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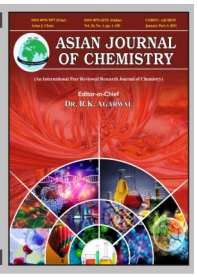

Antimicrobial and Antioxidant Properties of *Lamium galactophyllum* Boiss & Reuter, *L. macrodon* Boiss & Huet and *L. amplexicaule* from Turkish Flora

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Some Lamium species have been used in folk medicine worldwide in the treatment of several desorders. This work aimed to screen the possible antimicrobial and antioxidant properties as well as total phenolic, resveratrol and flavonoid contents of extracts of three different Lamium species. According to analysis results the highest total phenolic concent was determined at *Lamium galactophyllum* (112.87 mg GAE/g extre). Quercetin and katesin were the major flavonoid contents for *Lamium galactophyllum* (respectively 296.7 and 323.35 µg/mL), *Lamium macrodon* (respectively 445.75 and 338.6 µg/mL) and *Lamium amplexicaule* (respectively 101.6 and 330.9 µg/mL). ABTS free radical scavenginig activity of plant extracts was higher than DPPH free radical scavenginig activity, but all plant extracts had free radical scavenging activity against DPPH and ABTS. *Lamium galactophllum* and *Lamium macrodon* exhibited more effective antimicrobial activity than *Lamium amplexicaule*. These results suggested that *Lamium* species used in this work had antimicrobial and antioxidant properties.

Keywords: *Lamium galactophyllum*, *Lamium macrodon*, *Lamium amplexicaule*, Resveratrol-flavonoid contents.

INTRODUCTION

The genus *Lamium* L. (Lamiaceae) comprises about 40 species distributed in Europe, Asia and Africa. There are 30 *Lamium* species recorded in the flora of Turkey[1]. Some *Lamium* species have been used in folk medicine worldwide as remedy in the treatment of several disorders, such as trauma, fracture, paralysis, hypertension, menorrhagia and uterine hemorrhage[2,3].

Flavonoids are ubiquitous in photosynthesizing cells and therefore ocur widely in the plant kingdom[4]. They are found in the fruit, vegetables, nuts, seeds, stems and flowers as well as tea, wine[5], propolis and honey[6] and represent a common constituent of the human diet[7]. Historically, the biological actions of flavonoids, including those on the brain, have been attributed to their ability to exert antioxidant actions[8], through their ability to scavenge reactive species, or through their possible influences on intracellular redox status[9].

The main purpose of this study is to investigate the antimicrobial and antioxidant properties as well as total phenolic, resveratrol and flavonoid contents of extracts of three different *Lamium* species.

EXPERIMENTAL

Plant samples [*Lamium galactophyllum* Boiss & Reuter (Locality: Posof-Ardahan, Turkey, Coordinate: N41° 26.504 E042° 40.233, Altitude: 1694), *Lamium macrodon* Boiss & Huet (Locality: Ardahan District, Turkey, Coordinate: N41° 13,49 E042° 43.01, Altitude: 1960), *Lamium amplexicaule* (Locality: Ardahan District, Turkey, Coordinate: N41° 13, 49' E042° 43.01', Altitude: 1960)] were obtained from Ardahan region (eastern part of Turkey). Plant samples were identified by Flora of Turkey and The East Aegean Island by Dr. Ahmet Ilcim[10] and voucher specimens were deposited in herbarium of Depertmant of Biology, Faculty of Art and Science, Kahramanmaras Sutcu Imam University, Turkey. Different parts of plant such as root, leaf, stalk, flower and aerial parts were cleaned from debris, dried in the shate at room temperature and finally powdered.

Preparation of Extracts: Powdered plant materials such as root, leaf, stalk, flower and aerial parts (10 g) were loaded to Soxhlet apparatus. The extraction was carried out using five solvents such as purified chloroform (polarity index: 4.1), hexane (pi: 0), acetone (pi: 5.1), ethanol (pi: 5.2) and methanol (pi: 5.1) (300 mL) for 6 h. The resulting mixture was then

filtered and concentrated under vacuum at 40 °C (Buchi, Rotavapor R-210, Labortechnik, AG, Flavil, Switzerland). Filter-sterilized and concentrated extracts were refrigerated (-18 °C) until use.

Resveratrol and flavonoid contents: In order to chromatographic analysis of flavonoid content of *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule* (methanolic extracts of aerial parts), ALTIMA C18 (15x4.6 mm, GRACE, USA) HPLC colon was used. Methanol/water/acetonitrile mix (46/46/8, v/v/v) which include 1 % acetic acid was also used as mobile phase and this mobile phase was filtrated by 0.45 µm membrane filter. 280 nm (for catechin and naringin), 254 nm (for rutin, myricetin and quercetin), 306 nm (for resveratrol) and 265 nm (for kaemoferol) were used as wavelength and were done HPLC seperation. After these processes, flavonoids were measured by DAD (Diode-arrey Detector). All chromatographic prosesses were done at 25 °C.

Total phenolic contents: Total phenolic constituents of *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule* methanolic extracts (aerial parts) were performed employing methods from the literature involving Folin-Ciocalteu reagent and used gallic acid as standard[11].

Test of DPPH free radical scavenging activity: The scavenging of DPPH radical was determined according to a modified version of the method described by Blois[12]. 1 mM solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was used as free radical. 10, 20, 40, 80 and 200 µL methanolic extracts of aerial parts of *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule* were tested against DPPH. The reaction mixtures were incubated at room temperature and darkness for 0.5 h. The reduction of DPPH was followed by monitoring the decrease in absorbance at 517 nm. The percentage of free radical scavenging effect was calculated as follows: SC % = $[(A_{control}-A_{test})/A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus extracts). All tests were performed in triplicate and mean were centred.

Test of ABTS free radical scavenging activity: The $ABTS^+$ scavenging ability of methanolic extracts of *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule* aerial parts were determined according to method described by Re *et al.*[13]. $ABTS^+$ was generated by reaction an ABTS solution (7 mM) with $K_2S_2O_8$ (2.45 Mm) in the dark and room temperature for 16 h and adjusting the 734 nm. 10, 20, 40, 80 and 200 µL extracts were added to 4.0 mL $ABTS^+$ solution and absorbances were measured at 734 nm after 2 h. The percentage of free radical scavenging effect was calculated as follows: SC % = $[(A_{control}-A_{test})/A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control (ABTS solution without test sample) and A_{test} is the absorbance of the test sample (ABTS solution plus extracts). All tests were performed in triplicate and mean were centred.

Antimicrobial activity: The antimicrobial activities of extracts were determinated by the disc diffusione method. 100 µL of each extract was absorbed to sterile disc which is 12 mm diameter.

To inoculate the media for assay, 1 % rate of each microorganism from 10^6-10^7 cfu/mL suspension was added to 15 mL sterile media (for bacteria Muller-Hintone agar, for yeast Sabourand 2 % glucose agar). Each of these inoculated mediums was poured into petri dishes (9 cm) and left to +4 °C for 1 h. Subsequently discs prepared from *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule* extracts were added on these inoculated medias and left again to +4 °C for 1 h.

Seven standard antibiotic discs were used as the positive controls. Sensitivity was deduced by comparing the inhibition zone diameter produced by the erythromycin (E-15), gentamicin (CN-10), chloramphenicol (C-30), penicillin (P-10), cefoperazone (CEP-75), ceptazidime (CAZ-30) and ampicillin (AM-10).

The petri dishes were incubated at 35 °C for 18-24 h, except for *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* which were incubated at 27 °C. Inhibition zones were measured by vernier caliper and recorded as the mean diameter of 3 replications in mm. All tests were performed in triplicate and mean were centred.

Data analysis: One-Way ANOVA test (SPSS 16.0) was used to analysis data obtained from the zone of inhibition produced by different extracts.

RESULTS AND DISCUSSION

The results of resveratrol and flavonoid content of *Lamium* extracts were presented in Table-1. According to these results, quercetin and katesin were major flavonoid contents for *Lamium galactophyllum* Boiss & Reuter (respectively 296.7 and 323.35 µg/mL), *Lamium macrodon* Boiss & Huet (respectively 445.75 and 338.6 µg/mL) and *Lamium amplexicaule* (respectively 101.6 and 330.9 µg/mL), resveratrol was also major componds for *Lamium galactophyllum* Boiss & Reuter (396.35 µg/mL) and *Lamium amplexicaule* (165.65 µg/mL). Additionally, rutin, myricetin and kaempferol were minor falvonoid contents for *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule*.

Total phenolic contents: The amount of the total phenolics was the highest in *Lamium galactophyllum* (112.87 mg GAE/g extre), followed by *Lamium macrodon* (112.79 mg GAE/g extre). *Lamium amplexicaule* had the lowest total phenolic content (94.75 mg GAE/g extre).

TABLE-1
RESVERATROL AND FLAVONOID CONTENTS OF *Lamium* SPECIES

Plant samples	Rutin (µg/mL)	Myricetin (µg/mL)	Quercetin (µg/mL)	Kaempferol (µg/mL)	Katesin (µg/mL)	Naringin (µg/mL)	Naringenin (µg/mL)	Resveratrol (µg/mL)
L. galactophyllum Boiss & Reuter	0.5	0.5	296.7	0.1	323.35	61.7	0.5	396.35
L. macrodon Boiss & Huet	0.5	1.8	445.75	5.5	338.6	0.5	47.35	0.5
L. amplexicaule	0.5	0.5	101.6	0.5	330.9	0.5	3.4	165.65

Antioxidant activity: Antioxidant activities of *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule* methanolic extracts were screened by DPPH and ABTS methods. As listed in Table-2, the results showed that DPPH free radical scavenging activity rates of plant extracts dwindled to 200 µL concentration. *L. amplexicaule* extracts exhibited the highest free radical scavenging activiyt against DPPH (57.65-97.57 %). While DPPH free radical scavenging activity rates had great differences among concentrations, ABTS free radical scavenging activity rates didn't have. Free radical scavenging activity of *L. galactophyllum* extract was 99.80 % at all concentrations, this was the highest rate of free radical scavenging activity against ABTS. Consequently, ABTS free radical scavenging activity of *Lamium* extracts were higher than DPPH free radical scavenging activity, but all *Lamium* extracts had free radical scavenging activity against DPPH and ABTS.

Antimicrobial activity: Antimicrobial activities of root, stalk, leaf, flower and aerial parts of *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule* extracts were tested against test microorganisms. Antimicrobial activity results were demonstrated in Tables 3-5.

Experimental results showed that *Lamium galactophllum* and *Lamium macrodon* had more effective antimicrobial activity than *Lamium amplexicaule*. Antimicrobial activity of *Lamium galactophyllum* were given in Table-3 and these results showed that methanolic extracts of root, stalk, leaf, flower and aerial parts of *L. galactophyllum* had more effective antimicrobial activity than other extracts. However, chloroform and hexane extracts of any parts of *L. galactophyllum* and *L. amplexicaule* didn't show any inhibitory effects towards test microorganisms. Additionally, hexane extracts of any parts of *L. macrodon* didn't show antimicrobial activity against test microorganisms either. Some plants based solvent extracts used in this study revealed to have lower antibacterial effect compared to standard antibiotics (Fig. 1).

Unlike chloroform and hexane extracts; acetone, ethanol and methanol extracts of three species of *Lamium* displayed activity against a number of bacteria. *Bacillus subtilis* ATCC 6633 and *Enterobacter aerogenes* ATCC 13048 were more sensitive than other test microorganisms. For example, *L. galactophyllum* methanol extracts had activity against *B. subtilis* ATCC 6633, with inhibition diameter of 33 ± 0.00 mm (for root), 23 ± 0.57 mm (for stalk), 36 ± 1.15 mm (for leaf), 38 ± 0.33 mm (for flower) and 34 ± 0.57 (for aerial part). *L. galactophyllum* methanol extracts had correspondingly activity against *E. aerogenes* ATCC 13048, with inhibition diameter of 28 ± 1.15 mm (for root), 18 ± 1.45 mm (for stalk), 32 ± 0.57 mm (for leaf), 35 ± 0.00 mm (for flower) and 37 ± 0.33 mm (for aerial part).

The results show that ABTS free radical scavenginig activity of *Lamium* extracts was higher than DPPH free radical scavenginig activity, but all extracts had free radical scavenging activity against DPPH and ABTS. In a similar study, Yumrutas and Saygideger[14] were designed to determin the *in vitro* antioxidant activities of methanol and hexane extracts of *Lamium amplexicaule* L. The methanol extract of this plant exhibited significant antioxidant activity.

In this study, it is showed that quercetin and katesin are the major components for *Lamium* species used in this study. Many research groups have gone one step further and either isolated and identified the structure of flavonoids that possess antibacterial activity, or quantified the activity of commercially available flavonoids. Examples of such flavonoids are quercetin, 3-*O*-methylquercetin and various quercetin glycosides[15-17], apigenin[18], galangin[19-21], pinocembrin[22,23], ponciretin[24,25], genkwanin[26,27], sophoraflavanone G and its derivatives[28,29], naringin and naringenin[15,28,29], epigallocatechin gallate and its derivatives[30,31], luteolin and luteolin 7-glucoside[18,32] and kaempferol and its derivatives[15,33]. Other flavones [15,34,35], flavone glycosides[36-38], isoflavones[39,40], flavanones[28,34,40], isoflavanones[41], isoflavans[42], flavonols[43], flavonol glycosides[36,44,45] and chalcones[34,40] with antibacterial activity have also been identified.

Plant extracts obtained from *Lamium* species exhibited antibacterial activity against test bacteria at different rate. *B. subtilis* ATCC 6633 displayed the most sensitivity towards extracts of *Lamium* species. The greater sensitivity of gram positive bacteria to plant extracts was reported earlier[46,47].

In a similar study, the organic solvents and aqueous extracts obtained from the leaves, rootstock and the combined formulation of endemic *Lamium* tenuiflorum Fisch. & Mey. (Lamiaceae) were tested for their antimicrobial activity against Escherichia coli ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, Micrococcus luteus CCM 169 by the well-in-agar method. All the organic solvent extracts exhibited a strong antibacterial effect against the bacterial cultures except for the aqueous extracts, which had no effect[48].

None of the extracts obtained from *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule* displayed activity towards *Candida albicans* ATCC 10231 and *Sacharomyces cerevisiae*. However, in a earlier study studied by Dulger[49], the ethanol extracts obtained from the leaves, rootstock and combined formulation of endemic *Lamium tenuiflorum* Fisch. & Mey were investigated for their antifungal activities against medical yeast *Candida* and *Cryptococcus* species. All the extracts exhibited a strong antifungal effect against yeast cultures. The ectracts exhibited greater antifungal effect against *Candida* species than *Cryptococcus* species.

Several scientific reports have described the inhibitory effect of plants on a variety of microorganisms, although

TABLE-2
DPPH AND ABTS FREE RADICAL SCAVENGING ACTIVITY OF *Lamium* SPECIES

Plant samles	DPPH (%)					ABTS (%)				
	10 µL	20 µL	40 µL	80 µL	200 µL	10 µL	20 µL	40 µL	80 µL	200 µL
L. galactophyllum Boiss & Reuter	79.66	71.29	60.65	40.68	17.68	99.80	99.80	99.80	99.80	99.80
L. macrodon Boiss & Huet	92.02	91.45	82.51	60.27	11.98	98.57	98.37	98.78	99.18	99.56
L. amplexicaule	97.57	94.92	84.37	75.35	57.65	99.80	99.80	99.59	99.60	99.39

TABLE-3
ANTIMICROBIAL ACTIVITY OF *Lamium galactophllum* BOISS & REUTHER

	Antimicrobial activity (mm)																				
	Root					Stalk					Leaf					Flower					AP
Microorganisms	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	M
B. subtilis ATCC 6633	-[1]	-	17±0.33[2]	-	33±0.00	-	-	-	-	23±0.57	-	-	-	16±0.33	36±1.15	-	-	16±0.57	18±0.57	38±0.33	34±0.57
E. aerogenes ATCC 13048	-	-	-	14±0.00	28±1.15	-	-	-	-	18±1.45	-	-	-	-	32±0.57	-	-	15±0.57	17±0.33	35±0.0	37±0.33
E. cloacae ATCC 13047D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. luteus NRLL B-4375	-	-	-	-	20±0.00	-	-	-	-	-	-	-	-	-	18±0.88	-	-	-	-	24±2.88	35±0.88
S. aureus ATCC 25923	-	-	-	-	13±0.33	-	-	-	-	14±0.33	-	-	-	-	13±0.00	-	-	-	-	12±0.00	-
E. coli ATCC 11229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12±0.33	-
C. albicans ATCC 10231	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. cerevisiae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1]: No inhibtition zone, [2]: Inhibition zone (mm), AP: Aerial part, C: Chloroform, H: Hexane, A: Acetone, E: Ethanol, M: Methanol

TABLE-4
ANTIMICROBIAL ACTIVITY OF *Lamium macrodon* BOISS & HUET

	Antimicrobial activity (mm)																				
	Root					Stalk					Leaf					Flower					AP
Microorganisms	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	M
B. subtilis ATCC 6633	25±0.33[2]	-[1]	29±0.33	15±0.33	30±3.46	22±0.33	-	-	-	19±0.88	-	-	-	-	19±0.33	20±0.88	-	-	-	19±0.57	24±0.33
E. aerogenes ATCC 13048	-	-	19±0.57	-	25±1.73	-	-	-	-	18±0.33	-	-	-	-	18±0.33	-	-	-	-	16±0.33	22±0.57
E. cloacae ATCC 13047D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. luteus NRLL B-4375	-	-	-	-	18±0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21±0.57
S. aureus ATCC 25923	13±0.00	-	-	-	14±0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14±0.00	14±0.00
E. coli ATCC 11229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans ATCC 10231	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. cerevisiae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1]: No inhibtition zone, [2]: Inhibition zone (mm), AP: Aerial part, C: Chloroform, H: Hexane, A: Acetone, E: Ethanol, M: Methanol

TABLE-5
ANTIMICROBIAL ACTIVITY OF *Lamium amplexicaule*

	Antimicrobial activity (mm)																				
	Root					Stalk					Leaf					Flower					AP
Microorganisms	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	M
B. subtilis ATCC 6633	-[1]	-	19±0.00[2]	-	18±1.15	-	-	-	-	-	-	-	-	-	-	-	-	-	15±0.33	22±0.00	23±1.15
E. aerogenes ATCC 13048	-	-	14±0.33	-	15±0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15±0.33	23±0.33
E. cloacae ATCC 13047D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. luteus NRLL B-4375	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21±1.15
S. aureus ATCC 25923	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12±0.00	-
E. coli ATCC 11229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans ATCC 10231	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. cerevisiae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1]: No inhibtition zone, [2]: Inhibition zone (mm), AP: Aerial part, C: Chloroform, H: Hexane, A: Acetone, E: Ethanol, M: Methanol

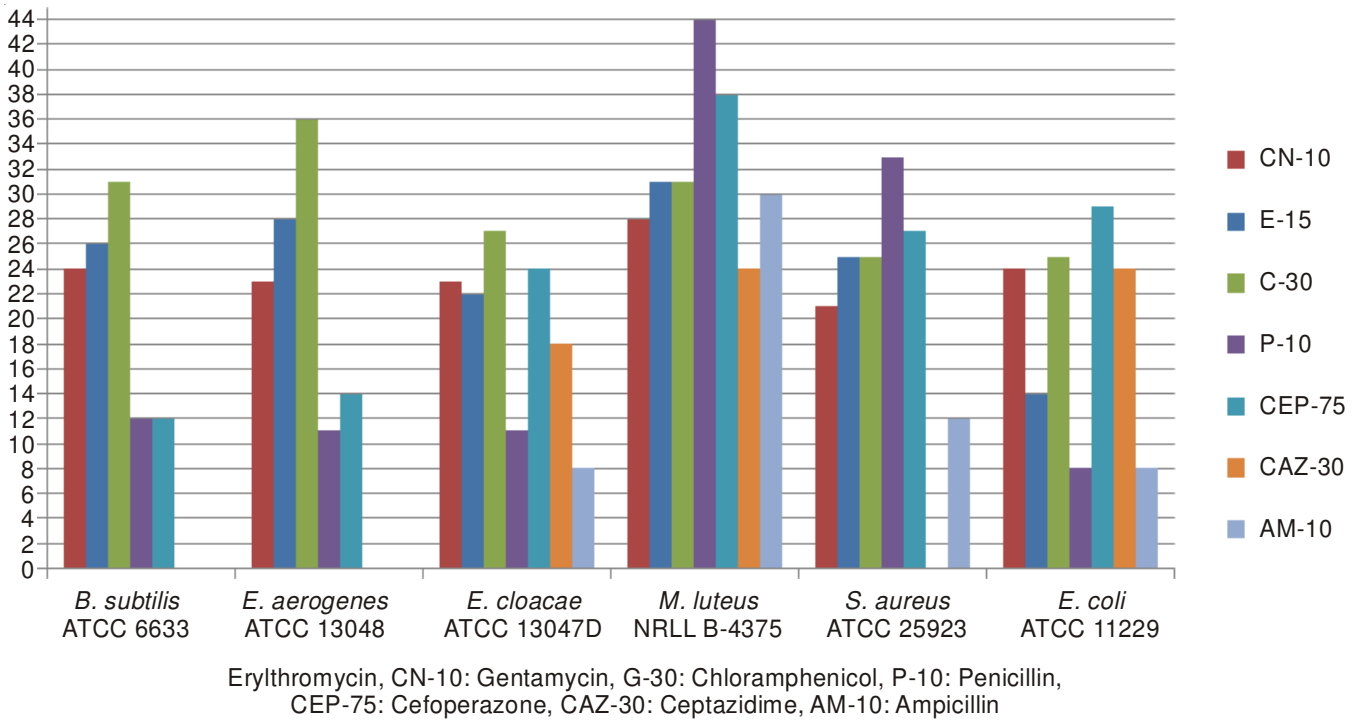

Fig. 1. Antimicrobial activities of standard antibiotics used as positive control

considerable variation for resistance of different microorganisms to a given plant and of the same microorganisms to different plants[50]. Differences in the activity of many species may be explained due to variations in the nature and combinations of phytocompounds present in the solvent extract, strain sensitivity, antimicrobial procedure adopted in tests, or may be largely depending on the plant species and/or geographical sites[51-53]. The extraction product also varied in terms of quality, quantity and composition according to climate, soil composition, plant organ, age *etc*.[54].

In conclusion, antimicrobial and antioxidant properties of various plants are of great interest in academia, food, cosmetic and pharmaceutical industries. This study has shown that *Lamium galactophyllum* Boiss & Reuter, *Lamium macrodon* Boiss & Huet and *Lamium amplexicaule* extracts exhibite antimicrobial and antioxidant activity. Methanolic extracts of three *Lamium* species compared to the chlorophorm, acetone, hexane and ethanol extracts exhibite more effective antibacterial activity against test bacteria.

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