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# Synergistic interactions between Labiatae species and antibiotics on gram positive and gram negative bacterial strains

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## Abstract

**Objective and methods:** This study was aimed to evaluate antibacterial activity; type of interaction between chloroform leaves extract of *Mentha piperita*, *Mentha longifolia* and *Ocimum basilicum* together and with antibiotics by agar well diffusion method on isolated bacterial strain and to determine active constituents responsible on antibacterial activity by agar overlay bioautographic method.
**Results:** *Mentha piperita* exhibited more pronounced inhibition zone (20mm) against *Staphylococcus aureus, Staphylococcus auricularis, Streptococcus mitis* and *Escherichia coli* followed by *Mentha longifolia* highest inhibition zone (15mm) against *Staphylococcus auricularis* and *Streptococcus mitis*, then *Ocimum basilicum* exhibited highest inhibition zone (14mm) only against *Staphylococcus auricularis*. As a result of combination chloroform leaves extract with each other the strongest synergistic activity exhibited during combination of *Mentha longifolia* with *Ocimum basilicum* (14 to 25mm) against *Escherichia coli*, while during combination of leaves extract with antibiotics the greatest synergistic activity resulted from combination of *Mentha piperita* with azithromycin and ampicillin separately (14 to 30mm) and (10 to 23mm) against *Escherichia coli* and *Streptococcus mitis* respectively. *Escherichia coli* revealed susceptibility to all leaves extract and antibiotics alone and pronounced increase in susceptibility to combination of leave extract with each other and with antibiotics. Bioautography assay showed presence number active constituents with antibacterial activity in leaves extract in which three of them identified as caffeic acid, luteolin and rosmarinic acid.
**Conclusion:** From the result concluded that *Mentha piperita* showed strongest activity followed by *Mentha longifolia* then *Ocimum basilicum* alone and there activity increased in combination form.
**Keywords:** Labiatae species, herbal-herbal interaction, herbal-drug interaction, agar well diffusion, bioautography.

## 1. Introduction

Multiple bacterial resistance is the ability of bacteria due to abuse antimicrobial agents by humanity over the world not only accept resistance against single drug but also against multiple antibacterial agents [1] and today the rate of development resistance higher than rate discovery of new antimicrobial agents to combat them [2]. Antibacterial activity of plant with their bioactive constituents since ancient time attracted great attention [3,4]. Now day activity plants have been evaluated not only for direct activity, but also as resistance modifying agents [5,6 ].

Number of study found that antimicrobial activity different class of synthetic drugs improved by combining them with various natural products such as against *Staphylococcus species, Escherichia coli* and *Pseudomonas aeruginosa* [7,8]. The type of effect expected by stimulate the activity of a antibacterial drugs may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms, enhancing the elimination of plasmids from bacteria such as *Escherichia coli*, prevent transport functions of the plasma membrane in regard to given drugs or modifying the resistance of specific bacteria to given drugs [9]. This study was aimed to evaluate in vitro antibacterial activity, influence of interaction between chloroform leaves extract *Mentha piperita, Mentha longifolia* and *Ocimum basilicum* together which belong to family Labiatae and with antibiotics such as

ampicillin and azithromycin by agar well diffusion method on susceptibility of gram positive and gram negative bacteria and to determine active constituents responsible on antibacterial activity by agar overlay bioautographic method.

## 2. Material and Methods

### 2.1 Materials

Ethanol and chloroform from Scharlab S.L. (Spain), muller Hinton agar, nutrient agar (Merck Co. Germany), dimethyl sulfoxide (Scharlau Chemie S.A.), azithromycin (Bioactive T, United Kingdom), ampicillin (Sandoz GmbH, Kundl, Austria), dimethyl thiazolyl diphenyl tetrazolium bromide (MTT) (Taizhou xianju pharma.co., China).

### 2.2 Preparation of leaves extract

Hundred gm of the dried powdered leaves were extracted with 100 ml (80%) ethanol for 1 hr using soxhlet extractor that yielded an extract, which was after drying dissolved in 20 ml (20%) HCl and refluxed for 30 min, followed by liquid-liquid fractionation using chloroform (10 x 3 ml) resulted an chloroform fraction on drying in vacuum that used for evaluation of antibacterial activity.

### 2.3 Microorganisms and inoculate preparation

The bacteria used in this study included *Staphylococcus aureus*, *Staphylococcus auricularis*, *Streptococcus mitis*, *Streptococcus pneumoniae*, *Klebseilla pneumoniae*, and *Escherichia coli*. These organisms were obtained from the department of Biology, Collage of Education, Salahadin University. The media used for the growth and maintenance of microorganisms were nutrient agar and blood agar. Each of the isolated bacteria was suspended in a saline solution (0.9 %, w/v) and matched with 0.5 McFarland standards to give a resultant concentration of $1.5 \times 10^8$ cfu/mL.

### 2.4 Antibacterial activity screening

#### 2.4.1 Agar well diffusion assay

The Antibacterial activity was measured using agar well diffusion method [10] by swabbing the surface of agar plates which contain 25ml Mueller-Hinton agar with the prepared saline suspension of each strain. Wells were then bored into the agar medium with heat sterilized 5 mm cork borer and filled with 50 µl of leaves extract alone, antibiotics alone and in case of synergism effect 50 µl of extract-extract and extract-antibiotic has been added into well. DMSO used as negative control. Replicate of each plate has been done; the plates were incubated at 37°C for 24 h. The antibacterial activity was assessed by measuring the inhibition zone diameter (mm) around the well.

#### 2.4.2 Evaluation of activity index

The significant use of chloroform leave extract of *Mentha piperita*, *Mentha longifolia* and *Ocimum basilicum* with standard antibiotics was estimated by calculating of activity index according to equation described by Singh *et al* [11]:

Activity index (AI) = Mean of inhibition zone of leave extract/ Mean of inhibition zone of standard antibiotics

#### 2.4.3 Agar overlay bioautography assay

The agar overlay method was used for identification bioactive constituents responsible on antibacterial activity of chloroform extract of *Mentha piperita*, *Mentha longifolia* and *Ocimum basilicum*. Leaves extract were applied on thin layer chromatography (TLC) plate (Merck, silica gel $GF_{254}$) and developed with suitable solvent system was Toluene: ethyl acetate: formic acid: water (10: 80: 2.5: 2.5), then chromatogram dried for complete evaporation of solvents. Developed chromatogram was placed in sterile plate then muller hinton agar seeded with colony suspension of isolated bacteria poured on chromatogram, after agar solidified the plate was incubated at $37^OC$ for 24hr, then sprayed with aqueous solution of 2mg/ml dimethylthiazolyl diphenyl tetrazolium bromide (MTT), the areas of inhibition were pale or yellow on a purple colored background indicated on antibacterial activity of constituents [12].

### 2.5 Statistical analysis

All procedures for antibacterial activity were repeated at least three times and the mean value was estimated using Microsoft Excel 2007.

## 3. Results

The results of table 1 showed that chloroform leaves extract of *Mentha piperita*, *Mentha longifolia* and *Ocimum basilicum* inhibited all isolated bacterial starin. *Mentha piperita* showed highest inhibition zone (20mm) against *Staphylococcus aureus*, *Staphylococcus auricularis*, *Streptococcus mitis* and *Escherichia coli*. *Mentha longifolia* exhibited highest inhibition zone (15mm) against *Staphylococcus auricularis* and *Streptococcus mitis*. The leaves extract of both Mentha species showed lowest inhibition zone (14mm) and (12mm) respectively against *Streptococcus pneumoniae*. While leaf extract of *Ocimum basilicum* exhibited highest inhibition zone (14mm) only against *Staphylococcus auricularis* and lowest activity (10mm) against *Klebseilla pneumoniae*.

The antibacterial activity of *Mentha piperita* in combination with *Mentha longifolia* increased from (20 to23mm), (14 to 15mm), (16 to 18mm), (20 to 23mm), against *Staphylococcus auricularis*,

*Streptococcus pneumoniae, Klebseilla pneumoniae* and *Escherichia coli* respectively. While the activity of *Mentha piperita* in combination with *Ocimum basilicum* increased from (20 to 22mm) only against *Escherichia coli*. The activity of *Mentha longifolia* in combination with *Ocimum basilicum* increased from (13 to 17mm), (15 to 17mm), (13 to 17mm), (14 to 25mm) against *Staphylococcus aureus, Staphylococcus auricularis, Klebseilla pneumoniae* and *Escherichia coli*. During combination of equal volume from three plants extract activity increased against all bacterial strain except *Streptococcus mitis*.

**Table 1: Antibacterial activity of *Mentha piperita, Mentha longifolia* and *Ocimum basilicum* leaves extract alone and in combination form by agar well diffusion assay**

| Bacteria | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mp | Ml | Ob | Mp & Ml | Mp & Ob | Ml & Ob | Mp, Ml & Ob |
| *Staphylococcus aureus* | 20 | 13 | 13 | 20 | 18 | 17 | 24 |
| *Staphylococcus auricularis* | 20 | 15 | 14 | 23 | 17 | 17 | 24 |
| *Streptococcus mitis* | 20 | 15 | 12 | 18 | 13 | 14 | 20 |
| *Streptococcus pneumoniae* | 14 | 12 | 11 | 15 | 12 | 12 | 17 |
| *Klebseilla pneumoniae* | 16 | 13 | 10 | 18 | 15 | 17 | 22 |
| *Escherichia coli* | 20 | 14 | 12 | 23 | 22 | 25 | 29 |

*Mp: *Mentha piperita*; Ml: *Mentha longifolia*; Ob: *Ocimum basilicum*

The activity of azithromycin and ampicillin tested against isolated bacterial strain result showed in table 2 produced inhibition zone ranged between (14-33mm) and (10-30mm) respectively. The result showed increase in activity of azithromycin and ampicillin when combined with leaves extract against most of isolated bacteria. When azithromycin combined with *Mentha piperita* and *Ocimum basilicum* separately exhibited pronounced increase in zone of inhibition from (14 to 30mm) and (14 to 20mm) against *Escherichia coli*.

On combination of azithromycin with *Mentha longifolia*, inhibition zone diameter increase from (33 to 34mm) and (14 to 15mm) only against *Klebseilla pneumoniae* and *Escherichia coli* respectively. During combination of ampicillin with *Mentha piperita* and *Mentha longifolia* separately the highest increase of inhibition zone from (10 to 23mm) and (30 to 38mm) against *Streptococcus mitis* and *Staphylococcus auricularis* respectively and lowest increase against *Staphylococcus aureus* (20 to 27 and 23mm). On combination of ampicillin with and *Ocimum basilicum* the highest increase of inhibition zone from (27 to 34mm) against and *Klebseilla pneumoniae*, while the lowest increase against *Streptococcus pneumoniae* (20 to 22 mm).

**Table 2: Antibacterial activity of azithromycin and ampicillin alone and in combination with *Mentha piperita, Mentha longifolia* and *Ocimum basilicum* leaves by agar well diffusion assay**

| Bacteria | Zone of inhibition (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ab | Chloroform extract & Ab | | | Ab | Chloroform extract & Ab | | |
| | Az | Mp & Az | Ml & Az | Ob & Az | Am | Mp & Am | Ml & Am | Ob & Am |
| *Staphylococcus aureus* | 25 | 23 | 23 | 25 | 20 | 27 | 23 | 25 |
| *Staphylococcus auricularis* | 25 | 22 | 23 | 25 | 30 | 40 | 38 | 36 |
| *Streptococcus mitis* | 20 | 27 | 16 | 23 | 10 | 23 | 16 | 13 |
| *Streptococcus pneumoniae* | 20 | 28 | 18 | 22 | 20 | 18 | 13 | 22 |
| *Klebseilla pneumoniae* | 33 | 35 | 34 | 37 | 27 | 35 | 30 | 34 |
| *Escherichia coli* | 14 | 30 | 15 | 20 | 11 | 20 | 17 | 15 |

*Az: Azithromycin; Am: Ampicillin; Mp: *Mentha piperita*; Ml: *Mentha longifolia*; Ob: *Ocimum basilicum*

Activity index of chloroform leaves extract with two standard antibiotics such as azithromycin and ampicillin was evaluated at concentration 100mg/ml against isolated bacterial strain the result as shown in figure 1 and 2. In comparison with azithromycin and ampicillin the strongest activity index revealed by *Mentha piperita* leaf extract (1.42) and (2) against *Escherichia coli* and *Streptococcus mitis* respectively, which indicated that antibacterial activity of *Mentha piperita* higher than activity of azithromycin and ampicillin against those bacteria.

**Figure 1: Activity index of *Mentha piperita* , *Mentha longifolia* and *Ocimum basilicum* extract with azithromycin against isolated bacteria.**

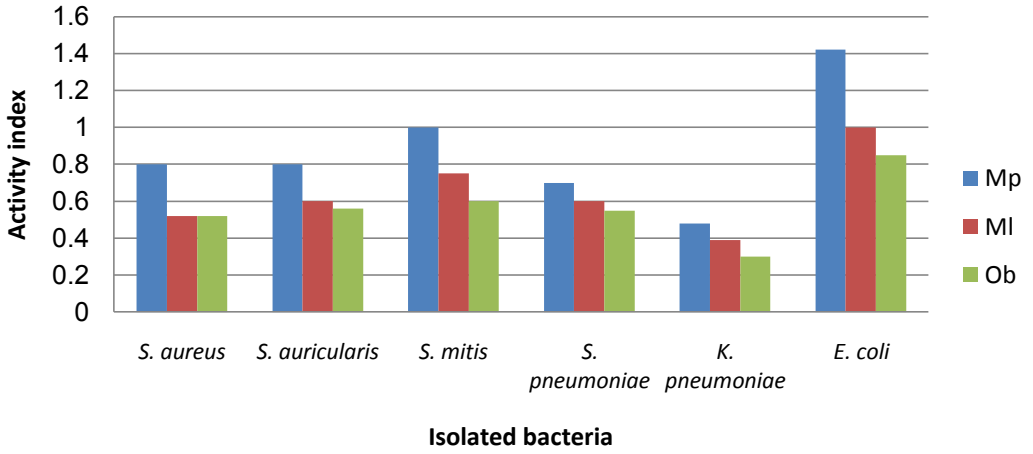
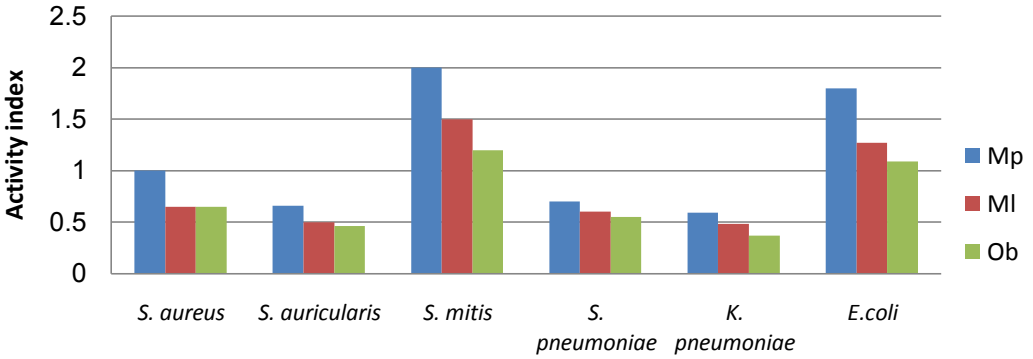



**Figure 2: Activity index of *Mentha piperita, Mentha longifolia* and *Ocimum basilicum* extract with ampicillin against isolated bacteria.**




TLC analysis revealed that *Mentha piperita* and *Mentha longifolia* contain eleven constituents, while *Ocimum basilicum* contain eight constituents and seven of their constituents have similar $R_f$ value but not all of them same according to their colour under UV 254 and 366nm the result shown in figure 3. About 5 to 7 constituents of *Mentha piperita* exhibited activity against different bacterial starin with $R_f$ value (0.43-0.91), while 4 to 7 constituents of *Mentha longifolia* showed activity with $R_f$ value (0.47-0.8) and 3 to 5 constituents of *Ocimum basilicum* showed activity with $R_f$ value (0.33-0.8) the result shown in figure 4, table 3 and 4.

**Table 3: $R_f$ value and inhibition zone of separated leaves constituents by TLC against *Staphylococcus aureus* and *Staphylococcus auricularis***

| ***Staphylococcus aureus*** | | | | | | ***Staphylococcus auricularis*** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **$R_f$ Mp** | **Act** | **$R_f$ Ml** | **Act** | **$R_f$ Ob** | **Act** | **$R_f$ Mp** | **Act** | **$R_f$ Ml** | **Act** | **$R_f$ Ob** | **Act** |
| 0.2 | -Ve | | | | | 0.2 | -Ve | | | | |
| 0.33 | -Ve | 0.33 | -Ve | 0.33 | +Ve | 0.33 | -Ve | 0.33 | -Ve | 0.33 | -Ve |
| 0.43 | -Ve | | | | | 0.43 | -Ve | | | | |
| | | 0.47 | -Ve | | | | | 0.47 | +Ve | | |
| 0.49 | -Ve | 0.49 | +Ve | 0.49 | -Ve | 0.49 | -Ve | 0.49 | +Ve | 0.49 | -Ve |
| | | 0.5 | -Ve | | | | | 0.5 | +Ve | | |
| 0.57 | +Ve | 0.57 | +Ve | 0.57 | +Ve | 0.57 | +Ve | 0.57 | +Ve | 0.57 | +Ve |
| | | 0.6 | +Ve | 0.6 | +Ve | | | 0.6 | -Ve | 0.6 | -Ve |
| 0.64 | +Ve | | | | | 0.64 | -Ve | | | | |
| | | 0.69 | +Ve | | | | | 0.69 | -Ve | | |
| 0.7 | +Ve | 0.7 | +Ve | 0.7 | +Ve | 0.7 | +Ve | 0.7 | +Ve | 0.7 | +Ve |
| 0.73 | +Ve | 0.73 | +Ve | 0.73 | +Ve | 0.73 | +Ve | 0.73 | +Ve | 0.73 | +Ve |
| 0.8 | +Ve | 0.8 | -Ve | 0.8 | -Ve | 0.8 | +Ve | 0.8 | +Ve | 0.8 | -Ve |
| 0.83 | +Ve | | | | | 0.83 | +Ve | | | | |
| 0.91 | +Ve | 0.91 | -Ve | 0.91 | -Ve | 0.91 | +Ve | 0.91 | -Ve | 0.91 | -Ve |

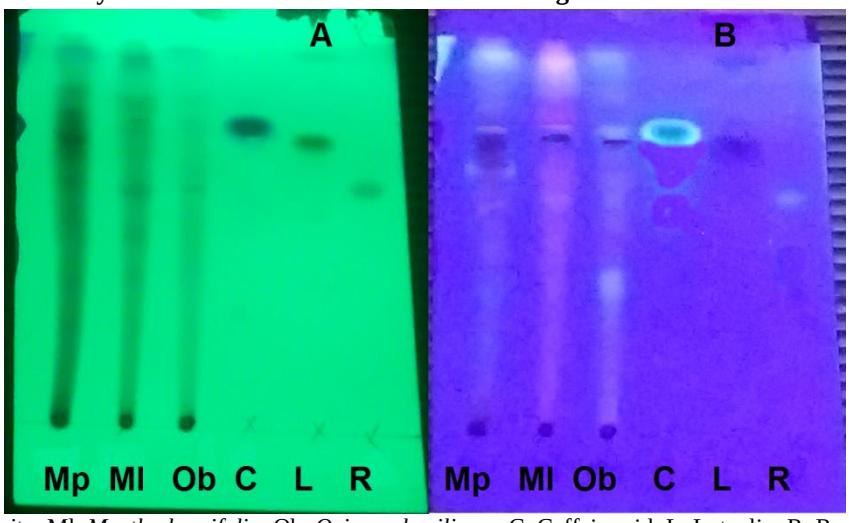
*Mp: *Mentha piperita*; Ml: *Mentha longifolia*; Ob: *Ocimum basilicum*; Act: Activity

**Table 4: $R_f$ value and inhibition zone of separated leaves constituents by TLC against *Streptococcus mitis* and *Escherichia coli***

| ***Streptococcus mitis*** | | | | | | ***Escherichia coli*** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **$R_f$ Mp** | **Act** | **$R_f$ Ml** | **Act** | **$R_f$ Ob** | **Act** | **$R_f$ Mp** | **Act** | **$R_f$ Ml** | **Act** | **$R_f$ Ob** | **Act** |
| 0.2 | -Ve | | | | | 0.2 | -Ve | | | | |
| 0.33 | -Ve | 0.33 | -Ve | 0.33 | -Ve | 0.33 | -Ve | 0.33 | -Ve | 0.33 | -Ve |
| 0.43 | +Ve | | | | | 0.43 | -Ve | | | | |
| | | 0.47 | +Ve | | | | | 0.47 | -Ve | | |
| 0.49 | -Ve | 0.49 | -Ve | 0.49 | -Ve | 0.49 | -Ve | 0.49 | -Ve | 0.49 | -Ve |
| | | 0.5 | -Ve | | | | | 0.5 | -Ve | | |
| 0.57 | +Ve | 0.57 | +Ve | 0.57 | +Ve | 0.57 | +Ve | 0.57 | +Ve | 0.57 | +Ve |
| | | 0.6 | -Ve | 0.6 | -Ve | | | 0.6 | -Ve | 0.6 | -Ve |
| 0.64 | -Ve | | | | | 0.64 | -Ve | | | | |
| | | 0.69 | -Ve | | | | | 0.69 | -Ve | | |
| 0.7 | +Ve | 0.7 | +Ve | 0.7 | +Ve | 0.7 | +Ve | 0.7 | +Ve | 0.7 | +Ve |
| 0.73 | +Ve | 0.73 | +Ve | 0.73 | +Ve | 0.73 | +Ve | 0.73 | +Ve | 0.73 | +Ve |
| 0.8 | +Ve | 0.8 | +Ve | 0.8 | +Ve | 0.8 | +Ve | 0.8 | +Ve | 0.8 | -Ve |
| 0.83 | +Ve | | | | | 0.83 | +Ve | | | | |
| 0.91 | +Ve | 0.91 | -Ve | 0.91 | -Ve | 0.91 | -Ve | 0.91 | -Ve | 0.91 | -Ve |

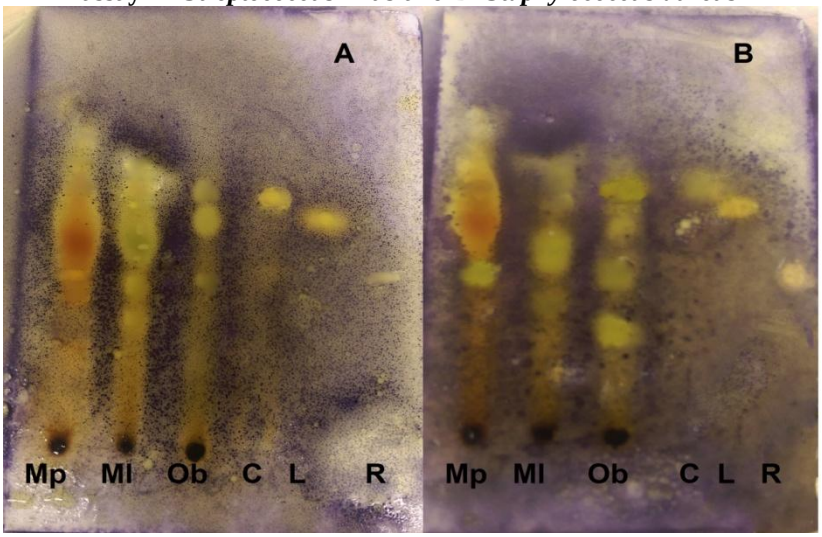
*Mp: *Mentha piperita*; Ml: *Mentha longifolia*; Ob: *Ocimum basilicum*; Act: Activity

**Figure 3: TLC analysis for chloroform leaves extract viewing under A-UV 254nm B- UV 366nm.**




*Mp: *Mentha piperita*; Ml: *Mentha longifolia*; Ob: *Ocimum basilicum*; C: Caffeic acid; L: Luteolin; R: Rosmarinic acid.

**Figure 4: Antibacterial activity of chloroform leaves extract constituents by agar overlay bioautography assay A- *Streptococcus mitis* and B- *Staphylococcus aureus***




*Mp: *Mentha piperita*; Ml: *Mentha longifolia*; Ob: *Ocimum basilicum*; C: Caffeic acid; L: Luteolin; R: Rosmarinic acid.

## 4. Discussion

Now days most of bacterial strains can easily accept resistance against different classes of antibiotic as a result require great attention to discovery alternative treatment such as combination of natural product with each other or with synthetic antibacterial drugs [13]. This study was carried out to evaluate antibacterial activity of *Mentha piperita, Mentha longifolia* and *Ocimum basilicum* leaf alone, in combination extract with each other and in combination of extract with the antibiotic (Azithromycin and ampicillin). All isolated bacterial strain showed sensitivity against chloroform extract of three Labiatae species and highest sensitivity showed by *Staphylococcus auricularis* against *Mentha piperita, Mentha longifolia* and *Ocimum basilicum* (20mm), (15mm) and (14mm) respectively. The results revealed that *Mentha piperita* exhibited highest antibacterial activity followed by *Mentha longifolia* then *Ocimum basilicum* (Table 1). These result in agreement with finding of Sujana *et al*., [14], who described antibacterial activity of chloroform leaf extract of *Mentha piperita* against *Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli* and *Klebsiella pneumonia*.

Also Ghderi *et al*., [15] reported antibacterial activity of *Mentha longifolia* against *Staphylococcus aureus*. But the result of our study on antibacterial activity of *Ocimum basilicum* in contrast with the study of Kaya *et al*.,[16] who also studied the antibacterial activity of chloroform leaf extract of *Ocimum basilicum* against different bacterial strain and exhibited no activity on *Staphylococcus aureus* and *Escherichia coli.* As a result of combination of leaves extract of three Labiatae species revealed synergistic activity against five bacterial strains, followed by combination of *Mentha piperita* with *Mentha longifolia* and *Mentha longifolia* with *Ocimum basilicum* synergistic activity against four bacterial strain, then *Mentha piperita* with *Ocimum basilicum* only against one bacterial strain. The strongest synergistic activity exhibited during combination of *Mentha longifolia* with *Ocimum basilicum* (14 to 25mm) against *Escherichia coli*.

The result of these study were in agreement with a previous research who mentioned a synergetic effect between plant extracts like *Mentha Piperita* with *Allium Sativum* against *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris* and *Pseudomonas aeruginosa* [17]. Staphylococcus species exhibit higher sensitivity to combination of *Mentha longifolia* with *Ocimum basilicum* than Streptococcus species and combination of *Mentha longifolia* with *Ocimum basilicum* showed stronger activity against gram negative than gram positive bacteria. *Escherichia coli* revealed susceptibility to all plant extract alone and in combination form while *Streptococcus mitis* revealed susceptibility to all plant extract alone and their susceptibility not increased with combining plant extract with each other. The combination of azithromycin with *Mentha piperita* and *Ocimum basilicum* revealed synergistic activity against four bacterial strain while with *Mentha longifolia* only against two isolated bacteria while the combination of ampicillin with *Ocimum basilicum* exhibit synergistic activity against all bacterial strain but with *Mentha piperita* and *Mentha longifolia* against five isolated bacteria. The greatest synergistic activity resulted from combination of *Mentha piperita* with azithromycin and ampicillin separately (14 to 30mm) and (10 to 23mm) against *Escherichia coli* and *Streptococcus mitis* (Table 2). The increase in the sizes of inhibition zones resulting from combinations of extract and antibiotic indicated the improved bactericidal potentials of the extract and the antibiotics as combined antibacterial agents. The synergistic effects from the combination of antibiotics with plant extracts were recorded by other workers [18]. Nascimento *et al*., [19] reported that the antibacterial activity of ampicillin improved on combinations with clove extract against *Klebsiella pneumonia*. Also antibacterial activity amoxacillin improved on combination with lemon grass essential oil, cardamom oil and Thyme extracts against *Staphylococcus aureus* [20].

One of the phytoconstituents of Labiatae species are polyphenols compound such as flavonoids, phenolic acid and others have antibacterial activity interacted with the antibiotics to enhance their action at the target sites, also have ability for modulating resistant properties in the bacteria to be more effective [21,22]. Sensitivity of most isolated bacteria increase to ampicillin when combined with *Mentha piperita, Mentha longifolia* and *Ocimum basilicum* leaves extract except *Streptococcus pneumoniae* on combination with *Mentha piperita* and *Mentha longifolia* decrease. While on combination of azithromycin with *Mentha piperita, Mentha longifolia* and *Ocimum basilicum*, gram negative bacteria exhibit pronounced increase in susceptibility to three combination forms, *Streptococcus mitis* and *Streptococcus pneumoniae* not exhibit any increase against combination with *Mentha longifolia*, while *Staphylococcus aureus* and *Staphylococcus auricularis* not showed any increase in susceptibility. The activity index value more than 1 indicated that the plant extract have higher activity than standard antibiotics against isolated bacterial strains. The result of these study showed that in comparison with azithromycin only *Mentha piperita* exhibited higher activity index (1.42) against *Escherichia coli*, while with ampicillin the higher activity exhibited by *Mentha piperita* (2) and (1.8), followed by *Mentha longifolia* (1.5) and (1.27), then *Ocimum basilicum* (1.2) and (1.09) against *Streptococcus mitis* and *Escherichia coli* respectively (Figure 1 and 2). Bioautography is one of the easiest and cheapest methods for identification constituents with antibacterial activity in partially purified extracts and the method is easy to run, reproducible and requires less equipments. Because of chloroform leaves extract showed the highest inhibition zone against *Staphylococcus aureus, Staphylococcus auricularis, Streptococcus mitis* and *Escherichia coli* we used those bacteria for bioautography assays in which combines TLC with bioassay in situ. The result of bioautography showed larger number constituents of *Mentha piperita* exhibited antibacterial activity

against *Staphylococcus aureus* and *Streptococcus mitis*, while of *Mentha longifolia* exhibited highest activity against *Staphylococcus auricularis* and *Ocimum basilicum* against *Staphylococcus aureus*. Among those constituents in each leaf extract with antibacterial activity three of them were identified by comparing $R_f$ values and color properties with reference substances as caffeic acid (0.73), luteolin (0.7) and rosmarinic acid (0.57) (Figure 3 and 4). The previous studies showed that phenolic compounds such as caffeic acid, rosmarinic acid and luteolin are widely distributed in *Mentha piperita*, *Mentha longifolia* and *Ocimum basilicum* [23,24].

## 5. Conclusion

*Mentha piperita*, *Mentha longifolia* and *Ocimum basilicum* leaves extract exhibited antibacterial activity against gram positive and gram negative bacterial strain alone and their activity increased in combination form. The zones of inhibition produced by the combinations of antibiotic with extract varied in size and were mostly wider than those obtained from combinations of leave extract with each other. Isolated bacteria showed high susceptibility to combination of ampicillin with chloroform leave extract than azithromycin. *Mentha piperita* revealed higher antibacterial activity than azithromycin and ampicillin against *Escherichia coli*. The most important constituents responsible on antibacterial activity of leave extract are phenolic acid and flavonoids.

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**Conflict of Interest:** Authors have declared that no competing interests exist.

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