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BIOLOGICAL ACTIVITIES OF LAURUS NOBILIS LEAVES

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ABSTRACT

The presence of phenolic compounds in spices and herbs, along with the essential oils, has been gaining attention due to their various functions like antioxidant capacity, antimicrobial properties, and flavoring properties. The Bay leaf belongsto Lauraceae family and is endemic in the Mediterranean region. Lauraceae, is an aromatic plant frequently used as a spice in Mediterranean cookery and as a traditional medicine for the treatment of several infectious disease. L. nobilis also belongs to Lauraceae. L. nobilis is aromatic tree, and is 2 m to 10 m high. L. nobilis contains about 1.3% essential oils and polar flavonoids mono, sesquiterpenes, alkoloids, glycosylated flavor-noids, megastigmane and phenolic components. It is known to have various pharmacological effects, including antimicrobial, cytotoxic and immune modulating. Its' essential oil containg eucalyptol, α-terpinyl acetate, linalool, methyl eugenol, sabinene and carvacrol. The property of every essential oil varies according to the harvest country, altitude, period of sunshine, conditions of harvest. These essential oil contents of L. nobilis are strong antibacterial activity against Gram negative and Gram positive foodorne pathogens (Salmonella, Staphylococcus aureus, Esherichia coli, Listeria monocytogenes like that), spoilage bacteria (Pseudomonas aeroginosa) as well as antifungal effects. The synergy between terpenes (linalool), lactones, oxides (1,8 cineole) and monoterpenes (camphene, alpa-pinene) gives to the essential oil of Laurel a good antibacterial activity. Its essential oils' various or single chemical compositions at different concentrations have different inhibition mechanisms that can affect a variety of pathogens by changing membrane permeability, denaturing proteins and inhibiting enzymes. The oils are not affecting on existing beneficial intestinal bacteria.

Keywords: Essential Oils, Laurus Nobilis, Antibacterial Activity, Review.

I. INTRODUCTION

Antibiotic or multiple antibiotic resistance (MDR) microorganism particularly pathogen bacteria has dramatically increased in human and animal. Therefore,resistance microorganism caused diseases have posed a risk in human and treated public health. Due to these resistance properties of microorganism, researchers started looking for alternative way for treatment or for preventing diseases. Nowadays, ingredients obtained from plants, like essential oil, can be used as alternatives to antibiotics. Bay laurel, cinnamon, oregano and clove like plants have antimicrobialactivity against both some Gram negative and positive microorganisms (1). In this review, L. nobilis (bay leaf) and its effects as antimicrobial properties against some microorganism are highlighted.

It is cultivated and endemic in the Mediterranean countries of Turkey, Spain, Morocco, Greece, Portugal, as well as in Mexico and other temperate and warm parts of the world. This aromatic tree is 2 m to 10 m high (2). The plants inherently cultivated in coastal areas to an altitude of 600- 800 meters. The plant's leaves and berries are commonly used as a spice aroma and enhancer for foods especially for meats, sauces and soups (3). Besides its special aroma, it is also used to cure diseases all over the world. Some compounds of this plant such as essential oils and organic acids have shown strong antibacterial activity against some foodborne pathogen microorganism besides spoilage bacteria (3, 4, 5, 6).

Laurus nobilis is a flowering plant that related to Lauraceae family. This family is consisting of about 2850 spp & 45 genera in the world. It's an aromatic evergreen shrub or tree that regarded as high-content spice found in Europe, South America, Asia, and endemic in Morocco, Spain &Turkey (13). Laurus nobilis leaves are used as preservative in the industry of food. Chemical analysis of the plant revealed the presence of many compound suchas flavonoids, volatile and non-volatile oils,tannins, alcohols, many minerals, alkaloids& different vitamins.

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Also it's containing many secondary metabolites as active constituents like hydroxyl group (3). Laurus nobilis leaves aqueous extracts have been used in herbal medicine for treatment of several dermatological & neurological disorders. It's also have antimicrobial activity against different pathogenic bacteria in vitro .Infection of urinary Tract (UTI) is defined as the invasion and spread of pathogenic microorganisms (M.O) in the tissues of urinarytract. Many factors may leads to UTI infections such as predisposition genetically,sexually intercourse, abnormalities in UTI structure, diabetes, low immunity, pregnancy, formation of stone, and use of catheterization (7). The bacteria are able to adhere, resistant tohost defenses, then colonization and infection of UTI by two important routes: the hematogenous and ascending pathways (15). Burn wound injuries are common nosocomial infections in the world. Burn wound area are predispose for colonization with M.O of exogenous and endogenous origin during thermal injury (11). Many factors like burnnature, patient immunity, age, depth & extent of injury, also number & type of M.O. at the burn site, enzyme and toxin production that dissemination systemically, all of these factors enhance burn wound infections (20). Burn wounds become colonized and infected with gram negative bacteria like P(22).

II. TOXICOLOGY

- **Laurel Leaves**

In a study to determine the concentration of some toxic chemical elements in eight medicinal plants, using instrumental neutron activation analysis (INAA), it was found that L. nobilis had the highest arsenic concentration . Arsenic toxicity is a widespread health concern affecting millions of individuals worldwide, and it can be found as a contaminant in several conventional remedies. Arsenic's toxic effects are due to its ability to inactivate nearly 200 enzymes, particularly those that play a role in cellular energy pathways and DNA synthesis and repair. Initial symptoms of acute arsenic poisoning include vomiting, abdominal pain, nausea, and severe diarrhea, with reported cases of encephalopathy and peripheral neuropathy. Chronic arsenic toxicity leads to a range of health issues affecting multiple body systems. Furthermore, arsenic is a well-established human carcinogen that impacts various organs .

- **Common Toxic Activity of Leaves and Fruits**

A positive sodium lauryl sulfate (SLS) patch test result may be a possible marker of fragrance allergy . Therefore, SLS in plants can induce allergic reactions, and in fact, some cases of allergic contact dermatitis to bay fruit oil have been reported. However, this condition of allergic contact dermatitis is rather rare and usually affects aromatherapists or traditional medicine users, who frequently expose themselves to L. nobilis . The Information Network of Departments of Dermatology (IVDK) indeed indicates that around 1% of patients with patch-tested dermatitis show a positive reaction to laurel leaf extract. In addition, systemic allergic dermatitis induced by SLS contained in plants has been reported, in which ingestion or inhalation of SLS can cause generalized exanthema or other systemic reactions.

III. ESSENTIAL OIL CONTENT

Essential oils are generally derived from one or more plant parts, such as flowers, leaves, stems, bark, wood, roots, seeds or fruits and the yield of the essential oil varies among different parts of the same plant. The amount of essential oil extracted from different plants ranges from 0.01 to 10%. The leaves of bay leaf collected from Lebanon yielded 35.15% 1,8- cineole while the essential oil obtained from young and old leaves collected from North Black Sea region of Turkey yielded 24.2 and 32.1%, respectively. The essential oils of the leaves and fruits from bay grown in Antakya, Yayladagi and Samandagi were isolated by solvent extraction and analysed by capillary gas chromatography (GC), gas chromatography and mass spectrometry (GC-MS).

Chemical Composition

The chemical composition of the EOs from laurel leaves, twigs, and fruits is listed in Table 1. The laurel EO was light yellow and had a specific odor. In the fruit EO of this study, 38 constituents representing 99.3% of the total content were identified. Twelve of the constituents were in concentrations over 1% of the EO. The main constituents in the fruit EO (above 3%) were 1,8-cineole (33.3%), α-terpinyl acetate (10.3%), α-pinene (11.0%), β-elemene (7.45%), sabinene (6.30%), β-phellandrene (5.2%), bornyl acetate (4.38%), and camphene (4.3%). Thirty-seven volatile constituents, representing 98.8% of the total composition were identified in the twig oil, 12 of them exceeding 1%. The most abundant constituents found in the twig EO (above 3%) were 1,8-

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cineole (48.5%), α-terpinyl acetate (13.1%), methyl eugenol (6.6%), β-linalool (3.8%), β -pinene (3.4%), sabinene (3.3%), and terpinene-4-ol (3.3%).

Results show that 40 constituents representing 98.93% of the total content were identified in the leaf EO, 11 of them being above 1%. The main constituents in the leaf EO (above 3%) were 1,8-cineole (41.0%), α-terpinyl acetate (14.4%), α-pinene (2.6%), β-elemene (0.78%), sabinene (8.8%), β-linalool (4.9%), α-terpineol (3.1%), α -pinene (2.6%), and terpinene-4-ol (2.4%).

IV. BIOLOGICAL ACTIVITIES

Biological activity is defined as the inherent capacity of a substance to alter chemical or physiological functions of cell, tissue or organism. The genus laural comprises of more than 100 species which are distributed throughout temperate regions of Europe and Asia. Several species of this genus are being used in traditional medicines in Asian countries. Despite many medicinal uses, the plants from this family are very well known to contain bioactive compounds that are useful to develop plant based pesticides. The compounds isolated from it are known to cause toxic effect against many pests causing damage to agricultural commodities or crops. The details of the biological activities exhibited by different parts of L. nobilis are listed below:

4.1 Nematicidal

Plant parasitic nematodes are the most destructive group of plant pathogens worldwide and their control is extremely challenging. The root-knot nematodes, Meloidogyne spp, are one of the most economically damaging genera of plant parasitic nematodes on horticultural and field crops (Andres et al. 2012. The nematicidal effects of the essential oils on J2 and eggs of Meloidogyne javanica at a concentration of 1000 µl/l were examined. In vitro, bay leaf essential oil immobilized more than 80% of J2 after 2 days of incubation (Oka et al. 2000). Nematicidal activity of bay essential oil against Meloidogyne incognita was investigated in tomato and pepper. There were no significant differences between nematode inoculums level and essential oil concentration used. However, the plant extract treatments restrained nematode populations in both tomatoes and pepper host plants (Cetintas and Qadir 2014).

4.2 Antioxidant

Ozcan et al. (2010) determined the potential antioxidant activity of the essential oil and methanolic extract of seed oil from L. nobilis by employing DPPH free radical scavenging and β-carotene/linoleic acid test systems. In both test systems the essential oil and the methanolic extract of seed oil of L. nobilis exhibited antioxidant properties. The 50% (IC50) inhibition activity of the essential oil on the free radical DPPH was determined as 94.65 mg/ml whereas IC50 value of methanolic extract of seed oil was found unstable. In the case of the linoleic acid system, oxidation of linoleic acid was inhibited by essential oil and methanolic extract of seed oil, which showed 64.28 and 88.76% inhibition, respectively. The inhibition value of the methanolic extract of seed oil was quite close to the synthetic antioxidant butylated hydroxytoulene (BHT), 92.46% inhibition. The extracts of cardamom, coriander seeds and dried bay leaves were prepared and iron(III) reduction, 1,1-diphenyl-2-picrylhydrazyl radicalscavenging, hydrogen peroxide, superoxide and nitric oxide radical scavenging, reducing power were assayed as antioxidant capacity. Bay leaves showed greater amount of phenols and high antioxidant activity as compared to cardamom and coriander extracts (Deepa et al. 2013). Al-Hashimi and Mahmood (2016) determined the reducing power and antioxidant activity of alcoholic extracts of bay leaves. The rates of antioxidant activity and reducing power increases as the concentrate of bay leaves extract increased. The in vitro and in vivo antioxidant activities of different extracts of Laurel leaves were studied by Kaurinovic et al. (2010). The results indicated that ethyl acetate extract of bay leaves exhibited the largest free radical scavenging capacity in neutralization of DPPH, NO, O2 •− and OH radicals. The in vivo effects were evaluated on some antioxidant systems (activities of GSHPx, LPx, Px, CAT and XOD, and GSH content) in the mice liver and bloodhemolysate after treatment with the examined laurel extracts, or in combination with carbon tetrachloride. On the basis of the results obtained it can be concluded that the examined extracts exhibited a certain protective effect, which is more pronounced on the liver than on blood hemolysate parameters and the ethyl acetate extract showed strongest protective effect. The antioxidant potentials of ethanolic and aqueous extracts of Hypericum perforatum, Ocimum basilicum and L. nobilis leaves were evaluated by 2, 2-diphenyl-l-picrylhydrazyl radical (DPPH) assay. The lowest radical scavenging capacity (RSC) was reported in the aqueous extract of L. nobilis as compared to H. perforatum and O. basilicum. The ethanolic

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extracts of L. nobilis showed more DPPH radical scavenging action than their aqueous extracts (Rukhkyan et al. 2013). El et al. (2014) obtained the laurel essential oil by using solvent-free microwave extraction (SFME) and hydrodistillation (HD) methods from L. nobilis leaves at 622 W (100%) and 249 W (40%) power levels and hydrodistillation inhibited oxidation generated by 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical by 93.88, 94.13 and 92.06%, respectively. Trolox equivalent antioxidant capacities (TEAC) of essential oils were 0.18 mm/ml oil for SFME at 622 W, 1.36 mm/ml oil or SFME at 249 W and 2.40 mm/ml oil for hydrodistillation ($p < 0.05$). Essential oils of L. nobilis were extracted by SFME at 100 and 40% power levels and hydrodistillation inhibited linoleic acid peroxidation by 70.57, 63.53 and 89.18% respectively. Inhibition effects of laurel essential oils obtained by SFME at different power levels and hydrodistillation on DPPH radical cation oxidation were not significantly different. The strongest antioxidant activity against DPPH radical was found in the essential oil obtained by SFME at 100% power level. Basak and Candan (2013) showed that the DPPH, hydroxyl and superoxide radical as well as hydrogen peroxide scavenging activities of bay leaf essential oil were greater than the positive controls and the three main components of the oil when tested independently. The inhibition of lipid peroxidation by the oil occurred less frequently than with 1,8- cineole and R-(+)- limonene alone, but the effects were more pronounced than those seen with 1-(S)-α-pinene and the positive controls. Antioxidant combination effect was assessed by DPPH free radical scavenging method (Bag 2015). The bay leaf essential oil was screened for possible antioxidant activity by DPPH (2,2-iphenylpicrylhydrazyl) free radical-scavenging and the β-carotene/linoleic acid assay. Both of these in vitro methods showed that the essential oil was a less powerful reducing agent than the well-known synthetic antioxidants, butylated hydroxytoluene and ascorbic acid (Yilmaz et al. 2013). Lyophilized aqueous and ethanol extract of L. nobilis were evaluated for their antioxidant activity, reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities to determine the total antioxidant capacity of both extracts. Both extracts showed strong total antioxidant activity in linoleic acid emulsion.

4.3 Insecticidal

Bay leaf essential oil was tested for its insecticidal activity against Tribolium castaneum at five different concentrations ranging from 4-12 mg/g. The polar fraction of bay leaf essential oil was found to be more active as insecticide as compared to non-polar fraction and essential oil. Moreover, the insecticidal potential was found to be both concentration and time dependent (Chahal et al. 2016). Jemaa et al. (2011) reported the chemical composition and the repellent activity of L. nobilis essential oil against 7-10 days old adults of Lasioderma serricorne. The results revealed that repellent action was highly dependent upon oil concentration and exposure time and the repellent efficacy was found to be high for high doses and short exposure period. So, L. nobilis essential oil may have potential as a control agent against cigratte beetle. L. nobilis essential oil from Morocco showed better insecticidal activity as compared to Tunisian and Algerian oils with RD50 values 0.013, 0.036 and 0.033 µl/cm2 for Rhyzopertha dominica whereas 0.045, 0.139 and 0.096 µl/cm2 for T. castaneum (Jeama et al. 2012) Salehi et al. (2014) reported the repellency effects of L. nobilis essential oil against adults of Ephestia kuehniella Zeller as bay leaf essential oil showed 82.4 percent repellency rate at highest tested concentration i.e. 2.00 µl/l. Repellency and toxicity of essential oil from L. nobilis against the rust-red flour beetle (T. castaneum Herbst) were reported by Andronikashvili and Reichmuth (2003) . The toxicity of ethanol extracts on the large diamond back moth, Plutella xylostella, was 55% (Erturk et al. 2004) . Essential oils from laurel were evaluated for fumigant toxicity against all developmental stages of the confused flour beetle (Tribolium confusum). The vapours of laurel essential oil were toxic to all the stages of T. confusum (Isikber et al. 2006) . The bruchid, Acanthoscelides obtectus, is one of the most damaging pests of kidney beans (Phaseolus vulgaris) worldwide. However, aromatic plants from the families Lamiaceae, Lauraceae, Myrtaceae and Poaceae can protect P. vulgaris by a direct or delayed insecticidal effect, through increased adult mortality and inhibition of reproduction (both oviposition and adult emergence). The results suggested that lipid, as well as nonlipid allelochemicals, such as phenolics, or non protein amino acids or flavonoids may be involved in the toxicity of extracts of aromatic plants to A. obtectus (Regnault- Roger and Hamraoui, 1995; Mackeen et al. 1997). Tayoub et al. (2012) investigated the fumigant toxicity of the bay and sage essential oils against larvae of Trogoderma granarium insect. Exposure to vapours of essential oil from bay laurel and sage for 48 h resulted in

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about 98 and 100% mortality of the larvae at a concentration of 60 and 90 µl/160 cm3 air, respectively. Essential oils of bay laurel showed a higher lethal activity than that of sage.

4.4 Antimicrobial

Essential oils displayed antimicrobial activity against Staphylococcus aureus 6538P, Escherichia coli O157:H7 and Salmonella typhimurium NRRL E 4463. The inhibitory effect on S. aureus 6538P of laurel oil obtained from SFME using lower power level was found to be lower than that obtained from SFME at 100% power level and hydrodisttilation method ($p < 0.05$) (El et al. 2014). The antimicrobial activity of the essential oil was tested against a panel of foodspoiling bacteria and one yeast strain. The minimum inhibitory concentration values for food-spoiling bacteria and yeast strain that were sensitive to L. nobilis essential oil ranged from 125-2000 µg/ml. E. coli O157:H7, Candida albicans ATCC 16231, Salmonella enteritidis ATCC 13076 and L. monocytogenes ATCC 7644 had MIC values of 125, 250 and 500 g/ml, respectively and were most sensitive to L. nobilis. They showed the largest growth inhibition halos in the agar well diffusion assays (33.0, 26.0, 24.0 and 22.0 mm, respectively (Yilmaz et al. 2013). The antimicrobial activities of essential oil were determined by disc diffusion and minimum inhibitory concentration methods. Both seed oil and methanolic extract of seed oil did not show activity against Gram-negative bacteria except for Haemophilus influenzae but they exhibited remarkable antimicrobial activity against Grampositive bacteria. The methanolic extract of seed oil exhibited more effective antimicrobial activity compared to the seed oil. (Ozcan et al. 2010) . Bouzouita et al. (2011) reported that the high content of 1,8-cineole in the essential oil of L. nobilis L. contributed to its weak antimicrobial activity on two bacteria (Lactobacillus plantarum and E. coli).. Methanolic extracts of bay leaf showed higher antimicrobial activity, except for Aspergillus niger, Aspergillus fumigatus and Penicillium verrucosum. The differences in bioactivity might be related to the higher phenolic compounds content (flavonols, flavones and even, total phenolic compounds) present in methanolic extracts (Dias et al. 2013).

4.5 Antibacterial

Bay leaf extract was assayed for antibacterial activity by agar well diffusion and agar dilution methods in order to determine the zone diameter of inhibition compared with tetracycline zone diameter of inhibition as control. The extract showed antibacterial activity against S. aureus. The results indicated the antibacterial use of the bay extract for the treatment of S. aureus infection (Ghadiri et al. 2014) . The antibacterial activity of the essential oil of bay on human pathogenic bacteria by disc diffusion method via average inhibition zone was studied against 9 bacteria strains such as three Gram positive bacteria: S. aureus, Staphylococcus epidermidis and Streptococcus faecalis and six Gram negative bacteria: Pseudomonas aeroginosa, Shigella flexneri, Klebsiella pneuomoniae, Salmonella typhi, Serratia marcescens and E. coli were studied. Effect of the essential oil of L. nobilis on bacteria tested was more than that of tetracycline antibiotic. The results showed that the essential oil of L. nobilis showed strong anti-bacterial effects (Moghtader and Farahmand 2013). Ouibrahim et al. (2013) evaluated the antibacterial activity of essential oils of L. nobilis L., Rosmarinus officinalis L. and Ocimum basilicum L. against twenty bacterial strains: Enterococcus faecalis ATCC 29212, S. aureus ATCC 25923, ~ 1158 ~ Journal of Pharmacognosy and Phytochemistry MRSA ATCC 31 (Méthicilino), S. aureus, S. epidermidis, Enterococcus avium, E. coli ATCC 25922, Salmonella OMA 04, E. coli, Klebsiella oxytoca, K. pneumoniae, Proteus mirabilis, Enterobacter sp., Citrobacter freundii, Pseudomonas aeruginosa, Acinetobacter baumanii, S. marcescens, Salmonella sp., Shigella sp. and Providencia alcalifaciens. The three oils showed good antibacterial activity against both Gram negative and Gram positive bacteria. Laurel oil is the most efficient, Shigella sp. showed the highest sensitivity to the three oils. Among the three oils, Laurel showed the lowest MIC against E. faecalis ATCC 29212, Enterobacter sp., Shigella sp., S. aureus and S. Epermidis (0.25%). Al-Hadi (2011) conducted in vitro antibacterial activity of extracts of peppermint and bay leaf against S. aureus and found that the peppermint extract exhibited more activity than bay leaf on S.aureus but both of them were found to have inhibitory effect against S. aureus. The minimum bactericidal concentration for peppermint and bay leaf extracts were 25 and 35%, respectively.

Bay Leaf Benefits

It adds minimal calories to your food while boosting the amount of fiber, vitamins, minerals, and **antioxidants**.

Some notable health benefits of bay leaf include:

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Immune system health. Bay leaf is a good source of vitamin A, vitamin B6, and vitamin C. These vitamins are all known to support a healthy immune system.

Digestive aid. Bay leaf tea can help ease bouts of upset stomach. The tea is also very aromatic, which can help relieve sinus pressure or stuffy nose.

Reduces type 2 diabetes risk factors. A pair of small studies suggested that taking ground bay leaf capsules or drinking tea brewed from Turkish bay leaf may lower your blood sugar levels. However, one of the studies was small and the other tested bay leaves on healthy volunteers, not people with diabetes

Sources

Bay leaves come from several plants, such as:

- Bay laurel (Laurus nobilis, Lauraceae). Fresh or dried bay leaves are used in cooking for their distinctive flavour and fragrance. The leaves should be removed from the cooked food before eating (see safety section below). The leaves are often used to flavour soups, stews, braises and pâtés in many countries. The fresh leaves are very mild and do not develop their full flavour until several weeks after picking and drying.[2]
- California bay leaf. The leaf of the California bay tree (Umbellularia californica, Lauraceae), also known as California laurel, Oregon myrtle, and pepperwood, is similar to the Mediterranean bay laurel, but contains the toxin umbellulone which can cause methemoglobinemia.
- Indonesian bay leaf or Indonesian laurel (salam leaf, Syzygium polyanthum, Myrtaceae) is not commonly found outside Indonesia; this herb is applied to meat and, less often, to rice and to vegetables.[3]
- West Indian bay leaf, the leaf of the West Indian bay tree (Pimenta racemosa, Myrtaceae) is used culinarily (especially in Caribbean cuisine) and to produce the cologne called bay rum.
- Mexican bay leaf (Litsea glaucescens, Lauraceae).

V. MATERIAL AND METHODE

Preparation of the L. nobilis leaves ethanol extract.

Fresh L. nobilis leaves ethanol extract (Daun Salam) weighing 5 kilograms (kgs) were collected from a farm in Malang Indonesia in October 2019. Its were washed under running tap water, air dried and finely grinded with a blender. 500g of the finely grinded leaves were then soaked in 300mls of 70% ethanol in an airtight container for 24 hours. The mixture was filtered using filter paper 11µm and the solute was extracted with a rotary evaporator at 45°C were the final volum of extract 100ml then stored in -20 °C before used.(7,8,9)

Antimicrobial assay by agar-well diffusion method

This study was an evaluation which was intended to assess the antimicrobial activity of L. nobilis leaves ethanol extract to S. aureus, S. typhi and E. coli. This research was conducted in the Laboratory a BSL 3 Universitas Airlangga from November 2019 to December 2019. The used

Antimicrobial activity was carried out using the agar well diffusion method according to Clinical Laboratory Standards Institute guidelines (CLSI).Three to five colonies of each bacterium were dissolved in 2 ml of physiological saline and the turbidity was adjusted to 0.5 Mac Farland's turbidity which is equivalent to 0.5×10^8 bacteria per ml of solution. a swab use to spread the bacteria on surface of MHA media and then applied 100µl of L.nobilis leaves ethanol extractat concentration 100% on labeled well and 100 µl of Dimethyl Sulfoxide put on another well as a negative control and Gentamicin (10µg) disc was used as a positive control. The plate were incubated at 37°C for 24 h and the antibacterial activity determined by an inhibition zone (IZ) that formed around the well. The IZ of L.nobilis leaves ethanol extract was measure using calipers and compared with IZ gentamicin.(10,11,12)

annii,also gram positive bacteria like S. aureus (22)

EVALUATION OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity of Bay leaves extracts was tested in vitro using the agar well-diffusion assay. This method was performed using freshly prepared Mueller Hinton agar inoculated with an overnight culture of bacteria suspended in sterile saline and adjusted to a 0.5 McFarland standard. After solidification, 6 mm diameter wells were punched into the Mueller Hinton agar plates [19]. Each well was filled with 100 µl of the

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extract solution at concentration 1 mg/ml and then incubated for 24 h at 37°C. The inhibition zones were measured in millimeters. 5% phenol was used as a standard. The controls were prepared without extract. The experiment was carried out in triplicate to ensure reproducible results. Dimethylsulfoxide (DMSO) was used as a negative control while phenol was used as a positive control.

FIG NO: 01 BAYE LEAF

VI. RESULT AND DISSCUTION

Through our experiments as shown in Fig.(1) and Table 1 the results of agar well diffusionassay showed the IZ of L.nobilis leaves ethanolextract to S. aureus (16.3 ±1.5 mm), followed by S. typhi (14.5 ± 0.5 mm) and E. coli (11.3± 1.1 mm). This results were shown that the S. aureus were most sensitive against L.nobilis leaves ethanol extract. This finding was in tandem with the results published by Al-Ogaili (2020) which highlighted the great inhibition activity of L.nobilis leaves ethanol extracts to this Gram- positive bacterium.29 As reported by Otsuka et al. (2008) the L. nobilis has antimicrobial activity against methicillin-resistant S. aureus (MRSA) through purified two compound flavonoids and kaempferol, that both compounds showed strong antimicrobial activity.

The active compound was seen against S. aureus, S. typhi and E. coli. One from this Flavonoid compound has antibacterial properties because it has the capability to produce transduction energy.

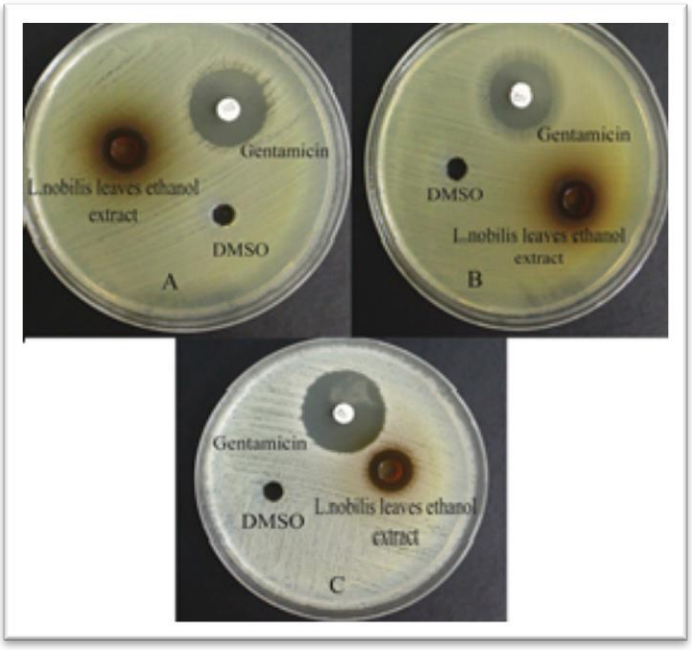

Figure 1. Antibacterial activity of L.nobilis leavesethanol extract against bacteria (A) Salmonella typhi (B) E. coli (C) S. aureus.

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that will affect the cytoplasm of the bacteria andslow down its motility, since it has an ability tointeract directly with the Deoxyribonucleic acid (DNA) of the bacteria.[32] Type of solvent used for extracting L.nobilis leave has a major impact ontheir antibacterial activity. Extraction of L.nobilis leave with ethanol resulted in a product with greater overall antibacterial activity. Study of Algabri that carried out on antibacterial activityof Libya bay leave extracted with methanol and n-hexane, it was observed that the n-hexane extract showed no antibacterial activity but the methanol extract had good inhibitory activity against S. aureus.[15] Also, El Malti and Amarouch (2009) found that the bay leave extract has a significant antimicrobial activity against wide range of human pathogen.

Therefore, the result that we found confirmed that L. nobilis leaves ethanol extract has antimicrobial activity against microorganism, it's that observed the antimicrobial activity during agar well diffusion and bactericidal activity experiment. These results concurred with the result of Aldhaher that found aqueous extract had good inhibitory activity against Streptococcus mutants with MBCs range 30-60mg/ml. Also concurred with study of Yilmaz who found that antimicrobial activity of the essential oil againstthe tested panel of food-spoiling bacteria and one yeast strain.[14,17] Also, the study of Sırıken who demonstrated that the essential oil of L. nobilis had strong antibacterial activity against Gram-negative and Gram-positive food-borne pathogens.[28] Study of Aljindan and Alkharsah, (2020) show, the resistance of Salmonella species to antimicrobial drugs increased from 24.6% in2011 to 37.8% in 2018. The research study by in 2018 all Salmonella isolates were completely resistant to Cefalotin, Cefuroxime, and Cefoxitin, while they found some susceptibility to other Cephalosporins and Ciprofloxacin.[17] While study of Patil and Mule they found S. typhi sensitive to Cefixime, Ceftriaxone, and Azithromycin and based on average Minimal Inhibitory Concentration and MIC breakpoints.[30] Throughthe experiment conducted on Rats by Qnais et al(2012) which found L. nobilis aqueous extract has antidiarrheal agent.(15)

Study of Nafis et al. exhibited notable potency regarding antimicrobial activity of (EOs) from L. nobilis leaves had the highest activity against

E. coli, with MIC: 22.2 mg/mL and IZ 9.00 mm. while it had activity against S. aureus with IZ 10.0mm and moderate MIC: 5.55 mg/mL.(15).

ANTIBACTERIAL EFFECTS OF ESSENTIAL OIL OF BAY LEAVES:

One of the important properties of essential oils and their components is their hydrophobicity, which allows them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and making them more permeable (20). The antimicrobial activity depends on not only the chemical composition of the essential oil, but also on lipophilic properties and power of functional groups or aqueous solubility. The mixture of compounds with different biochemical properties can improve the effectiveness of essential oils (11).Commonly, essential oil of bay leaves is more effective against Gram negative bacteria than Gram positive bacteria (12). This resistance is due to bacterial cellular membranes' nature group. Hence, their external structures make them to highly hydrophobic surface (13).There are some studies according to essential oils of L. nobilis' antimicrobial activities. One of them, (13)'sstudies. They report that the essential oil of L. nobilis L. had demonstrated a strong activity on the majority of tested. 22 strains; the highest sensitivity was in Enterobacter species having an inhibition diameter of 22.4 mm, 16.8 mm pure oil and 1/8 dilution. The most resistant strain was P. aeruginosa.

VII. CONCLUSION

The results of this investigation demonstrate the antibacterial and antioxidant properties of bay leaf extracts. However, a quantitative DPPH assay is required to validate the results, and additional research on a variety of pathogens is advised to gauge the effectiveness of bay leaf extracts. Specifically, the essential oil of Laurus nobilis has potent antibacterial activities against Gramme negative and Gramme positive foodborne pathogens, spoilage bacteria, and fungal impacts. It has not been usual to use nanoparticles as an antibacterial agent in research now being conducted with metal nanoparticles such as silver, gold, copper, iron, and metal oxide nanoparticles such as zinc oxide, etc. Zinc oxide nanoparticles from the Laurus nobilis plant show antibacterial properties, particularly against Gramme positive bacteria. Nanoparticles and plant-based drugs are two

alternatives to spoiling.

VIII. REFERENCE

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