

## Antibacterial activity and GC-MS analysis of baltic amber against pathogenic bacteria

Wijdan H. Al-Tamimi,

*Biology Department, College of Science, University of Basrah, Basrah, Iraq*

Sahar A.A. Malik Al-Saadi,

*Biology Department, College of Science, University of Basrah, Basrah, Iraq*

Ahmed A. Burghal

*University of Basrah, Basrah, Iraq, Biology Department, College of Science*

### Abstract

Amber is a fossil residue from an etched resin from the ancient, used as a medicinal agent to control microbial infections, due to containing chemical compounds that detected by GC-MS analysis. Antibacterial activity was detected for both Dimethyl sulfoxide and ethanolic extracts of orange and brown amber. The largest zones of inhibition were 19 mm and 16 mm for Dimethyl sulfoxide extract of orange and brown amber at 250 mg/ml on *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively, while the largest inhibition zones of the ethanolic extracts were 20 and 22 mm for orange and brown amber at 100 mg/ml on *E. coli*. The GC-MS analysis revealed a total of 35 compounds in Baltic amber. Major chemical components identified in orange and brown amber included borneol (16.80% and 17.60%), isopimar acid methyl ester (17% and 13.65%), camphor (8.15% and 7.04%), 2- Fenchanol (7.44% and 7.76%), and m-cymene (6.24% and 5.40 %), respectively. Orange amber contains seven monoterpenes, six sesquiterpenes, and three diterpenes, while brown amber contains seven monoterpenes, four sesquiterpenes, and one diterpene.

*Keywords: Baltic amber, GC-MS analysis, Antibacterial activity*

### I. INTRODUCTION

The discovery of antimicrobial agents is related to natural products and their derivatives which have historically been invaluable as a source of therapeutic agents [1]. At least 18 strains of bacteria have now evolved resistance to most of our drugs, and by 2050, an estimated 10 million people worldwide will die as a result of drug-resistant bacterial infections. Robert Koch the pioneer of modern bacteriology, analyzed succinic acid from Baltic amber in 1886, and discovered that there is no risk of the accumulation of surplus amounts of succinic acid in humans and confirmed the positive health effects of this substance [2]. [3] Showed that amber the fossil remains of resin exuded by ancient and extinct conifers was once considered one of the top six medicines and was used to cure a myriad of ills. Baltic amber has been believed to have medicinal and therapeutic powers since time immemorial. Baltic amber contains 3 to 8% succinic acid, and studies have demonstrated the beneficial effect of this material on living organisms. Amber has anti-bacterial, antiseptic and strengthen the body's natural immunity properties; because it decrease cold symptoms, fever, and rheumatic and muscle pain.

Amber resin has antibiotic properties and has been used as a wound dressing or plaster, thereby helping to stop bleeding and aiding healing. The history of amber's use as a medicine is long; it continues to be used as a medicinal agent in Europe and the Middle East, where it is used to alleviate stomach aches and rheumatic pains [4-5]. Baltic amber tinctures have antimicrobial properties and can be used to control bacterial, fungal, and viral infections [6-7] studied Conifers of the Baltic amber forest and their palaeoecological significance. [8] Have described the chemical profile and paleoenvironment of Baltic amber. Amber occurs in a range of different colors, including whitish color, lemon yellow, brown, black, red, green and even blue [9]. In the present study we used orange and brown Baltic amber as a bioactive agent against some clinical bacterial isolates and determined the chemical components of the amber extract by GC-MS analysis.

### II- MATERIAL AND METHODS

Two types of amber were used in the present study. The necklaces of orange and brown Baltic amber were obtained from the local market, imported from Germany (Figure 1).

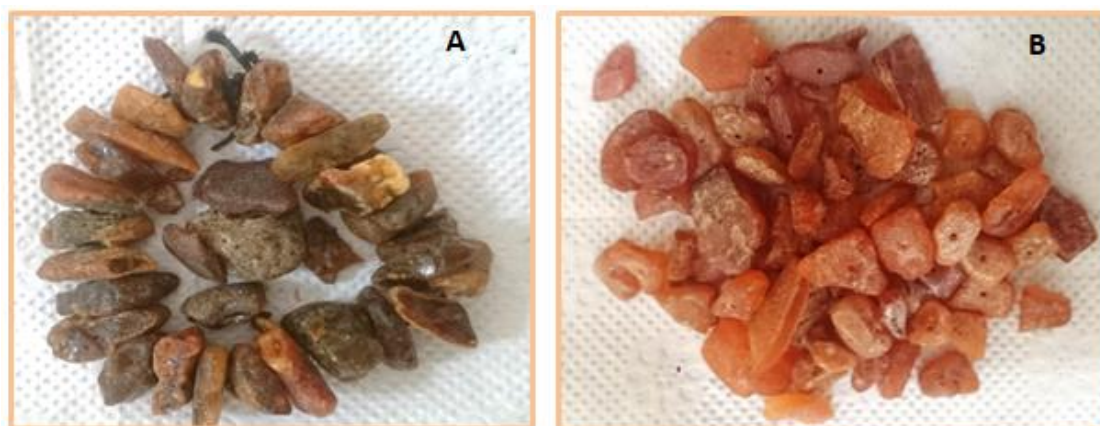


Figure 1. The necklaces of Baltic amber (A) brown amber and (B) orang amber.

### 2.1 DMSO extraction

The amber was crushed using a ceramic mortar and dissolved in dimethyl sulfoxide (DMSO) to obtain the required concentrations of 250, 100, and 50 mg/ml.

### 2.2 Alcohol extraction

The alcohol extract was prepared by dissolving 5 g of amber powder in 100 ml of ethanol. After 24 h with continuous stirring on the magnetic stirrer, the solution was filtered using filtration paper and dried on a Petri dish. The resulting dry product was then dissolved in DMSO to obtain concentrations of 100, 50 and 25 mg/ml [10].

### 2.3 GC-MS analysis

The GC-MS analysis was performed at the University of Basrah, College of Agriculture, Iraq. The GC-MS analysis was performed using Shimadzu GC-QP 2010 Ultra gas chromatography; the GC oven temperature was programmed to increase from 40°C to 300°C at 49.5 kPa; linear velocity was 36.1 cm/sec. The column flow was 1.00 mL/min; injector temperature, 280°C; and injection mode, split. The MS scan conditions were source temperature, 200 °C; interface temperature, 250 °C; Detector gain, 0.69 kV + 0.10 kV; Scan speed, 1000. Start, 50 m/z; End, 500 m/z. The amber components were detected by comparing the spectra with those of known compounds in the NIST library (2005).

### 2.4 Biological activity test

Isolates of bacteria (*Klebsiella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*) were activated in nutrient broth for 24 h. Next, the microbes were swabbed on sterile Mueller Hilton Agar (MHA) plates, and 50 µl of amber extract (Ethanollic and DMSO) at three concentrations (50,100 and 250 mg/ml for DMSO extraction and 25, 50 and 100 mg/ml for ethanollic extraction) was dropped on to the wells, which were punched over the agar plates using a sterile cork borer (7 mm diameter). After 24 hr. incubation at 37 °C the zones of clearance were measured [11]. The results obtained using these two extracts of two amber types were compared.

## III- RESULTS AND DISCUSSION

### 3.1 GC-MS analysis

The GC-MS analysis of the amber extract revealed the presence of 35 compounds (phytochemical constituents) (Figures 2 and 3; Table 1) that could contribute as bioactive agents in Baltic amber. Twenty-seven compounds in orange amber and the same number of compounds in brown amber but differing in quantity and quality.

We identified five major components in the orange and brown amber extracts: borneol (16.80% and 17.60%), isopimar acid methyl ester (17% and 13.65%), camphor (8.15% and 7.04%), 2- Fenchanol (7.44% and 7.76%), and m-cymene (6.24% and 5.40 %) respectively, as well as saturated hydrocarbon camphane, camphene, and isoborneol (Table 1). These findings are in line with the literature [12-13].

There are several resin acids identified in this study; these constituents contain a less percentage compared with other compounds such as agathic acid (1.28%) and abietic acid (1.36%), identified in orange amber only, as well as succinic acid monomethyl ester (3.17%), identified in brown amber.

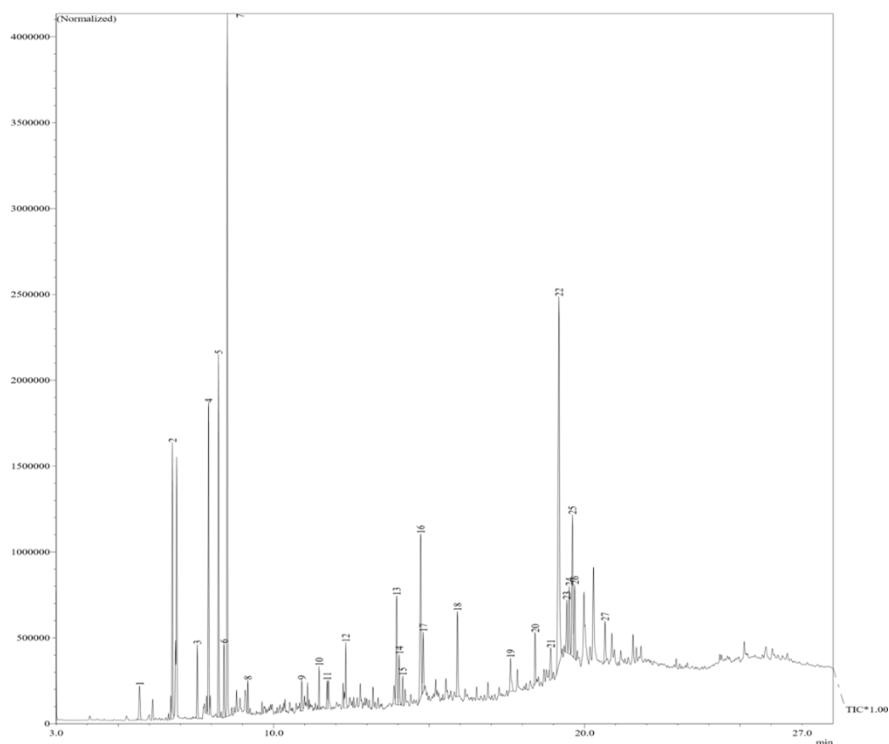


Figure 2. Chromatogram of chemical compounds of orang amber.

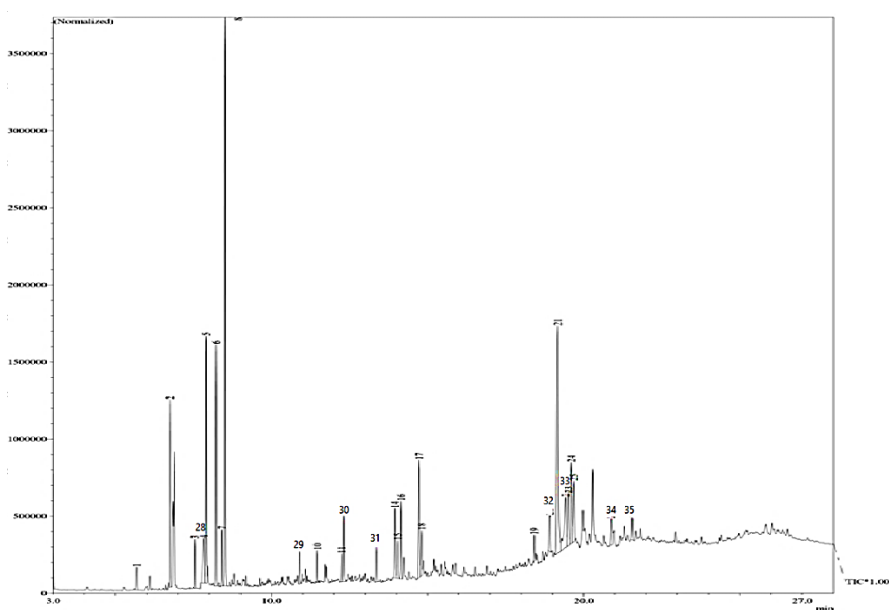
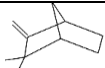
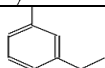
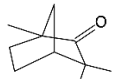
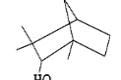
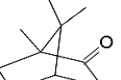
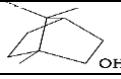
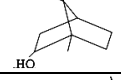
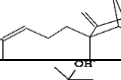
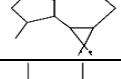
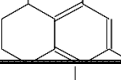
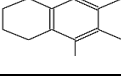
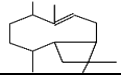
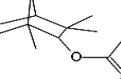
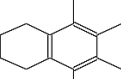
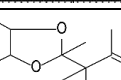
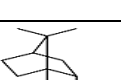
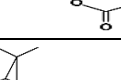
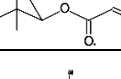
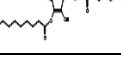
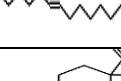
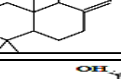
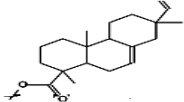
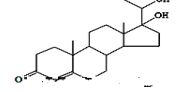
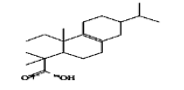
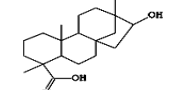
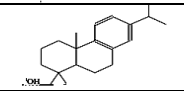
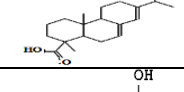
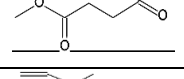
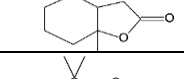
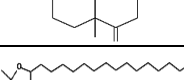
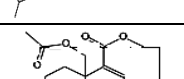
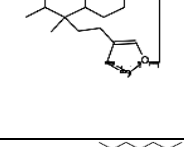
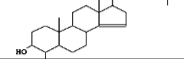
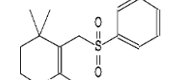
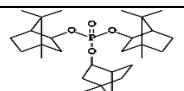


Figure 3. Chromatogram of chemical compounds of brown amber.

**Table -1 The chemical constituents of amber extract using gas chromatography mass spectrometry (GC-MS).**

Chemical structure	Formula	Components	Peak No.	Chemical structure	Content (%)		Retention time	
					Brown	Orange		
	5.686	1.09	1.25		C <sub>10</sub> H <sub>16</sub>	Camphene		1
	6.742	5.40	6.24		C <sub>10</sub> H <sub>14</sub>	m-Cymene		2

7.543	1.45	1.71		C <sub>10</sub> H <sub>16</sub> O	L-Fenchone	3
7.904	7.76	7.44		C <sub>10</sub> H <sub>18</sub> O	2-Fenchanol	4
8.226	7.04	8.15		C <sub>10</sub> H <sub>16</sub> O	Camphor	5
8.404	1.83	1.87		C <sub>10</sub> H <sub>18</sub> O	Isoborneol (Isocamphol)	6
8.506	17.60	16.80		C <sub>10</sub> H <sub>18</sub> O	Borneol	7
9.169	-	0.72		C <sub>15</sub> H <sub>24</sub> O	Santalol, trans-beta	8
10.902	-	0.93		C <sub>15</sub> H <sub>26</sub> O	Viridiflorol	9
11.460	0.92	0.97		C <sub>13</sub> H <sub>18</sub>	Naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl-	10
11.718	-	0.72		C <sub>14</sub> H <sub>20</sub>	Naphthalene, 1,2,3,4-tetrahydro-5,6,7,8-tetramethyl-	11
12.317	0.94	1.94		C <sub>15</sub> H <sub>26</sub> O	Caryophyllenyl alcohol	12
13.954	2.96	3.75		C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acetate, (1S-exo)-	13
14.039	1.53	1.59		C <sub>14</sub> H <sub>20</sub>	Naphthalene, 1,2,3,4-tetrahydro-5,6,7,8-tetramethyl-	14
14.149	2.66	0.86		C <sub>13</sub> H <sub>22</sub> O <sub>2</sub>	1,3-Dioxolane, 2-(2,3-dimethyl-1-cyclopenten-3-yl)-2,4,5-trimethyl-	15
14.729	5.80	6.68		C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	Acetic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester	16
14.810	1.95	1.67		C <sub>13</sub> H <sub>20</sub> O <sub>2</sub>	Isobornyl acrylate	17
15.911	-	2.60		C <sub>38</sub> H <sub>68</sub> O <sub>8</sub>	1-(+)-Ascorbic acid 2,6-dihexadecanoate	18
17.616	-	1.43		C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	Octadec-9-enoic acid	19
18.412	0.97	1.54		C <sub>20</sub> H <sub>30</sub> O	Cryptopinone \$\$ Dextropimarinal \$\$ Pimaral	20
18.913	-	1.28		C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>	Agathic acid	21

19.176	13.65	17.00		C <sub>21</sub> H <sub>32</sub> O <sub>2</sub>	Isopimaric acid methyl ester	22
19.427	-	1.67		C <sub>21</sub> H <sub>32</sub> O <sub>3</sub>	Pregn-4-en-17(alpha),20(alpha)-diol-3-one	23
19.509	2.81	2.34		C <sub>20</sub> H <sub>32</sub> O <sub>2</sub>	13Alpha-delta(8)-dihydroabietic acid (Abiet-8-en-18-oic acid)	24
19.610	3.57	5.01		C <sub>20</sub> H <sub>32</sub> O <sub>3</sub>	Dihydroxyisosteviol	25
19.685	3.11	2.48		C <sub>20</sub> H <sub>30</sub> O	1-methylet Abieta-8,11,13-trien-18-ol	26
20.659	-	1.36		C <sub>20</sub> H <sub>30</sub> O <sub>2</sub>	Abietic acid	27
7.828	3.17	-		C <sub>5</sub> H <sub>8</sub> O <sub>4</sub>	Succinic acid monomethyl ester	28
10.903	1.11	-		C <sub>12</sub> H <sub>18</sub> O <sub>2</sub>	Epianastrephin	29
12.316	2.24	-		C <sub>13</sub> H <sub>22</sub> O	5,5,8a-Trimethyldecalin-1-one	30
13.359	1.24	-		C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	1,3-Dioxolane, 4,4,5-trimethyl-2-pentadecyl-	31
18.907	1.84	-		C <sub>23</sub> H <sub>32</sub> O <sub>5</sub>	1.alpha-(Acetoxymethyl)-7.alpha.,8.alpha.-dimethyl-7-(2-(3-furyl)ethyl)bicyclo(4.4.0)dec-2-ene-2-carboxylic acid methyl ester	32
19.416	4.89	-		C <sub>28</sub> H <sub>48</sub> O	Cholest-14-en-3-ol, 4-methyl-, (3.beta.,4.alpha.,5.alpha.)-(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl) benzene	33
20.881	1.62	-		C <sub>16</sub> H <sub>22</sub> O <sub>2</sub> S	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl) benzene	34
21.560	0.86	-		C <sub>30</sub> H <sub>51</sub> O <sub>4</sub> P	Phosphoric acid, tribornyl ester	35

Also, isopimaric acid methyl ester and dehydroabietic acid were found in orange and brown amber, and these results are consistent with the literature ([14-17] report that succinite did not contain free succinic acid, but ester formation might induce cross-linking with ester, forming succinic acid monomethyl ester, which agrees with our study, whereas [18] reported succinic acid as free and identified six different esters could be in extracts of amber. Our results assured that abietic acid in amber found in lower concentrations which disagreed with [6]. Our GC-MS analyses identified naphthalene components, such as naphthalene, and 1,2,3,4-tetrahydro-5,6,7,8-tetramethyl- and naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl, which is consistent with the findings of [19]. Based on GC-MS spectroscopy studies, succinite has a botanical origin because it contains succinic acid, fenchol, borneol, isborneol, sesquiterpenes, diterpenes, and monoterpenes compounds [20], and supported that Baltic amber reflects source plant metabolism or diagenetic alteration, [21]. On the other hand, a less concentration of succinic acid supported that amber derived from coniferous

trees, which agreed with [22-23] reported that no extant conifer produces succinic acid and related compounds as copiously as is found in Baltic amber.

### 3.2 Biological activity test

Different types of pathogen (positive, negative and biofilm-forming) bacteria are used to estimate the biological activity of the extracted compounds [24]. Here we report antimicrobial effects for both orange and brown amber (Tables 2 and 3). The highest inhibition activity for the brown amber DMSO extraction was 16 mm at a concentration of 250 mg/ml on *P. aeruginosa*; the lowest inhibition activity was 11 mm at 50 mg/ml on *E. coli* and *S. aureus* (Table 2). For orange amber, the largest inhibition zone was 19 mm at 250 on *S. aureus*, and the lowest was 9 mm at 50 mg/ml on *E. coli* (Table 3). Furthermore, there was no effect of two types of amber on bacteria of *Klebsiella sp.*

For the brown and orange amber ethanolic extractions, the largest inhibition zones were 22 and 20 mm respectively, at 100 mg/ml on *E. coli* (Table 4,5 and Figures 4,5), while there was no effect for the ethanolic extractions against *Klebsiella sp.* and *Pseudomonas aeruginosa*.

**Table 2 Antimicrobial activity of DMSO extract of brown amber**

Con. mg/ml	Inhibition zone (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Klebsiella sp.</i>
50	11	11	12	0
100	13	12	14	0
250	14	13	16	0

**Table 3 Antimicrobial activity of DMSO extract of orange amber**

Con. mg/ml	Inhibition zone (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Klebsiella sp.</i>
50	14	9	12	0
100	15	11	17	0
250	19	16	18	0

Amber has bioactivity against clinically relevant bacteria, which is consistent with the findings of [2], who showed that for treating viral, fungal or bacterial infections, 1 or 2 drops of amber tincture per day in water daily for 3 weeks can be an effective dose. Also, these results agreed with those of [6] who reported that Baltic amber has potent antibacterial and antifungal properties against pathogenic microorganisms. [25] reported that abietic acid, pinene, and camphene are effective anti-acne agents.

Many succinate components act as antioxidants [26], antibacterial, nematocides, antivirals and antifungals [27-29], repellents and insecticides [30]. Camphore, camphene, borneol, terpinene-4-ol, p-cymene, fenchol, isoborneol, abietic acid, pimaric acid and isopimaric acid inhibit the biological and biochemical functions of organisms, as well as inhibiting the growth of bacteria (*P. aeruginosa*, *Escherichia coli*, *S. aureus* and *K. pneumoniae*) [31]. Also, these compounds available in the amber make it potentially useful for the preparation of modern medicines because they exhibit antibacterial, antifungal, anti-inflammatory, insecticidal and antioxidant potential, P-cymene, limonene, terpinen-4-ol and pulegone exhibit antioxidative properties. Also, fenchone totally inhibits the growth of *Rhizoctonia solani* [30]. Given the potential activity of Baltic amber on clinical bacteria, it might be useful agent against multiple drug resistant bacteria.

The results of antibacterial activity revealed that both types of amber affected clinical bacterial isolates and that the ethanolic extract was more potent than the DMSO extract. The GC MS analysis found that the orange amber contains seven components monoterpenes, six compounds sesquiterpenes, and three diterpenes, while the brown amber contains seven components monoterpenes, four compounds sesquiterpenes, and one diterpenes. These results are consistent with those reported by [6]. [36] who noted that amber is rich in terpenes, esters, hydrocarbons, acids, alcohols, and phenols, identifying five monoterpenes, seven sesquiterpenes and two diterpenes.

**Table- 4 Antimicrobial activity of ethanolic extract of brown amber**

Con. mg/ml	Inhibition zone (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Klebsiella sp.</i>
25	11	13	0	0
50	13	15	0	0
100	20	22	0	0

**Table- 5 Antimicrobial activity of ethanolic extract of orang amber**

Con. mg/ml	Inhibition zone (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Klebsiella sp.</i>
25	15	12	0	0
50	16	16	0	0
100	16	20	0	0

#### IV. CONCLUSION

Natural substances are a safe source for the treatment of many microbial infections. Effective substances were extracted from orange and brown amber, the GC, the Mass spectrometry analysis showed that they contain biologically active compounds that have the potential to inhibit pathogen microbes.

#### REFERENCE

- [1] T. Aikaterini, F. Nikolas and L. S. Alexios, "Natural Resins and Bioactive Natural Products thereof as Potential Antimicrobial Agents", *Current Pharmaceutical Design*, vol. 17, pp.1267-1290, 2011.
- [2] G. F. Edwards, "The medicinal use, preparation and application of baltic amber," *Benedicaria–The Blessing Way of Southern Italian Folk Medicine–Part 2 – Gail Faith Edwards*. 2015.
- [3] G. C. Granai, "The Magic of Amber". Copyright Gary C. Granai 2104. All Rights Reserved [www.natural-baltic-amber.com](http://www.natural-baltic-amber.com).pp.35, 2014.
- [4] S. Dharmananda, "Succinum (Amber) use in Chinese Medicine," *ITM's START Group Index - May 2003*.
- [5] C. Dietz, G. Catanzariti, S. Quintero and A. Jimeno, "Roman amber identified as Siegburgite. *Archaeol. Anthropol. Sci.*, vol. 6, pp. 63-72, 2014.
- [6] P. Tumiłowicz, L. Synoradzki, A. Sobiecka, J. A. Katarzyna, K. Pytkowska and S. Safarzyński, "Bioactivity of Baltic amber-fossil resin," *Polimery*, vol. 61, ( 5), pp. 347- 356, 2016.
- [7] E. M. Sadowski, A. R. Schmidt, I. J. Seyfullah and L. kunzmann, "Conifers of the 'Baltic amber forest' and their palaeoecological significance," *Stapfia* vol. 106, 1–73, 2017.
- [8] V. Friedman, J.B. Lambert, T.A. Contreras, E. Stout S. Kaur and H. Mitamura, "Late Cretaceous Amber in Texas: Chemical Characterization and Paleoenvironment," *Life: The Excitement of Biology*, vol. 5(3), 132-154, 2018.
- [9] P. Bowersock, B.P. Brown, and O. Grabar, "Amber," 19 In G. W. (eds.) *Late Antiquity: A Guide to the Postclassical World*, Harvard University Press, ISBN 0674511735, 1999.
- [10] J. P. F. Angeli, L. R. Ribeiro, C.M. Camellini, M. M. de Mendonca, and M. S. Mantovani, "Evaluation of the antigenotoxicity of oligosaccharides and B-glucans from *Agaricus blazei*, a model study with the single cell gel electrophoresis/Hep G2 assay," *J. Food Compos Anal*, vol. 22, pp. 699-703, 2009.
- [11] M. T. Madigan, J. M. Martinko, P. V. Dunlap and D. P. Clark, 2009. "Brock biology of microorganisms," Pearson International Edition, U.S.A.
- [12] F. Czechowski, B.R.T. Simoneit, M. Sachanbinski, J. Chojcan and S. Wołowiec, "Physicochemical structural characterization of ambers from deposits in Poland," *Applied Geochemistry*, vol, 11, pp. 811-834, 1996.
- [13] M. Sachabiński, F. Czechowski, J. Chojcan and S. Wołowiec, "Acta Universitatis Wratislaviensis. Vol. 55, 175, 1997.
- [14] R. Nicoletti, "Analisi di ambre: un nuovo approccio. – Studi e ricerche sulla problematica dell'ambra. – pp. 299-305, Rome (Consiglio Nazionale delle Ricerche), 1975.
- [15] J. S. Mills, R. White and L. J. Gough, "The chemical composition of Baltic amber," *Chem. Geol.* Vol. 47, pp. 15–39, 1984.

- [16] N. Vávra, "The Chemistry of Amber – Facts, Findings and Opinions," *Ann. Naturhist. Mus. Wien.* Vol. 3, pp. 445–474, 2009.
- [17] R.C.A. Rottländer, "Einführung in die naturwissenschaftlichen Methoden in der Archäologie," (= *Archaeologica Venatoria*, Vol. 6). – 604 p., In: Rottländer, R. (ed.): *Archaeologica venatoria*. – Tübingen (*Archaeologica Venatoria*, Institut für Vorgeschichte der Universität Tübingen). 1983.
- [18] S. Yamamoto, A. Otto, G. Krumbiegel and B.R.T. Simoneit, "The natural product biomarkers in succinite, glessite and stantienite ambers from Bitterfeld, Germany," *Review of Palaeobotany and Palynology*, vol. 140, pp. 27-49, 2006.
- [19] A. Matuszewska, "Bursztyn (sukcynit), inne żywice kopalne, subfosylne współczesne," *Uniwersytet Śląski Oficyna Wydawnicza Waclaw Walasek, Katowice*, 2010.
- [20] C. W. Beck, E. Wilbur, S. Meret, D. Kossove and K. Kermani, "The infrared spectra of amber and the identification of Baltic amber," *Archaeometry*, vol. 8, pp. 96–109, 1965.
- [21] A.P. Wolfe, R. Tappert, K. Muehlenbachs, M. Boudreau, R.C. McKellar, G.F. Basinger and A. Garrett, "A new proposal concerning the botanical origin of Baltic amber," *Proc. R. Soc. B.* vol. 276, pp. 3403–3412, 2009.
- [22] A. Otto, and V. Wilde, "Sesqui-, di-, and triterpenoids as chemosystematic markers in extant conifers," a review. *Bot. Rev.*, vol. 67, pp. 141–248, 2001.
- [23] A. Otto, B. R. Simoneit and V. Wilde, "Terpenoids as chemosystematic markers in selected fossil and extant species of pine (*Pinus*, Pinaceae)," *Bot. J. Linn. Soc.* Vol. 154, pp. 129-140, 2007.
- [24] G.M. Saleh, S.A. Alash, H.Y. Fadil and H.B. Ali, "The effect of termites extract on inhibition of growth of some pathogenic bacteria and synthesis of biofilm," *Iraqi Journal of Agricultural Sciences*, vol. 51, pp. 176-183, 2020.
- [25] C.D. Carpenter, T. O'Neill, N. Picot, J.A. Johnson, G.A. Robichaud, D. Webster, and C.A. Gray, "Anti-mycobacterial natural products from the Canadian medicinal plant *Juniperus communis*," *J. Ethnopharmacol.* vol. 143(2), 695-700, 2012.
- [26] M.K.Lee, H. Yang and J.S. Yoon, "Antifibrotic activity of diterpenes from *Biota orientalis* leaves on hepatic stellate cells," *Arch. Pharm. Res.*, vol. 31 pp. 866–7, 2008.
- [27] I. Kubo, H. Muroi and A. Kubo, "Naturally Occurring Antiacne Agents," *J. Nat. Prod.*, vol. 57(1), pp. 9-17, 1994.
- [28] N. Filipowicz, M. Kamiński, J. Kurlenda, M. Asztemborska and J.R. Ochocka, "Antibacterial and antifungal activity of juniper berry oil and its selected components," *Phytother. Res.*, vol. 17, pp. 227-23, 2003.
- [29] A. Angioni, A. Barra, V. Coroneo, S. Dessi and P. Cabras, "Chemical composition, seasonal variability and antifungal activity of *Lavandula stoechas* L. ssp. *stoechas* essential oils from stem / leaves and flowers," *J. Agricul. Food Chem.*, vol. 54, pp. 4364- 4370, 2006.
- [30] M. Hori, "Repellency of rosemary oil against *Myzus persicae* in a laboratory and in a greenhouse," *J. Chem. Ecol.*, vol. 24, pp. 1425–1432, 1998.
- [31] S. Chen, Q. Xin, W.X. Kong and J.I. Min, "Anxiolytic-like effect of succinic acid in mice," *Life Sciences*, vol. 73, pp. 3257-3264. 2003.
- [32] D. W. Armstrong, E. Y., Zhou, J. Zukowski and B. Kosmowska-Ceranowicz, "Enantiomeric composition and prevalence of some bicyclic monoterpenoids in amber," *Chirality*, vol. 8, pp. 39-48. 1996.
- [33] J. Zou, and R.G. Cates, "Effects of terpenes and phenolic and flavonoid glycosides from Douglas fir on western spruce budworm larval growth, pupal weight and adult weight," *Journal of Chemical Ecology*, vol. 23, pp. 2313-2326, 1997.
- [34] K.A. Hammer, C.F. Carson and T.V. Riley, "Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil," *J. Appl. Microbiology*, vol. 95(4), pp. 853-860, 2003.
- [35] M. Popova, I. Chinou, I. Marekov and V. Bankova, "Terpenes with antimicrobial activity from Cretan propolis," *Phytochemistry*, vol. 70, pp. 1262–1271. 2009.
- [36] G. Alexander, I. Hazai, J. Grimalt, and J. Albaigés, "Occurrence and transformation of phyllocladanes in brown coals from Norgrad Basin, Hungary," *Geochimica et Cosmochimica Acta*, vol. 51, pp. 2065-2073. 1987.