Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

Study of Biologically Active Compounds and Inhibitory Activity of Bay Leaves Laurus nobilis L.

Zainab A. Ali[1], Raghad Saad Al Musa1, Esraa S. Ethafa[2], and Widad S. Hannosh[3]
[1,2] Department of Food Science, College of Agriculture, University of Basrah
[3] Chemistry Department, College of Science University of Basrah, Basrah, Iraq

Email: zainab.abdali@uobasrah.edu.iq

Abstract. The global demand for medicinal plants has increased recently as food source for functional, healthy and sensory properties. This study allows the identification of active compounds found in alcoholic bay leaf extracts by GC-MS technique that give the plant functional properties. The inhibitory activity of extracts at concentrations of 0.25, 0.50, 0.75 and 1 mg/ml were tested against four types of bacteria: Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa. The results found the emergence of a number of active compounds, volatile oils and flavor compounds with a retention time (RT) ranging between 4.861-40.551 minutes, including compounds that gave the highest area of 24.96% such as Eucalyptol and alpha.-Terpinyl acetate compound by Area 10.87%. The results also showed that S. aureus recorded the highest inhibitory diameter at a concentration of 1 mg / ml was 10.7 mm, while E. coli recorded the lowest inhibitory diameter compared to the rest bacteria at the same concentration was 9.5 mm. B. cereus and P. aeruginosa, showed the highest inhibitory diameter were 10.4 and 9.8 mm at a concentration of 1 mg / ml. Which indicates the possibility of introducing bay leaves in many diets, improving its health and functional properties and prolonging the storage period due to rich in biologically active compounds

Highlights:

1. Active Compounds Identified: Eucalyptol (24.96%), α-Terpinyl Acetate (10.87%) via GC-MS.
2. Antibacterial Activity: S. aureus (10.7 mm), E. coli (9.5 mm) at 1 mg/ml.
3. Potential Applications: Functional food ingredient, health benefits, extended shelf life..

Keywords: active compounds, Bay leaves, GC-MS, extracts, Laurus nobilis L

Introduction

Bay (Laurus nobilis L.) is a native plant belonging to the Lauraceae family, it is also known as bay or laurel leaves, with spear-shaped leaves and aromatic wavy edges. It's one of the most common medicinal plants, used to treat digestive problems as epigastric pain, bloating and eructation difficulties [1]. Bay leaves are used in the treatment of rheumatism, rash, dermatitis, digestive problems such as poor digestion and flatulence, and in the treatment of type II diabetes [2,3]. Leaves are used as flavoring materials for many foods. The essential oil of the leaves of the laurel plant, which is widely used in

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

the food industry such as meat and fish products, as well as in the manufacture of perfumes, soaps and cosmetic purposes [4]. Bay leaf extracts are used in the treatment of hemorrhoids, diuresis, anti-snake bites, wound healing, as well as lowering the level of total cholesterol and low-density lipoprotein (LDL) and raising high-density lipoprotein (HDL) [5]. Bay leaves enhance the physiological properties of the liver and reduce oxidative stress and toxicity as one of its functions is the metabolization of digestible components such as food, medicines, alcohol and nutritional supplements, some of which lead to impaired functions [5, 6]. Bay leaf extends the shelf life of perishable foods as well as being non-contaminated by bacteria due to its low water content [7]. The chemical compounds found in the Laurus nobilis plant are antifungal, antibacterial and anti-cancer compounds because the plant contains active compounds, including flavonoids, alkaloids, phenols and tannins, minerals such as potassium, iron, zinc, magnesium and calcium, as well as important vitamins such as vitamin A, B and C [8,9]. The inhibitory activity of bay leaves is due to the fact that it contains active compounds with biological benefits[10]. Several studies have indicated that the phenolic extract of bay leaf has an antibacterial effect that poses a threat to health, especially the bacterium Escherichia coli and Staphylococcus aureus [11]. The current study aims to diagnose the active compounds in bay leaf extracts and test the antimicrobial activity as a plant commonly used in the world.

Methods

Sample collection

Dried bay leaves were obtained from local markets in Basra Governorate / Iraq. Rinsed with distilled water, then cut into small pieces, air dried for 24 hours, grinded with a Silver Crest type blender (Germany), samples were preserved in opaque packaging and kept at room temperature [5].

Preparation of extract

The methanol extract was prepared by mixing 1 g of bay leaf powder with 200 ml of methanol and distilled water mixture 50:50 V/V with a magnetic stirrer Shin Saeng (Korea) and left in an opaque package for 24 hours at 5 ° C and centrifuged by Brookfield (USA) 2900 rpm for 10 minutes. Prepare the alcoholic extract by mixing 1 g of bay leaf powder with 200 ml of water at 60 ° C for 30 minutes and filtered with filter papers No.1

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

Whatman. Evaporation of extracts with rotary evaporator Heidolph (Germany) and was frozen with a cryometer Crest (Germany) and kept at -20 ° C until tests [12].

Diagnosis of active compounds of bay leaf extracts using GC-MS technique

The active compounds in bay leaf extracts were diagnosed with GC-MS using the HP-5ms capillary column with an injection volume of 1 µL and helium gas at a flow rate of 0.5 ml / sec and the temperature of the injector and inter conveyor 250 ° C and set the furnace program to 80 ° C for 4 minutes and raised to 280 ° C for 20 minutes at a rate of 10 ° C / min and the spectra of the separated vertices of the components were compared with the spectra database of the NIST 2014 program library[13].

Bacterial cultures:

Inhibitory activity of the alcoholic extracts of bay leaf against four types of Pathological bacterial cultures Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Pseudomonas aeruginosa were obtained from the Microbiology Laboratory of the Faculty of Agriculture, Basra, Iraq. That identified using the technique VITEK 2. They activated on Nutrient broth medium Hi-media (India) company at a temperature of 37 ° C for 24 hours .It was compared with McFarland solution with a turbidity of 0.5 and giving an approximate number of bacterial cells of 1.5 × 108 units/ml after measuring the turbidity with spectrophotometer at 600 nm, then incubated on 37 ° C for 24 hours [12].

Determination of inhibition Zone:

According to the method mentioned by [8], the antimicrobial activity was estimated by spreading the pathogenic bacteria (Staphylococcus aureus, Bacillus cereus, E. coli, and Pseudomonas aeruginosa) spreading on Mueller-Hinton agar using sterilized L-glass rod, left to dry. Then holes were made by sterile metal drill with a diameter 6 mm and 50 µl of Bay leave extract at concentrations of 0.25, 0.50, 0.75 and 1 mg/ml and was compared with distilled water. The plates were incubated at 37°C for 24-48 hours, after that recording the Inhibition zone using a ruler assessed on based the diameter of clear zone surrounding the well.

Statistical analysis:

The Statistical Package for social scientists (SPSS) version 23.0 was utilized to analysis data. The differences in the mean values between groups were analyzed using the analysis of variance (ANOVA) and considered statistically significant at $p<0.05$. [14]..

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

Result and Discussion

Active compounds of bay leaf extracts with GC-MS technology

GC-MS technology is one of the most promising technologies that allows the qualitative diagnosis of bioactive compounds resulting from biosynthesis processes and extraction techniques for plant raw materials. Active compounds and essential oils contribute to the desirable flavor of the food products included in their composition, antibacterial and antifungal activity and antioxidant efficacy [15]. This study diagnosed the active compounds in the alcoholic extract of bay leaf using GC-MS technique as shown in Table 1 and Figure 1. As it was noted the emergence of a number of peaks due to active compounds, volatile oils and flavor compounds with a RT ranging between 4.861-40.551 minutes, including compounds that gave the highest area of 24.96%, such as Eucalyptol compound with a detention time of 10.91 minutes and a mass to charge ratio (m/z) 81.07, which gives quantitative and qualitative data as shown in Figure 2 and alpha.-Terpinyl acetate compound with an area ratio of 10.87% m/z 121.1 as shown in Figure 3. As well as other active compounds, including alpha.-terpineol and methyl eugenol, Area accounts for 2.82% and 3.38%, respectively.

[15] noted that Eucalyptol 12.30% is the most abundant compound in bay leaf oil along with 2-Octyl-Cyclopropaneoctanal, 7-Tetradecyne, Cis-9-Hexadecenal and Eugenol in proportions of 9.16, 6.68, 5.14 and 4.15% respectively. Other compounds have been observed, including 4-Hydroxy-4-methylcyclohex-2-enyl)propan-2-yl acetate 2- ((1R,4R)- by Area 2.78% and 1S- (1Alpha,2alpha,4beta))-1-isopropenyl-4-methyl-1,2-cyclohexanediol by Area 2.60%, 11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol by Area 2.14%, Sobrerol 8-acetate by 0.91%, in addition to many compounds that were present in small percentages. The results showed the emergence of Terpinen-4-ol, Furandimethanol, Vanillin and Caryophyllene oxide compound by 1.40, 1.11, 1.55 and 1.58%.

[16] showed that essential oils in bay leaves such as α-terpinyl acetate oil have a negative effect on pathogenic bacteria and fungi. The main part of bay leaf oils is represented by aromatic oils used for flavor used in diets as well as monoterpenes, phenylpropanoid, sesquiterpenes, 1,8-cineole, linalool and other compounds with health benefits[17].

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

[10] found the emergence of many volatile compounds, including 1, 8-cineole, α-piene, γ-elemene, cubenol, Verrucarol and Norethynodrel with different molecular weights and detention times. [18] proved that bay leaf extract contains chlorodifluoromethane which is used to control the quality of food products that bay leaves enter into their composition and stability and consists of terpenes by 25.07%, terpenoids 40.50% and fatty acids 7.93%. Noted the presence of large amounts of sabinen and linolenic acid.

Table 1. Active Compounds in Alcoholic Bay Leaf Extract

RT/ min	**Name**	**% Area**
4.861	Carbonyl sulfide	0.6911
8.456	l-Alanine, N-methoxycarbonyl-, methyl ester	0.5089
10.784	o-Cymene	0.8668
10.91	Eucalyptol	24.963
11.596	2,4-Dioxohexahydro-1,3,5-triazine	0.5511
11.617	Bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	0.7050
11.708	4-(Methylthio)-1-butene	1.5455
11.772	Furaneol	0.5226
12.177	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	0.8106
13.162	1-Methyl-5-fluorouracil	0.9859
13.181	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	0.5615
13.33	Cyclohexanemethanol,.alpha.,.alpha.-dimethyl-4-methylene-	1.0675
13.495	Terpinen-4-ol	1.4047
13.603	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0.6242
13.705	alpha.-Terpineol	2.8158
13.808	(-)-Myrtenol	0.3705
14.138	Benzofuran, 2,3-dihydro-	0.3708
14.438	Furandimethanol	1.1132
14.953	1,7-Octadiene-3,6-diol, 2,6-dimethyl-	0.5410
14.979	trans-Ascaridol glycol	0.6276
15.191	Benzene, 2-methoxy-1,3,4-trimethyl-	0.3595
15.211	p-Mentha-1,5-dien-8-ol	0.8617
15.324	Furyl hydroxymethyl ketone	0.6094
16	alpha.-Terpinyl acetate	10.865
16.112	Eugenol	1.6401
16.67	Pentanoic acid	0.5384

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

16.689	Methyleugenol	3.3840
16.75	Vanillin	1.5497
16.874	3,4-Dimethyl-1,2-cyclopentadione, Ac derivative	0.5665
17.126	2-((1R,4R)-4-Hydroxy-4-methylcyclohex-2-enyl)propan-2-yl acetate	2.7867
17.825	(1S,2S,4S)-Trihydroxy-p-menthane	0.7336
17.881	Benzene, 1,2-dimethoxy-4-(1-propenyl)-, (E)-	0.2899
17.999	3-Cyclohexene-1-methanol, 5-hydroxy-.alpha.,.alpha.,4-trimethyl-	1.5620
18.285	Sobrerol 8-acetate	0.9095
18.45	Homovanillyl alcohol	0.3629
18.477	(1S-(1Alpha,2alpha,4beta))-1-isopropenyl-4-methyl-1,2	2.6028
18.616	Sobrerol 8-acetate	0.3912
18.792	8-Acetoxycarvotanacetone	0.8518
19.101	Caryophyllene oxide	1.5839
19.242	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen	0.3088
19.425	Longifolenaldehyde	0.3263
19.617	Agarospirol	0.2899
19.705	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	2.1446
19.887	2-Azafluorenone	0.2985
19.945	Longifolenaldehyde	0.3343
20.07	14-Hydroxycaryophyllene	0.4819
20.257	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	1.1885
21.248	Cyclohexanol, 3,3,5-trimethyl-	0.5942
21.704	10-Epigazaniolide	0.3359
22.438	14-Hydroxycaryophyllene	0.3507
22.86	n-Hexadecanoic acid	0.4263
23.287	Spirafolide	0.3557
23.532	Dehydrocostus lactone	1.4799
40.551	1-Triethylsilyloxyheptadecane	0.6460

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

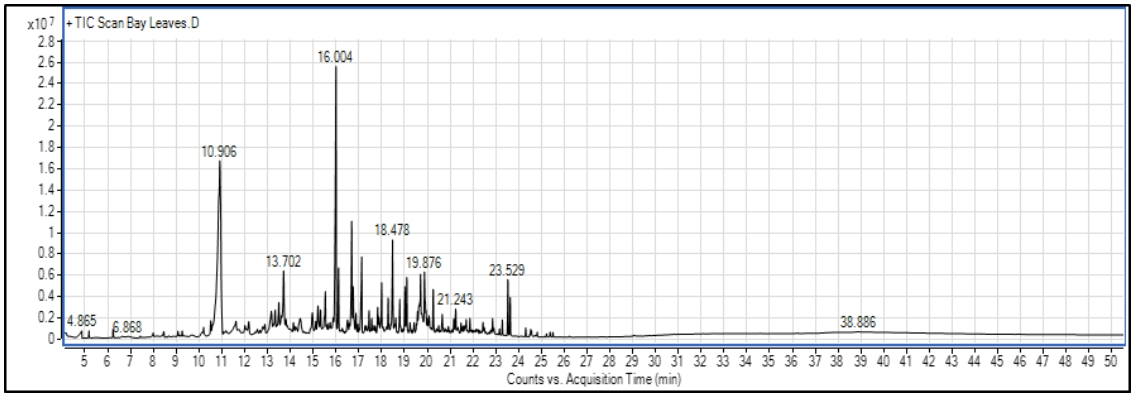

Figure 1. Chromatogram Active Compounds in Alcoholic Bay Leaf Extract

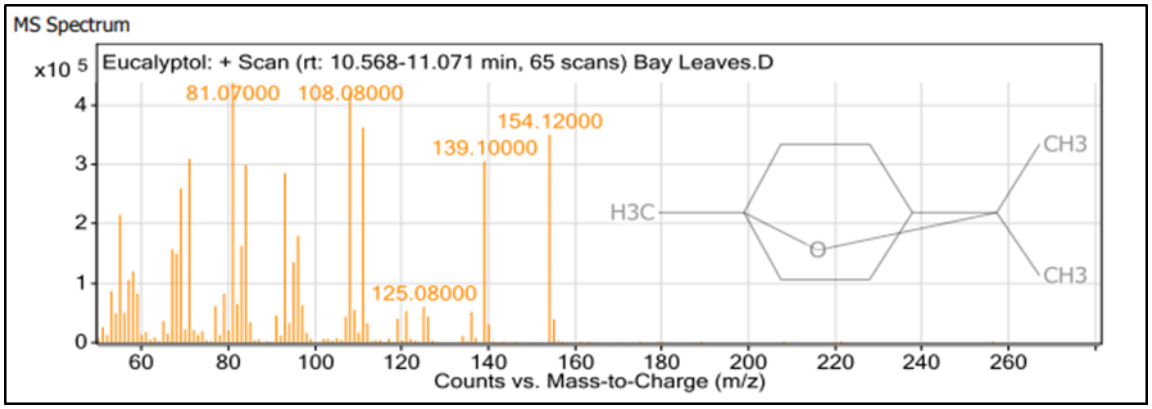

Figure 2. Mass to charge ratio m/z plotter for Eucalyptol

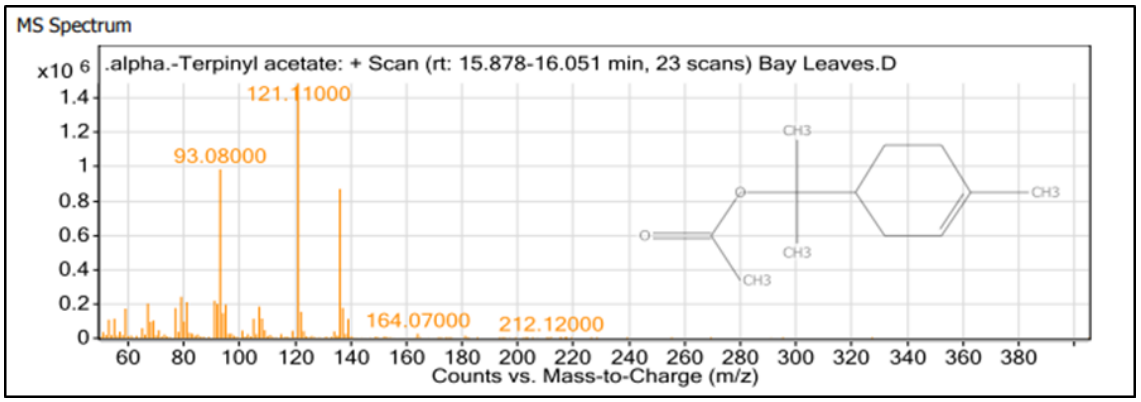

Figure 3. Mass to charge ratio m/z for Alpha-Terpinyl acetate

Antibacterial activity:

The antibacterial activity of S. aureus, B. cereus, E.coli and P. aeruginosa was tested for alcoholic bay leaf extract at concentrations of 0.25, 0.50, 0.75 and 1 mg/ml

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

and was compared with distilled water in which no activity was observed as a control sample and after incubation the diameters of the growth-free zone were recorded. The results shown in Figure 4 A, B, C and D showed the inhibitory activity of alcoholic bay leaf extract against Gram positive and Gram negative bacteria and increased inhibitory activity was observed with increasing concentration of the extract. The results of the statistical analysis showed significant differences $P \leq 0.05$ between the gram positive bacteria S. aureus and B. cereus, which significantly outperformed the negative bacteria E. coli and P. aeruginosa and the presence of significant differences in different concentration. The effectiveness of alcoholic bay leaf extract at 1 mg/ml was significantly higher inhibitory diameter for all types of bacteria tested compared to the rest of the concentrations 0.25, 0.50 and 0.75 mg/ml. Figure 4-A shows the inhibitory activity of S. aureus, Figure 4-B B. cereus, Figure 4-C E. coli and Figure 4-D P. aeruginosa. S. aureus bacteria were observed to have the highest inhibitory diameter at 1 mg/ml concentration of 10.7 mm due to their high sensitivity to active compounds found in bay leaf extract. While E.coli recorded 9.5 mm the lowest inhibitory diameter at the same concentration compared to the rest of the bacteria, as they were more resistant to the active compounds in bay leaf extract. B. cereus and P. aeruginosa, showed a higher inhibitory diameter of 10.4 and 9.8 mm at the same concentration, respectively. This is because the components of the negative bacterial cell wall contain 80% of polysaccarides that prevent antimicrobial substances from reaching the peptidoglycan layer underneath, which represents 20% of cell wall components [19]. [8] indicated that the E. coli was less sensitive to bay leaf extracts compared to the positive S. aureus. He also indicated that bay leaf reduces E. coli and thus prolongs the shelf life of food products that bay leaves enter into their composition, due to the presence of many effective compounds that have the ability to inhibit bacteria, especially Gram positive bacteria [20].

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

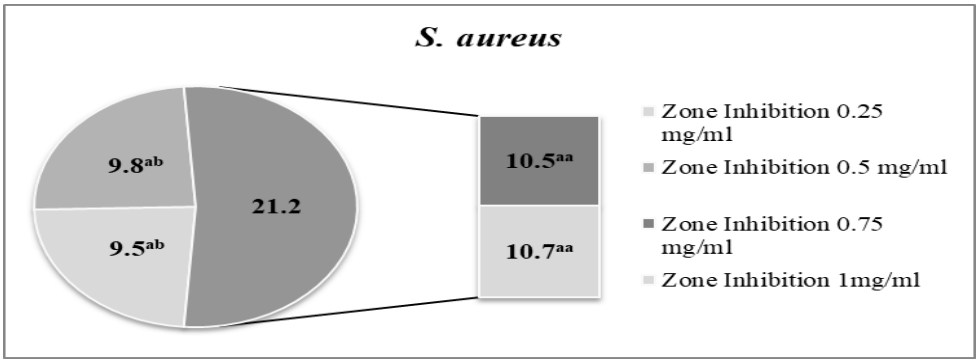

Figure 4-A. Inhibitory activity of S. aureus

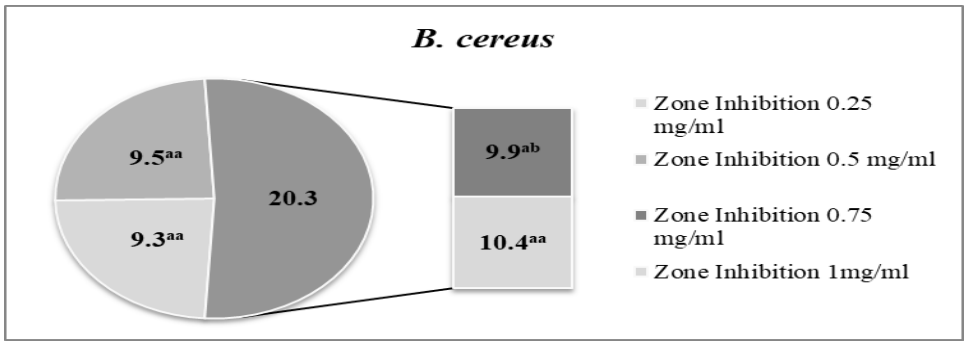

Figure 4-B. Inhibitory activity of B. cereus

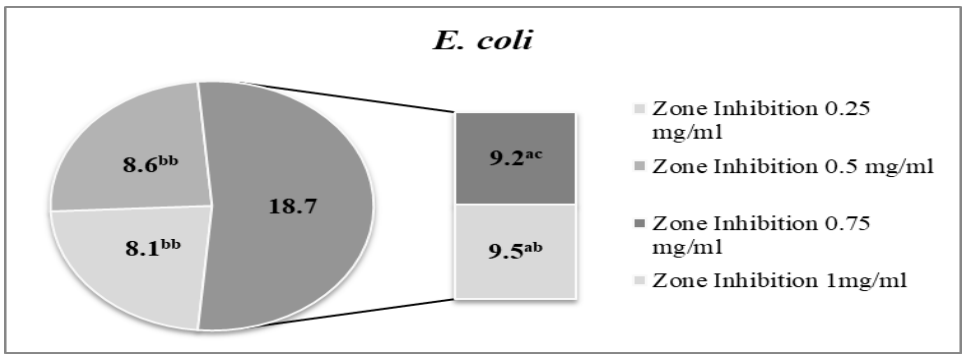

Figure 4-C. Inhibitory activity of E. coli

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

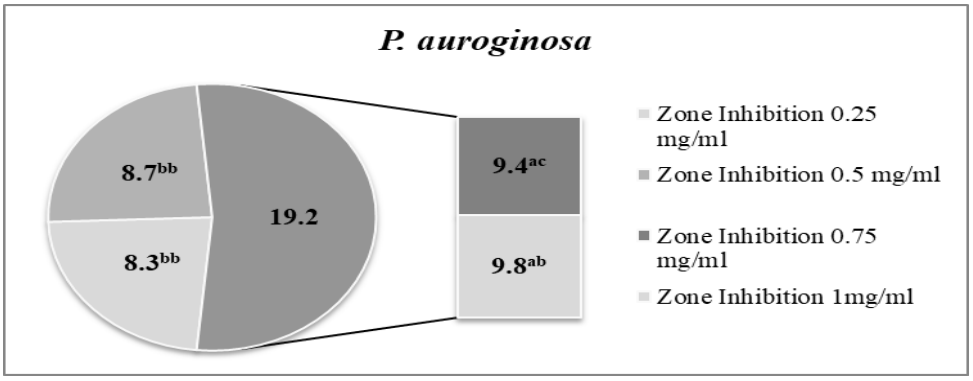

Figure 4-D. Inhibitory activity of P. aeruginosa

*Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability ($P<0.05$).

Conclusion

The results of this study showed the emergence of a different group of metabolites (biologically active compounds) in the alcoholic extract of bay leaves, including Eucalyptol, alpha.-Terpinyl acetate, alpha.-Terpineol and Methyleugenol by different areas and detention times, which give the food products included in their composition functional benefits as well as prolong the preservation of these foods. It was also noted that its antimicrobial activity was high, especially to the Gram positive bacteria which allows the possibility of investing bay leaves and extracts in the treatment of many diseases as well as protecting food from spoilage, so more research must be conducted on improving medicinal plant materials, notice the interactions between food components and active compounds in it to develop new products with health benefits.

Acknowledgements

We would like to gratitude to the Department of Food Science, College of Agriculture, Basrah University for supporting this research work

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

References

[1] A. O. Falade et al., "Aqueous Extract of Bay Leaf (Laurus nobilis) Ameliorates Testicular Toxicity Induced by Aluminum Chloride in Rats," Veterinary World, vol. 15, no. 11, pp. 2525–2532, 2022.

[2] K. Singletary, "Bay Leaf: Potential Health Benefits," Nutrition Today, vol. 56, no. 4, pp. 202–208, 2021.

[3] S. Tahar et al., "Chemical Composition, Antioxidant and Anticholinesterase Activity of the Essential Oil of Algerian Cachrys sicula L.," Natural Product Research, vol. 36, no. 16, pp. 4094–4102, 2022.

[4] A. Sharma et al., "Importance of Essential Oils and Current Trends in Use of Essential Oils (Aroma Therapy, Agrofood, and Medicinal Usage)," in Essential Oils, Academic Press, 2023, pp. 53–83.

[5] C. Obiandu et al., "Evaluation of the Effects of Extracts of Laurus nobilis on Some Biochemical Parameters of Wistar Rats," Scholars International Journal of Anatomy and Physiology, vol. 6, no. 4, pp. 37–41, 2023.

[6] R. Srivastava, "Nutritional and Pharmacological Properties of Bay Leaves (Laurus nobilis L.)," in Ethnopharmacological Investigation of Indian Spices, IGI Global, 2020, pp. 114–123.

[7] G. Masaki, F. Santoso, and M. D. P. G. Puteri, "Optimization of Aqueous Extraction of Indonesian Bay Leaf (Syzygium polyanthum Wight) as Powder Seasoning," in Proceedings of the 6th International Conference of Food, Agriculture, and Natural Resource (IC-FANRES 2021), Atlantis Press, 2022.

[8] Y. K. Khodja et al., "In Vitro Antioxidant and Antibacterial Activities of Phenolic and Alkaloid Extracts of Laurus nobilis," South Asian Journal of Experimental Biology, vol. 11, no. 3, pp. 45–53, 2021.

[9] V. A. Wahyudi et al., "The Bay Leaves Active Compounds and Its Lipid Oxidative Inhibition Activity in Bulk Cooking Oil," Planta Tropika, vol. 9, no. 1, pp. 71–81, 2021.

[10] V. J. Albaradeih, "Evaluation of Biological Activities and Phytochemical Screening of Laurus nobilis Leaves Extract," International Journal of Pharmacognosy and Phytochemistry, vol. 10, no. 1, pp. 55–63, 2024.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.63

[11] O. Mansour et al., "Review Study on the Physiological Properties and Chemical Composition of the Laurus nobilis," Pharmaceutical Chemistry Journal, vol. 5, pp. 225–231, 2018.

[12] L. P. Kose et al., "Anticholinergic and Antioxidant Activities of Avocado (Folium perseae) Leaves–Phytochemical Content by LC-MS/MS Analysis," International Journal of Food Properties, vol. 23, no. 1, pp. 878–893, 2020.

[13] U. U. George et al., "Ethno-Botanical Verification and Phytochemical Profile of Ethanolic Leaves Extract of Two Medicinal Plants (Phragmenthera capitata and Lantana camara) Used in Nigeria Using GC-MS Technique," Acta Biology Forum, vol. 5, no. 2, pp. 78–89, 2023.

[14] D. George and P. Mallery, IBM SPSS Statistics 26 Step by Step: A Simple Guide and Reference, 16th ed. New York, NY, USA: Routledge, 2019.

[15] I. O. Lawal et al., "Chemical Composition and Effectiveness of Bay Leaf and Cinnamon Essential Oils Against Aflatoxigenic Fungi," Ceylon Journal of Science, vol. 53, no. 3, pp. 245–254, 2024.

[16] V. Singh, G. P. Agrawal, and B. Singh, "GC-MS Analysis of Bio-Active Compound α-Terpinyl Acetate Extract of Bay Leaf Having Antimicrobial and Antifungal Properties," Journal of Essential Oil Research, vol. 35, no. 1, pp. 22–30, 2024.

[17] I. Peris and M. A. Blázquez, "Comparative GC-MS Analysis of Bay Leaf (Laurus nobilis L.) Essential Oils in Commercial Samples," International Journal of Food Properties, vol. 18, no. 4, pp. 757–772, 2015.

[18] V. V. Evlach et al., "Analysis of Chlorodifluoromethane Extract of Bay Leaf by Gas Chromatography With Different Kinds of Detection," Journal of Chemistry and Technologies, vol. 29, no. 2, pp. 321–330, 2021.

[19] B. Shams, N. G. Ebrahimi, and F. Khodaiyan, "Development of Antibacterial Nanocomposite: Whey Protein-Gelatin-Nanoclay Films With Orange Peel Extract and Tripolyphosphate as Potential Food Packaging," Advances in Polymer Technology, vol. 2019, no. 1, p. 1973184, 2019.

[20] S. O. Algabri et al., "Bay Leaves Have Antimicrobial and Antioxidant Activities," Journal of Pathogen Research, vol. 1, no. 1, pp. 3–10, 2018.