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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

The Correlation of Some Nutrient Elements and Antibacterial Activity of the Basil (*Ocimum basilicum)*

Fesleğen (*Ocimum basilicum*) Bitkisinde Bazı Bitki Besin Elementleri ile Antibakteriyel Aktivitesinin İlişkisi

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Abstract

This study aimed to analyze the correlation of some macro and micro nutritional elements of the basil plant (*Ocimum basilicum*) with the antibacterial activity of the basil extract on two Gram positive and two Gram negative bacteria. The extract of the basil plants grown in laboratory conditions was obtained by solid-liquid extraction with ethanol after drying and burning of the freshly harvested above ground parts. The macro (P, K, Ca, and Mg), micro (Fe, Cu, Zn, Mn, and B) nutrients and a functional element (Al) amounts were detected by ICP-OES. The results revealed that the basil extract was compatible with herbaceous perennial plants in terms of macro nutrient elements P, K, Ca and Mg. Moreover, some micronutrients (Fe, Mn, Zn, B) and the functional element Al was found to be significantly high. The basil extract was significantly effective on inhibiting the Gram negative *E. coli*, *P. aeruginosa*, and Gram positive *S. aureus* and *E. faecium*. The element B was found to be positively correlated with the antibacterial activity of the basil plant suggesting as the B content increases, the antibacterial activity of basil extract is supposed to increase, too. The P and K contents were found to be positively correlated with antibacterial activity, while Ca and Mg contents were negatively correlated. The correlation analysis of the nutritional elements, this extract can be suggested to be more preferrable against Gram positive bacteria, especially when the Fe, Cu, Zn, Mn levels are high. These results revealed the importance of the macro and micro nutrient contents of medicinal plants which can be used traditionally against various diseases. In basil cultivation the soils low in lime may convert the negative correlation of Ca and Mg into a positive correlation in terms of antibacterial effect. Conscious and proper fertilization therefore is suggested to be effective on antibacterial activity of the basil plant.

Keywords: *Ocimum basilicum,* Antibacterial activity, Macro elements, Micro elements, Correlation

Özet

Bu çalışmada fesleğen bitkisinin (*Ocimum basilicum*) ekstraktında bulunan bazı makro ve mikro besin elementleri ile bu ekstraktın iki Gram pozitif ve iki Gram negatif bakteri üzerine antibakteriyel etkisinin ilişkisi araştırılmıştır. Laboratuvar ortamında saksılarda yetiştirilen fesleğen bitkisinin toprak üstü aksamının tamamı kurutma ve yakma işlemleri sonrasında ekstrakt çıkarılması için kullanılmış ve ekstrakt etanol ile katı-sıvı ekstraksiyonu ile elde edilmiştir. Makro (P, K, Ca ve Mg), mikro (Fe, Cu, Zn, Mn ve B) besin ve bir fonksiyonel element (Al) içerik ve miktarları ICP-OES cihazı ile belirlenmiştir. Sonuçlar fesleğen bitkisinin makro besin elementlerinden olan P, K, Ca ve Mg elementlerinin miktarlarının çok yıllık otsu bitkilerin besin elementlerinin miktarları ile uyumlu çıktığını göstermiştir. Ayrıca, bu çalışmada yetiştirilen fesleğen bitkisinde bazı mikro besin elementlerinin (Fe, Mn, Zn, B)

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içeriği ve bir fonksiyonel element olan Al'nin içeriği de benzer bitkilerle karşılaştırıldığında önemli miktarda yüksek bulunmuştur. Fesleğen ekstraktının Gram negatif bakterilerden olan *E. coli*, *P. aeruginosa*, ve Gram pozitif bakterilerden olan *S. aureus* ve *E. faecium* bakterileri üzerinde bakteri büyümesini engelleme anlamında hepsi üzerinde etkili olan bir antibiyotikle karşılaştırıldığında etkili olduğu gösterilmiştir. Bitkinin makro besin elementlerinden P ve K miktarlarının antibakteriyel etki ile pozitif ilişkili, Ca ve Mg elementlerinin de negatif ilişkili olduğu belirlenmiştir. Mikro besin elementleri arasında da Mn, Zn ve B; ve fonksiyonel bir element olan Al'nin de antibakteriyel etki ile pozitif ilişkili olduğu gösterilmiştir. Sonuçlar geleneksel olarak farklı bir çok hastalığın tedavisinde toplumda sıklıkla kullanılan tıbbi bitkilerin makro ve mikro element içeriklerinin önemini vurgulamaktadır. Fesleğen yetiştiriciliğinde kireç bakımından düşük toprakların kullanılmasının bu bitkilerin antibakteriyel etkisi Ca ve Mg elementlerinin negatif olan etkisini pozitife çevirebileceği düşünülmektedir. Dolayısıyla doğru ve bilinçli gübreleme yöntemlerinin bu ve buna benzer bitkilerin antibakteriyel aktivitesi üzerine olumlu bir etki oluşturacağı düşünülmektedir.

Anahtar Kelimeler: *Ocimum basilicum,* Antibakteriyel aktivite, Bitki besleme, Makro element, Mikro element, Korelasyon

1. Introduction

Ocimum basilicum, also known as sweet basil, is a perennial herbaceous plant belonging to the Lamiaceae family. It is preferred all over the world as a kitchen herb since it has high nutritional value and low calorific value. It contains several vitamins such as vitamin A, C, B6, thiamine and carotene (Dumbravă et al., 2012). Besides these, basil contains important amounts of micro and macro nutrients. Such nutrients in a plant are vital for proper functioning of plants, animals and humans (Soetan et al., 2010). Basil can be cultivated in a wide range of geographies all over the world, hence can be highly accessible. It is also frequently used in traditional medicine due to its rich secondary metabolites. Basil is rich in phenolics and has many benefits due to its antioxidant, anti-inflammatory, anti-hyperlipidemic, insecticidal and many other effects (Patil et al. 2011; Purushothaman et al. 2018). Besides these advantageous properties, basil has antimicrobial effects, similar to other aroma therapeutic plants (Nascimento et al. 2000). Recent antibiotic resistance problem all over the world drove researches to search for new and alternative antimicrobial agents that can replace synthetic antibiotics, especially for developing countries where access to hospitalization and synthetic drugs constitutes a problem. Several frequently used medicinal plants such as mint, thyme, clove, oregano, rosemary, cinnamon etc. have been tried for their antimicrobial effects (Estetlili et al., 2014; Elansary et al. 2016; Sakkas & Papadopoulou 2017; Antolak et al. 2018; Gonelimali et al. 2018). More plants are continuously being tested for their antibacterial activities (Razmavar et al. 2014; Manandhar et al. 2019; Diarra et al. 2020) Basil (*Ocimum basilicum*) is one of the medicinal plants whose extracts and essential oil have been shown to have antibacterial and antifungal properties (Suppakul et al., 2003; Adigüzel et al. 2005; Patil et al. 2011). Ethanolic solvent of basil was tested on diarrhea causing *E. coli* strain and was found to be inhibiting on this bacterium (Shweash et al., 2014). In another study, rosmarinic acid was extracted from the hairy roots of basil and tested on several soil borne microorganisms. This metabolite was found to be inhibiting on especially *Pseudomonas aeruginosa* (Bais et al., 2002). Ethanol, methanol and distilled water extracts of basil was also tested against pathogenic *Staphylococcus aureus* and different *Staphylococci* strains and ethanol and methanol extracts were found to be inhibiting on these bacteria (Okmen & Balpınar 2018). There are many recent researches about nutritional contents of basil (Li et al. 2017; Joshi 2014; Skrypnik et al. 2019) and other medicinal plants such as thyme, clove, mint etc. (Lorenzo et al. 2019; Batiha et al. 2020; Barboza et al. 2018; Janpen et al. 2019), or their heavy metal contents in different conditions (Georgiadou et al. 2018; Zahedifar et al. 2019; Mirosławski & Paukszto 2018; Adamczyk-Szabela et al. 2017). However, to the best of our knowledge there is no research about the correlation of nutrient content with the microbial inhibitory effect of *Ocimum basilicum*. It is important to collect information about the antibacterial and other medicinal properties of basil so that more conscious cultivation can be performed to ensure the quality of agricultural products having medicinal importance. Therefore, in this study, the correlation of antibacterial activity of whole aboveground parts of basil with the macro and micro nutrients of the plant has been studied.

2. Materials and Methods

The basil plants (*Ocimum basilicum*) were grown in 20 L pots in the laboratory conditions in the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Tekirdağ Namık Kemal University. Plant seeds (Miracle, Turkey) were first germinated in viols and transferred to pots after germination to be 10 plants/pot. Peat (Klasmann-Deilmann, Potground H, Germany) was used as the production material in this study. Some specifications of the used peat are: 160-260 mg L^{-1} N, 180-280 mg L^{-1} P_2O_5, 200-150 mg L^{-1} K_2O, 80-150 mg L^{-1} Mg, pH: 6, 70% organic matter, and 35% C. The plants were watered once a week and harvested after about four months. Freshly harvested aboveground parts of the plants were washed with pure water, dried as a whole at 65°C for 48 hours and then grinded. Elemental analyses were carried out on the dry grinded plant material by using ICP-OES device according to Kacar and İnal (2010) in Tekirdağ Namık Kemal University Central Laboratory (NABILTEM). Triplicate measurements were taken. Moreover, 20 g of the grinded dry basil was mixed with 500 mL of 96% ethanol (Merck, Darmstadt, Germany) and the solid-liquid extraction was carried out with a Soxhlet apparatus for 8 hours. Later, the extract was evaporated using a rotary evaporator. The liquid phase obtained from the total evaporation of ethanol was tested against four strains of bacteria. The reference strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Eenterococcus faecium* were obtained from Turkish Republic General Directorate of Public Health, Ankara, Turkey and Middle East Technical University, Ankara, Turkey. The bacteria were grown on blood agar containing 5% sheep blood. Bacterial cell concentrations were adjusted to 0.5 McFarland using sterile saline solution, and inoculated on Mueller-Hinton agar plates using sterile cotton swabs under aseptic conditions. The antibacterial activity of *Ocimum basilicum* extracts were tested using agar well diffusion method in triplicates (Balouiri et al., 2016) where sterile pipette tips were used to form wells of 8 mm size. 100µL of basil extracts were added to the wells, while 10µg of Gentamicin sulphate (Sigma-Aldrich, Darmstadt, Germany) was used as the standard antibiotic agent. Gentamicin is a broad spectrum aminoglycoside antibiotic which binds to the 30S ribosomal subunit of bacteria and therefore interferes with protein synthesis. The plates were kept at 37 ± 0.1°C for 24 h in an incubator and afterwards the inhibition zones around the wells were measured in mm as an indicator of antibacterial activity. The triplicate zones of basil extract were compared with the zone of Gentamicin. The results of the experiment was analyzed using SPSS 21 Statistical Software (IBM 2016) using Pearson correlation analysis.

3. Results and Discussion

In this study, the correlation of some micro and macro nutrient elements of whole aboveground parts of the basil plants (*Ocimum basilicum*) grown in laboratory conditions with its antibacterial effect on four different bacteria was investigated. An image of the growth of basil plants in viols belonging to this study can be seen in Figure 1. Whole plants were dried and grinded properly, and some micro and macro nutrient elemental contents were determined with ICP-OES. The amounts of the micro and macro elements determined in basil plants used in this study is given in Table 1.

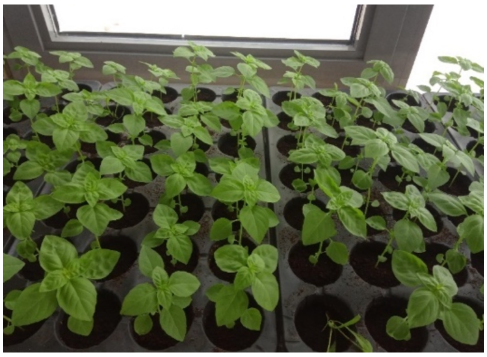

Figure 1. An image of Ocimum basilicum during the germination

Table 1. Some macro- and micro-nutrient elements of Ocimum basilicum

	Nutrient Elements	Amount
Macro Nutrients	P (%)	0.3221±0.03
	K (%)	3.529±0.23
	Ca (%)	1.842±3.49
	Mg (%)	0.372±0.04
Micro Nutrients	Fe (mg/kg)	127.43±0.64
	Cu (mg/kg)	7.47±0.03
	Zn (mg/kg)	32.80±0.23
	Mn (mg/kg)	174.10±0.79
	B (mg/kg)	47.83±0.03
Functional Elements	Al (mg/kg)	76.93±1.60

The macro nutrients of *Ocimum basilicum* used in this study are in an acceptable range (Table 1). The basil in this study is rich in micro nutrients when compared with herbaceous perennials stated in the literature (Mills and Jones, 1996)

The correlation between the macro and micro nutrients are given in Table 2. There is a strong positive correlation between phosphorus and potassium, but negative correlation between these two macro nutrients with calcium and magnesium. Potassium is a vital element for plant development and normal biochemical reactions, and it is referred as the quality element as it has crucial roles in quality parameters (Çalişkan & Çalişkan, 2018). It is also known that potassium inhibits calcium absorption (Johansen et al., 1968). Therefore this negative correlation was expected. A similar result was obtained by Dobrin et al. (2018), where they found a strong negative correlation between Mg and K, and strong positive correlation between Mg and Ca in hydroponic system for growing *Ocimum basilicum*.

Table 2. *Pearson's correlation coefficients between macro, micro nutrient and functional elements of Ocimum basilicum*

	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B	Al
P	1									
K	0.999**	1								
Ca	-.925	-.926	1							
Mg	-.634	-.638	.881	1						
Fe	.976	.974	-.818	-.449	1					
Cu	.618	.622	-.871	-0.999*	.430	1				
Zn	.982	.983	-.980	-.769	.916	.756	1			
Mn	.854	.857	-.988	-.944	.719	.937	.937	1		
B	-.618	-.622	.871	0.999*	-.430	-0,999**	-.756	-.937	1	
Al	.808	.805	-.522	-.056	.918	.036	.681	.384	-.036	1

* = P < 0.05; ** = P < 0.01

The elements with significant amount in basil in this study were detected to be Fe, Cu, Zn, Mn, B and Al. These micro nutrition elements were also detected in the study of Estetlili et al. (2014). The aluminum content of basil in the mentioned study was very high (574 mg/kg), while it was 77 mg/kg in this study. The difference may be resulted from the experimental parameters such as the soil and growing conditions. Our study was carried out in controlled lab conditions, whereas in the study of Estetlili et al. (2014) the plant materials was collected from markets. The soil of the plant materials collected from markets might have contaminated with Al. The nutrient element determination is therefore important for consumption of plant materials for human. Similarly, micro nutrition elements zinc and copper are positively correlated with potassium and phosphorus. However, when excess Cu and Zn was applied to the soil where *Ocimum basilicum* was grown, it was found that they have adverse correlation with Fe, Mn, P and K (Lajayer et al., 2014). This may show that basil might have a limiting mechanism for uptake of contaminants which are also micro nutrient elements.

The antibacterial effect of basil extract was tested on two Gram negative (*E. coli* and *P. aeruginosa*) and two Gram positive (*S. aureus* and *E. faecium*) bacteria. The agar plate photos are shown in Figure 2, where the zones in the middle of the plate belongs to the standard antibiotic Gentamicin. The inhibition zones were measured in mm and given in Table 3.

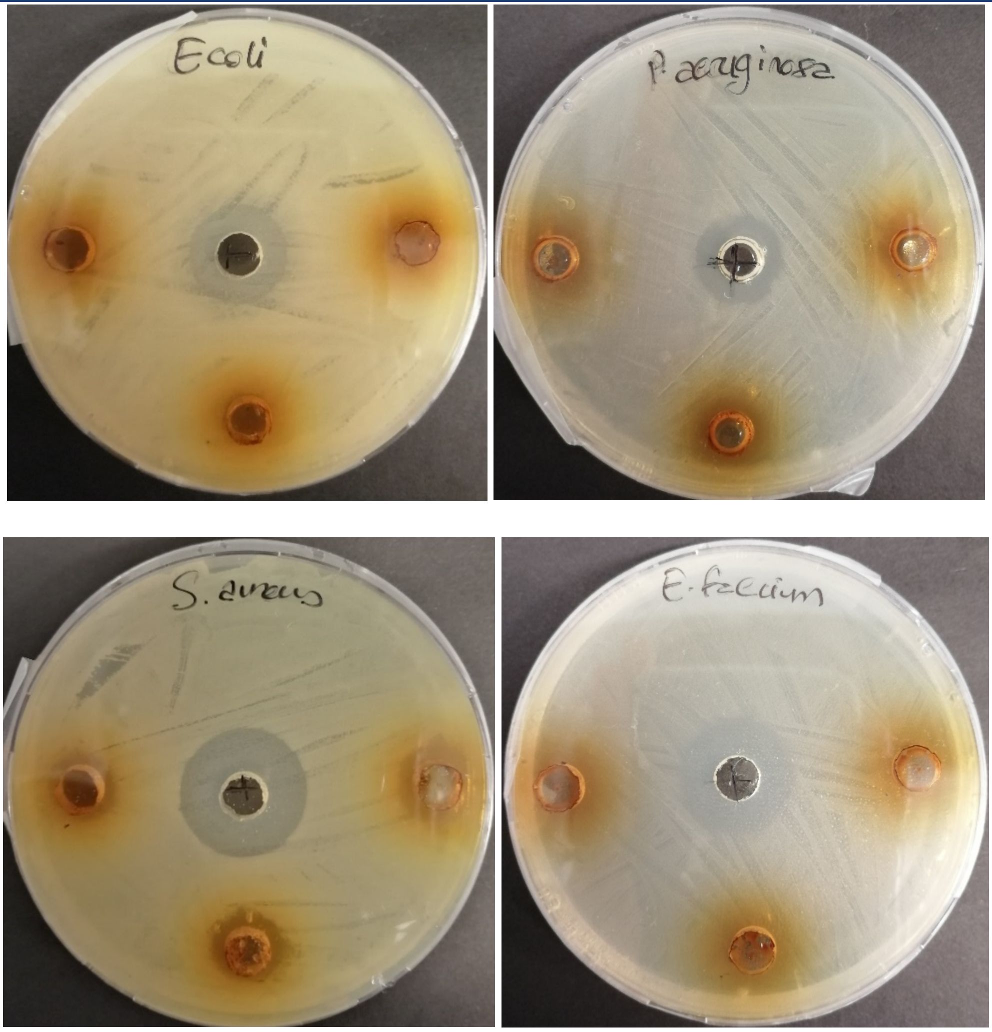

Figure 2. Agar plate images of the Ocimum bacilicum on different bacteria

Table 3. Antibacterial effect of Ocimum basilicum on different bacterial strains

Bacterium	Inhibition zone (mm)	Zone of Gentamicin	Inhibition degree
E. coli	9.67±0.58	18	++
P. aeruginosa	14.33±0.58	16	++++
S. aureus	14.67±1.15	23	+++
E. faecium	13.33±1.15	21	+++

++++ Excellent activity (100% inhibition), +++ Good activity (60-70% inhibition), ++ Significant activity (30-50% inhibition), + Negligible activity (10-20% inhibition), - no activity (<10% inhibition), Gentamicin: standard antibiotic

When compared with the positive control Gentamicin zones, the highest inhibitory effect of basil extract was on *P. aeruginosa*. This is a promising result considering that *P. aeruginosa* is an opportunistic human pathogen and a source of nosocomial infections which may cause death at a high rate (Moradali et al., 2017). A similar result was obtained by the rosmarinic acid extracted from hairy roots of basil and it was highly effected on *P. aeruginosa* (Bais et al. 2002). Except from *P. aeruginosa*, basil extract had good inhibitory activity on Gram positive bacteria. This might result from the presence of an outer membrane in Gram negative bacteria provides an extra barrier, which is lacking in Gram positive bacteria (Delcour 2009). Nevertheless, the antibacterial effect of basil extract on *E. coli* is significant. It is important as *E. coli* is associated with intestinal and extra intestinal infections and some strains are highly pathogenic (Toval et al. 2014). Even though both *E. coli* and *P. aeruginosa* are Gram negative bacteria, there are some molecular differences between species, caused by the genetic background. For instance, the composition in surrounding biofilm, the biomolecules produced by the organisms, the lipid contents of the membranes may differ. These may cause different reactions of the microorganism to different organic and inorganic molecules (Gugala et al. 2019; Harrison et al. 2007). The correlations between the nutrient elements of basil and the antibacterial activity measure by inhibition zones are given in Table 4. The correlation of boron with *E. coli* and *E. faecium* were strongly positive, and it was mild positive with other two bacteria. Boron is known to be essential for normal growth of plants. Moreover, it is a beneficial element for animal developmental processes, as this element plays role in stabilizing some biochemical molecules, but can be impairing in high concentration (Bolaños et al. 2004; Uluisik et al. 2018). Compounds and derivatives of B were found to be effective as bacterial growth and biofilm inhibitor (Sayin et al. 2016; Sopchenski et al. 2018). A new boron based antibacterial agent was tested on *E. coli* and *P. aeruginosa* and was suggested to have a medicinal potential (Hernandez et al. 2013). The B content of basil can therefore be significant in terms of antibacterial effect, as the present study suggests. Some heavy metals such as Fe, Cu and Zn are essential for bacteria in low amounts, but they are toxic in excess amounts. Bacteria have developed coping mechanisms for toxic heavy metal levels. Gram negative bacteria can be advantageous against heavy metals (Mounaouer et al. 2014). The more positive correlation of the metals with Gram positive bacteria in this study can be explained by the cell wall structure difference. On the other hand, the results revealed that the correlation of the metals, such as Cu, Zn and Mn, with the growth inhibition of *E. coli* growth is less negative than that of *P. aeruginosa*. This results from elevated resiliency of *P. aeruginosa* as it is primarily an environmental organism found in soil and water, while *E. coli* is an enteric bacterium where it does not usually exposed metals in a level that *P. aeruginosa* does (Teitzel et al. 2006).

Phosphorus has a strong positive correlation on Gram positive bacteria this means as the P content of the basil increases, the inhibitory effect of the plant extract on Gram positive bacteria increases. Similar correlation was obtained for K and Gram positive bacteria. However, this group of bacteria has a negative correlation with Ca and Mg contents, stronger for Ca. While Gram positive bacterial growth are mildly negatively correlated with Ca and Mg, Gram negative bacterial growth inhibition is positively correlated with Mg. The difference can be attributed to the different cell wall structures of Gram positive and negative bacteria. Mg ions play a role in cell wall stability and prevention of the cell from lysis in Gram positive bacteria, since Gram positive bacterial cell walls have a high binding affinity for Ca and Mg (Thomas & Rice 2014). Moreover, Mg and Ca were supposed to be more effective on stationary phase *S. aureus* as these ions cause membrane destabilization of the membrane when present in <40 mM (Xie & Yang 2016). In our study the Ca content was greater than 40 mM which explains the negative correlation of Ca amount and antibacterial activity. Therefore, cultivation of basil in neutral pH or slight acidic soils is preferred in order for basil to have more antibacterial activity. On the other hand, Mn and Zn were showed to be strongly and positively correlated with Gram positive bacteria. Zinc deficiency in soil can be up to %30 worldwide and 50% in Turkey. This is a serious problem in plant cultivation (Adiloglu & Adiloglu 2006) and our results suggest that Zn and B should be high for higher antibacterial activity.

Table 4. Pearson's correlation coefficients between the antimicrobial activity and macro and micro nutrient elements of Ocimum basilicum

	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B	Al
E. coli	.372	.367	.010	.482	.566	-.500	.189	-.165	0.99**	-.036
P. aeruginosa	-.618	-.622	.871	0.99*	-.430	-0.99**	-.756	-.937	.500	.847
S. aureus	.990	.989	-.861	-.517	.997	.500	.945	.771	.500	.847
E. faecium	.990	.989	-.861	-.517	.997	.500	.945	.771	0.99*	-.036

* = P < 0.05; ** = P < 0.01

The correlation between the concentrations of the functional element Al with the bacterial growth inhibition is strong positive or very weakly negative, the latter therefore can be neglected. This suggests that as the Al content in basil increases, the inhibitory zones increase. The inhibitory effect of Al on bacteria is already known and it increases with the increasing concentrations (Kurniawan et al., 2018).

4. Conclusions

This study aimed to investigate the correlation between the macro and micro nutrient elements of the basil extract with antibacterial activity of them on four different bacterial strains. The results showed that the basil plant used in this study was rich in some macro, micro nutrients, especially Fe, Zn, Mn, B; and a functional element (Al). The basil extract was significantly effective on inhibiting bacteria in this study, whose pathogenic strains and are responsible for nosocomial infections. The macro and micro nutrition elements might be the reason of antibacterial activity along with the phenolic content of this medicinal plant. Especially B was found to be positively correlated with the antibacterial activity of the basil plant. As the B content increases, the antibacterial activity of basil extract is supposed to increase, too. Phosphorus and potassium contents had a positive correlation with antibacterial activity, while calcium and magnesium had negative correlation. Moreover, the micronutrients Mn, Zn, B and Al contents were positively correlated with the antibacterial activity of the basil plant. The difference between Gram positive and Gram negative bacterial cell walls should be taken into account together with the elemental composition of the basil extract. Even though the antibacterial activity tests revealed that basil extract is effective on both Gram positive and Gram negative bacteria, the correlation analysis of the nutritional elements, this extract can be more preferred against Gram positive bacteria, especially when the Fe, Cu, Zn, Mn levels are high. Supplementary pharmaceuticals can be produced using the extracts of basil and similar plants. The investigation of the macro and micro nutrients with the antibiotic activity is important as basil and many other medicinal plants are frequently used in traditional medicine especially in developing countries. Basil and its extracts can be used as antibacterial agents, too. However, the metal contents of the extracts should be taken into consideration for medicinal products. The results also suggest the importance of the properties and contamination status of soils where the medicinal plants are grown as their correlation affect the antibacterial activity.

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