Research Article

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Journal of Pathogen Research

2018
Vol.1 No.1:3

Bay Leaves have Antimicrobial and Antioxidant Activities

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Received date: July 06, 2018; **Accepted date:** July 28, 2018; **Published date:** August 02, 2018

Citation: Algabri SO, Doro BM, Abadi AM, Shiba MA, Salem AH (2018) Bay Leaves have Antimicrobial and Antioxidant Activities. J Pathogen Res Vol.1 No.1:3.

Abstract

Background: Bay, *Laurus nobilis* L is a native plant and is one of the most frequently used cooking spices. The dry Bay leaves are used to treat several digestive problems with anticonvulsive, narcotic and antibacterial properties. Thus, this study was aimed to investigate the *in-vitro* antimicrobial and antioxidant activities of different extracts of Bay leaves.

Methods: The dried Bay leaves were extracted sequentially with n-hexane, dichloromethane and methanol by Soxhlet apparatus. The extracts were concentrated and evaluated for antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeroginosa, E. coli* and *Candida albicans* using well diffusion method to determine the diameter of zone of inhibition. Also, Bay leaves extracts were evaluated for antioxidant activity using qualitative DPPH assay.

Results: The findings of antimicrobial assay showed that methanolic extract of Bay leaves has an antibacterial activity against *Staphylococcus aureus* with zone of inhibition of 18 ± 0.8 mm, which is higher than phenol inhibition zone (10 ± 1.0 mm) whereas, no antibacterial inhibition against other tested bacteria was detected. The dichloromethane extracts inhibited *E. coli* growth with zone of inhibition of 14 ± 0.6 mm and with *Staphylococcus aureus* of 18 ± 0.8 mm, while, the n-hexane extract has no antibacterial activity with all of the tested organisms. However, all of Bay leaves extracts displayed no antifungal effect on *Candida albicans*. In terms of antioxidant activity, all of Bay leaves extracts exhibited antioxidant activity, but the methanolic extract displayed the most prominent level.

Conclusion: The bay leaves extracts have antibacterial and antioxidant activity and further investigations to assess these effects are recommended.

Keywords: Bay leaves; Staphylococcus; Qualitative DPPH; Antimicrobial; Antioxidant

Introduction

One of the most well-known plants from the *Lauraceae* family is *Laurus nobilis L*, which is also known as Bay or laurel leaves. Bay is one of the most frequently used cooking spices for flavouring meat products, fishes and soups. It is a native plant in the Southern Mediterranean area, found in warm climate regions, but it is used as a decorative plant in Europe and USA. In addition, it is commercially grown in, Algeria, Morocco, Portugal, Spain, Italy, France, Turkey and Mexico [1-3].

Traditionally, the dry Bay leaves and their infusions are used to treat digestive difficulties as epigastric pain, flatulence, bloating, and eructation problems. Leaves and fruits of Bay plant have been used as astringent, diaphoretic, stimulant and emetic as well as emmenagogue, abortifacient and insect repellent. In addition, as it is an aromatic plant, its essential oil is added in the cosmetic products like soaps, creams and perfumes [4].

Phytochemical studies on Bay leaves and its fruits have indicated various secondary metabolites including alkaloids, flavonols (kaempferol, myricetin, and quercitin), flavones (apigenin and luteolin), glycosylated flavonoids, sesquiterpene lactones, monoterpene and germacrane alcohols [5-10].

Interestingly, there is a worldwide concern around that use of antibiotics to treat bacterial and fungal infections can lead to the rise and spread of organisms resistant to broad-spectrum antibiotics, opening ways to use plants as natural sources for novel antimicrobial agents with a similar activity [11-13]. Natural medicinal plants, as *L. nobilis*, are rich sources of bioactive compounds. Thus, the biological properties of Bay extracts and its essential oil are documented, specifically their antimicrobial, antifungal and antioxidant effect. A previous study has reported that the aqueous decoction of bay leaf showed 53.4% of bactericidal effect against 176 bacterial isolates belonging to 12 different genera of bacterial

population isolated from oral cavity of 200 individuals [14]. Also, another study found that the bay leaf essential oil (EO) was able to decrease the population of total coliforms (2.8 log CFU/g) and to prolong the shelf life of fresh Tuscan sausage stored at 7°C for 14 days for two days [15]. In a Turkish research, the in vitro antibacterial against three Gram-positive (*Bacillus subtilis, Staphylococcus aureus* and *S. epidermidis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), using agar dilution methods was assayed. Furthermore, its potential toxicity to *Candida albicans* and *Aspergillus niger* was examined by using both disc-diffusion and agar dilution methods. The findings of this study showed the minimum inhibition concentration (MIC) of the *L. nobilis* extract was 5 mg/mL for all the bacteria tested. Also, the extract of *L. nobilis*, showed higher inhibitory activity against the yeast *C. albicans* and the fungus *A. niger* than the standard antifungal nystatin that used as a positive control [16].

In addition, the potential antioxidant effect bay leaves extract has been reported. The methanolic extract of seed oil exhibited antioxidant properties in both 2, 2-diphenyl-l-picrylhydrazyl (DPPH) free radical scavenging and β-carotene/linoleic acid test systems [17]. In another study, the antioxidant possibility of ethanolic and aqueous extracts of *Hypericum perforatum, Ocimum basilicum* and *L. nobilis* leaves were assessed by DPPH assay. The aqueous extract of *L. nobilis* showed the lowest radical scavenging capacity (RSC) as compared to *H. perforatum* and *O. basilicum*. However, the ethanolic extracts of *L. nobilis* showed more DPPH radical scavenging effect than their aqueous extracts [18].

As in Libya the bay leaves commonly used in tradition meals, the aim of this study was to explore the *in-vitro* antimicrobial and antioxidant activities of different extracts of Bay leaves collected from local Libyan store.

Materials and Methods

Chemicals and reagents

2,2 diphenyl -1- picryl hydrazyl (DPPH) and ascorbic acid (Vitamin C) were obtained from Sigma Aldrich (Germany), Silica gel F254 TLC plates was obtained from Merck (Germany), metathanol (MeOH), dichloromethane (DCM) and hexane are of HPLC grade and obtained for Fisher Scientific.

Plant material

The dried Bay leaves were obtained from local market in Tripoli, Libya, in 27 November 2014 ready for grinding and extraction. The plant was identified and authenticated as *Laurus nobilis*. Dried leaves by Botanists in the Herbarium of Sciences College, University of Tripoli, Libya where a voucher specimen was deposited.

Preparation of plant extracts

The dried Bay leaves were finely ground using a coffee grinder and 196 gm were placed in a cellulose thimble and extracted with a Soxhlet apparatus sequentially with n-hexane, dichloromethane (DCM) and methanol (MeOH) extracts were concentrated using a rotary evaporator at 45°C and stored in pre-weighed glass jars for further analysis.

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 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.

Bacterial strains and media

The antimicrobial activity of Bay leaves extracts was tested against two Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 29138) and *Escherichia coli* (ATCC 25922), and one Gram- positive bacteria *Staphylococcus aureus* (ATCC 29213). In addition, the same extracts were tested against the yeast *Candida albicans* (ATCC 10231). The standard bacterial strains were streaked onto nutrient agar, incubated for 24 hours at 37°C then stored at 4°C. The media used in this study were nutrient broth (NB), nutrient agar (NA) and Mueller Hilton agar (Oxoid). The media was prepared according to the manufacturer's instructions.

Evaluation of antioxidant activity

Qualitative DPPH assay: Antioxidant activity of dried Bay leaves extracts was analyzed qualitatively by using 2, 2,diphenyl-1-picrylhydrazyl (DPPH) assay on thin layer chromatography (TLC) plates [20]. DPPH assay was used as a screening test for the radical scavenging ability of the compounds present in different extracts of Bay leaves. Silica gel F_{254} TLC plates were used to separate Bay leaves extracts where 10 µl aliquots of each of the n-hexane, DCM and MeOH extracts (10 mg/ml) were applied to Silica gel plates using capillary tubes, left to dry, then; The plates were dried in the fume hood for the detection of antioxidant activity, chromatograms were sprayed with 0.2% DPPH in methanol, as an indicator. It was allowed to develop for 30 min. The presence of antioxidant compounds was detected by observation of yellow spots against a purple background on TLC plates sprayed with 0.2% DPPH in methanol. Vitamin C (2 mg/100 ml methanol) was used as a positive standard [20,21].

Results and Discussion

Antimicrobial activity

In **Table 1**, the antimicrobial activity of Bay leaves in terms of zone of inhibition (in mm diameter) of n-hexane, dichloromethane and methanolic extracts at concentrations 0.5 mg/ml against the tested microorganisms were shown. The findings indicated that the methanolic extract of Bay leaves has an antibacterial activity against *Staphylococcus aureus* with zone of inhibition 18 ± 0.8 mm, which is much higher than the positive control (phenol) inhibition zone (10 ± 1.0 mm), whereas, there was no antibacterial inhibition against other tested bacteria. The Dichloromethane extract inhibited *E. coli* growth with an inhibition 14 ± 0.6 mm and *Staphylococcus aureus* with 13 ± 0.5 mm, while the n-hexane extract has no antibacterial activity with all the tested organisms. However, all of Bay leaves extracts displayed no antifungal effect on *Candida albicans* (**Table 1**).

Table 1 Average diameter, in millimeters, of the bacterial inhibition zones of Bay leaves extracts at concentration 0.5 mg per ml against tested microorganism.

Microorganism	Mean diameter of zone of inhibition (mm ± SE)			
	Extract (0.5 mg/ml)			
	n-hexane extract	DCM Extract	MeOH Extract	5% Phenol
Staphylococcus aureus	0.0 ± 0.0	13 ± 0.5*	18 ± 0.8*	10 ± 1.0
Pseudomonas aeruginosa	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	27 ± 0.5
E. coli	0.0 ± 0.0	14 ± 0.6	0.0 ± 0.0	12 ± 2.0
Candida albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	11 ± 2.5

**Statistically significant difference by t-test.*

In a previous study, the *in vitro* antimicrobial and antioxidant activities of the essential oil, seed oil and methanolic extract of seed oil of Bay leaves were showed that the extract of seed oil has more effective antibacterial activity compared with essential oil and seed oil [22]. Furthermore, GC-MS analysis of essential oil resulted in identification of 25 compounds. 1.8-Cineol (44.72%), a-Terpinyl acetate (12.95%), Sabinene (12.82%) were the main components. In addition, other study reported that *Laurus nobilis* extract has antibacterial activity against *Staphylococcus aureus* where the zone diameter of inhibition of *Laurus nobilis* extract was comparable to zone diameter of the inhibition of tetracycline [23]. That may support the findings of this study in terms of the antibacterial effect.

Antioxidant activity

Qualitative DPPH assay: Various methods have been used to evaluate antioxidant capacity of some compounds in different plant extracts, one of the most widely used methods are those involve the generation of free radicals which then neutralized by antioxidant compounds [24]. The DPPH antioxidant assay is based on the ability of DPPH to decolorize in the presence of antioxidants. The odd electron in the DPPH radical is responsible for the deep purple color. When DPPH accept an electron from antioxidant compound, the DPPH decolorize [20].

In the present study, only TLC based qualitative DPPH assay was performed to evaluate antioxidant activity of bay leaves extracts which provide a rapid, flexible and efficient screening method for antioxidant activity [25]. The results showed the presence of antioxidant activity in all the tested Bay leaves extracts as it is given in **Figure 1**. The activity was indicated by the presence of yellow spots against a purple background on the chromatograms. The degree of activity of all the samples tested was also determined qualitatively from observation of the yellow color intensity. It is observed that methanolic extract of Bay leaves displayed the most prominent level of antioxidant activity where it has an intense yellow color compared with the control using vitamin C.

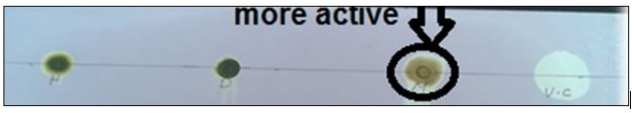

Figure 1 Chromatogram of Bay leaves extracts sprayed with 0.2% DPPH in methanol yellow zones indicate antioxidant activity.

Several previous studies assessed the quantitative antioxidant activity of different extracts of Bay leaves by using different models, all of them indicated that Bay leaves extracts provide both In vitro and In vivo antioxidant effect [26-30].

Although, in this research only the qualitative antioxidant effect was tested, and this effect may be related to the presence of phenolic secondary metabolites like phenols.

Conclusion

The findings of this study show that the bay leaves extracts have antibacterial and antioxidant effects. However,

quantitative DPPH assay is needed to confirm the obtained results and more investigations on wide range of pathogen to assess the spectrum of bay leaves extracts are recommended.

Acknowledgements

The authors would like to thank Prof. Fathi M Sherif, Department of Pharmacology, for his help in critical reviewing the manuscript and valuable comments. Also, thanks go to the laboratory staff of Microbiology and Immunology for their help.

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