. 8, pp. 3375-3383, 2009.

[38] B. Aslim and S. Ozturk, "Phenolic composition and antimicrobial and antioxidant activities of *Leucoagaricus leucothites* (Vittad.) Wasser," *J. Med. Food*, vol. 14, no. 11, pp. 1419-1424, 2011.

[39] M. Sevindik, A. Rasul, G. Hussain, H. Anwar, M. K. Zahoor, I. Sarfraz, K S. Kamran, H. Akgul, I. Akata, and Z. Selamoglu, "Determination of anti-oxidative, anti-microbial activity and heavy metal contents of *Leucoagaricus leucothites*," *Pak. J. Pharm. Sci.*, vol. 31, no. 5, pp. 2163-2168, 2018.