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

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***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 aster (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).

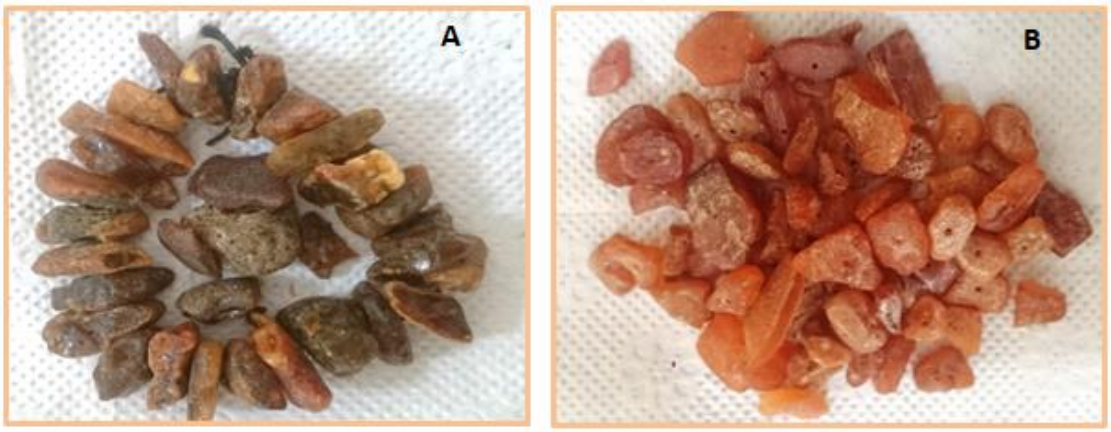



Figure 1. The necklaces of Baltic amber (A) brown amber and (B) orang amber.

### 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 (Ethanolic and DMSO) at three concentrations (50,100 and 250 mg/ml for DMSO extraction and 25, 50 and 100 mg/ml for ethanolic 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 ºC 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 aster (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.

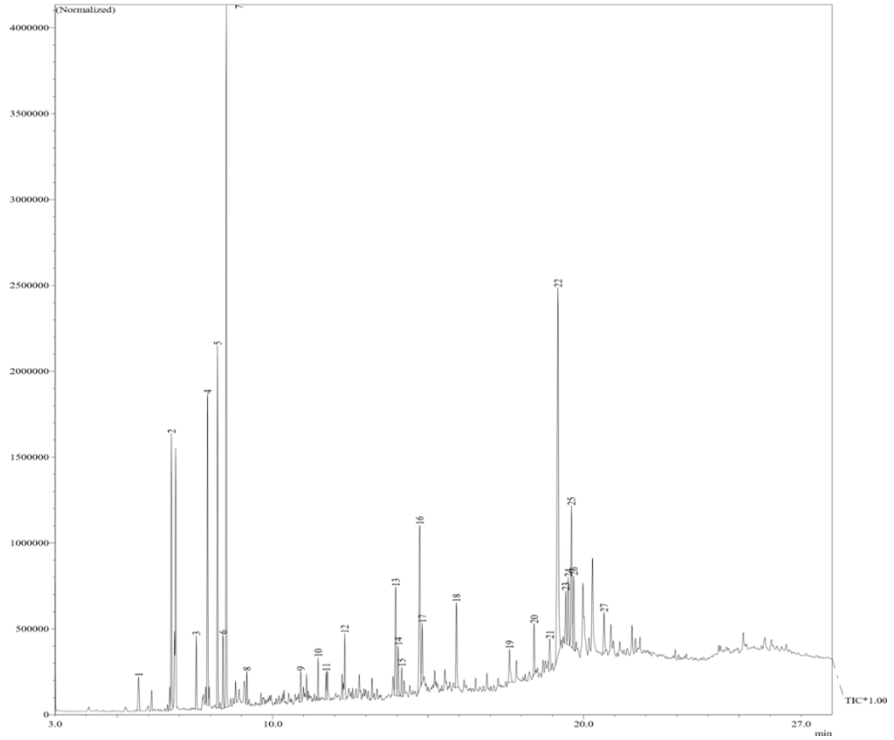



Figure 2. Chromatogram of chemical compounds of orang amber.

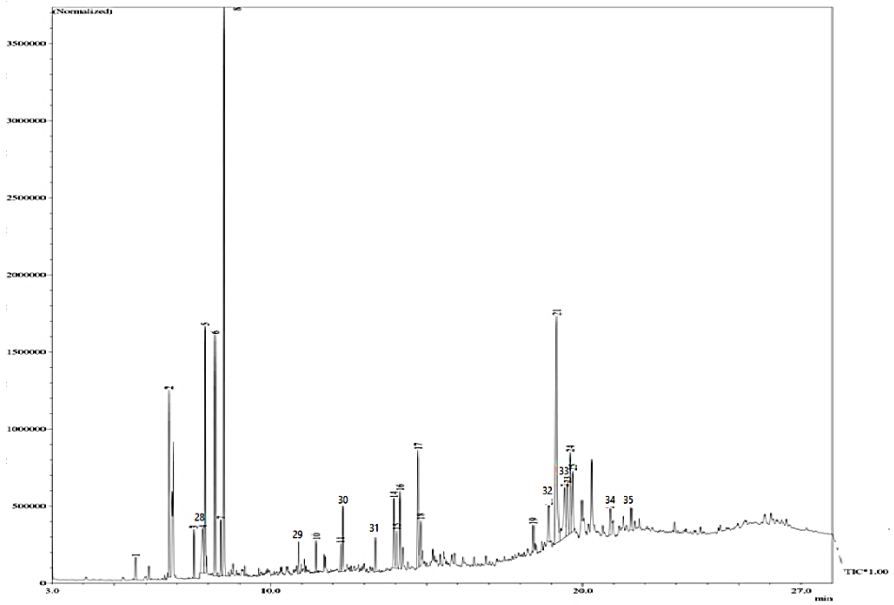
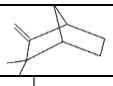
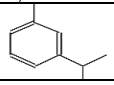
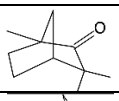
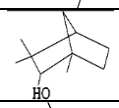
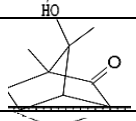
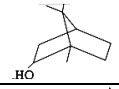
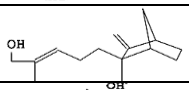
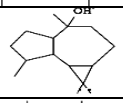
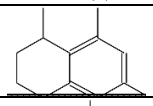
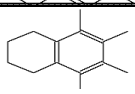
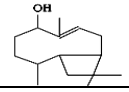
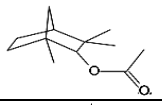
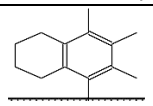
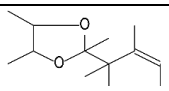
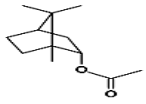
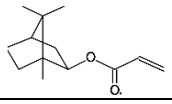
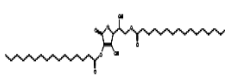
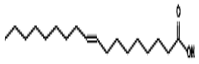
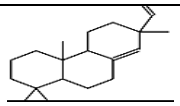
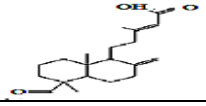
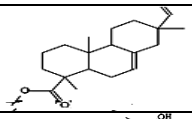
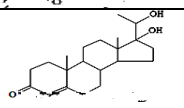
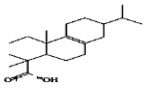
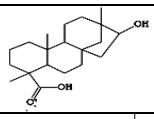
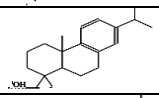
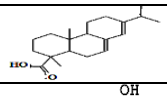
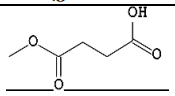
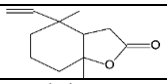
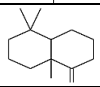
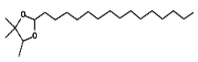
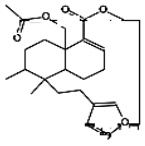
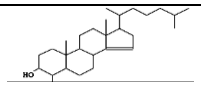
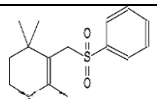
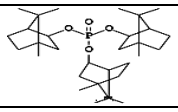



Figure 3. Chromatogram of chemical compounds of brown amber.

**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 |  | $C_{10}H_{16}$ | Camphene | | 1 |
| 6.742 | 5.40 | 6.24 |  | $C_{10}H_{14}$ | m-Cymene | | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7.543 | 1.45 | 1.71 |  | $C_{10}H_{16}O$ | L-Fenchone | 3 |
| 7.904 | 7.76 | 7.44 |  | $C_{10}H_{18}O$ | 2-Fenchanol | 4 |
| 8.226 | 7.04 | 8.15 |  | $C_{10}H_{16}O$ | Camphor | 5 |
| 8.404 | 1.83 | 1.87 |  | $C_{10}H_{18}O$ | Isoborneol (Isocamphol) | 6 |
| 8.506 | 17.60 | 16.80 |  | $C_{10}H_{18}O$ | Borneol | 7 |
| 9.169 | - | 0.72 |  | $C_{15}H_{24}O$ | Santalol, trans-beta | 8 |
| 10.902 | - | 0.93 |  | $C_{15}H_{26}O$ | Viridiflorol | 9 |
| 11.460 | 0.92 | 0.97 |  | $C_{13}H_{18}$ | Naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl- | 10 |
| 11.718 | - | 0.72 |  | $C_{14}H_{20}$ | Naphthalene, 1,2,3,4-tetrahydro-5,6,7,8-tetramethyl- | 11 |
| 12.317 | 0.94 | 1.94 |  | $C_{15}H_{26}O$ | Caryophyllenyl alcohol | 12 |
| 13.954 | 2.96 | 3.75 |  | $C_{12}H_{20}O_2$ | Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acetate, (1S-exo)- | 13 |
| 14.039 | 1.53 | 1.59 |  | $C_{14}H_{20}$ | Naphthalene, 1,2,3,4-tetrahydro-5,6,7,8-tetramethyl- | 14 |
| 14.149 | 2.66 | 0.86 |  | $C_{13}H_{22}O_2$ | 1,3-Dioxolane, 2-(2,3-dimethyl-1-cyclopenten-3-yl)-2,4,5-trimethyl- | 15 |
| 14.729 | 5.80 | 6.68 |  | $C_{12}H_{20}O_2$ | Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester | 16 |
| 14.810 | 1.95 | 1.67 |  | $C_{13}H_{20}O_2$ | Isobornyl acrylate | 17 |
| 15.911 | - | 2.60 |  | $C_{38}H_{68}O_8$ | l-(+)-Ascorbic acid 2,6-dihexadecanoate | 18 |
| 17.616 | - | 1.43 |  | $C_{18}H_6O_2$ | Octadec-9-enoic acid | 19 |
| 18.412 | 0.97 | 1.54 |  | $C_{20}H_{30}O$ | Cryptopinone $$ Dextropimarinal $$ Pimaral $$ Pimarinal | 20 |
| 18.913 | - | 1.28 |  | $C_{20}H_{30}O_4$ | Agathic acid | 21 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 19.176 | 13.65 | 17.00 |  | $C_{21}H_{32}O_2$ | Isopimaric acid methyl ester | 22 |
| 19.427 | - | 1.67 |  | $C_{21}H_{32}O_3$ | Pregn-4-en-17(alpha),20(alpha)-diol-3-one | 23 |
| 19.509 | 2.81 | 2.34 |  | $C_{20}H_{32}O_2$ | 13Alpha-delta(8)-dihydroabietic acid (Abiet-8-en-18-oic acid) | 24 |
| 19.610 | 3.57 | 5.01 |  | $C_{20}H_{32}O_3$ | Dihydroxyisosteviol | 25 |
| 19.685 | 3.11 | 2.48 |  | $C_{20}H_{30}O$ | 1-methylet Abieta-8,11,13-trien-18-ol | 26 |
| 20.659 | - | 1.36 |  | $C_{20}H_{30}O_2$ | Abietic acid | 27 |
| 7.828 | 3.17 | - |  | $C_5H_8O_4$ | Succinic acid monomethyl ester | 28 |
| 10.903 | 1.11 | - |  | $C_{12}H_{18}O_2$ | Epianastrephin | 29 |
| 12.316 | 2.24 | - |  | $C_{13}H_{22}O$ | 5,5,8a-Trimethyldecalin-1-one | 30 |
| 13.359 | 1.24 | - |  | $C_{21}H_{42}O_2$ | 1,3-Dioxolane, 4,4,5-trimethyl-2-pentadecyl- | 31 |
| 18.907 | 1.84 | - |  | $C_{23}H_{32}O_5$ | 1.alpha-(Acetoxymethyl)-7alpha.,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 | - |  | $C_{28}H_{48}O$ | Cholest-14-en-3-ol, 4-methyl-, (3.beta.,4.alpha.,5.alpha.)- | 33 |
| 20.881 | 1.62 | - |  | $C_{16}H_{22}O_2S$ | (2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl) benzene | 34 |
| 21.560 | 0.86 | - |  | $C_{30}H_{51}O_4P$ | 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, isoborneol, 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 Klepseilla sp. and Pseudomonas aeruginosa.

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

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

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

| **Con. mg/ml** | **Inhibition zone (mm)** | | | |
|---|---|---|---|---|
| | ***S. aureus*** | ***E. coli*** | ***P. aeruginosa*** | ***Klebsiella sp.*** |
| 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, nematicides, 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) | | | |
|---|---|---|---|---|
| | ***S. aureus*** | ***E. coli*** | ***P. aeruginosa*** | ***Klebsiella sp.*** |
| 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) | | | |
|---|---|---|---|---|
| | ***S. aureus*** | ***E. coli*** | ***P. aeruginosa*** | ***Klebsiella sp.*** |
| 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.

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