



The examination of the water concentrates of *Laurus nobilis* leaves antibacterial activity utilizing various strategies for extraction (In Vitro)

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ABSTRACT

The utilization of antimicrobials is essential to battle bacterial ailment. Yet, because of the expanding abuse of antimicrobial medications that are utilized in the treatment of infectious diseases, resistance by bacteria developed. The objective of the investigation is to detect the *in vitro* antibacterial impact of bay leaf water concentrates utilizing distinctive extraction strategies. The impact of water concentrates of bay leaf (*Laurus nobilis*) employing maceration and decoction extraction method against *Staphylococcus aureus* and *Escherichia coli* were tested by "agar well diffusion" technique. The consequences of the investigation demonstrated insignificant differences between techniques for extractions. The study concludes that the *in vitro* antibacterial effect might be potentiated against test strains by using different methods for extraction and solvent systems, which might be helpful to defeat antimicrobial resistance.

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showed cytotoxic action (Kivçak and Mert, 2002). The antimicrobial inhibitory activity of essential oil of *Laurus nobilis* were tested by the minimum inhibitory concentration (MIC) method against bacterium and yeast. The volatile oils extracted from nobilis leaf had antimicrobial action (Raharivelomanana et al., 1989). Volatile oil of the leaves is incredibly valuable in averting headache, producing soap, treat acne, skin ulcers, and abscesses. Plus, the water infusion of bay leaves used for a very long time by European ladies to relieve post-partum pain while the decoction of *L. nobilis* as a wash for bruises and to expel lice from the head.

INTRODUCTION

Bay leaf (*Laurus nobilis* L., Lauraceae) is a culinary plant and has a long conventional use in the Chinese and Ayurvedic practice of therapy. Traditionally, the analgesic activity of bay leaf used to ease pain in many illnesses, neuralgia, and digestive symptoms. Bay leaf extract investigated for bactericidal action has demonstrated to be vital against *Staphylococcus aureus* and *E. coli* (Friedman et al., 2002). Ethanol, water, and n-Hexane concentrates of *L. Nobilis* leaves have been assessed for cytotoxic properties using the saline solution shrimp bioassay. This assessment revealed only the n-hexane concentrate

Staphylococcus aureus is a gram (+) spherical shaped bacteria found in the respiratory tract causing sinusitis; on the skin causes skin diseases like cankers, food contamination, and bone and joint contaminations (Masalha et al., 2001). *Escherichia coli* is a gram (-) rod-shaped bacteria found in the lower digestive system of warm-blooded animals (endotherms). Normally cause gastroenteritis, urinary tract contaminations, and neonatal meningitis (Singleton, 1999). The aim of the study is to determine the *in vitro* antibacterial activity of nobilis leaf aqueous concentrates utilizing two methods of extraction (decoction and maceration).

MATERIALS AND METHODS

Preparation of aqueous extracts

The leaves of *Laurus nobilis* purchased from a local market in Baghdad, Iraq. The leaves powdered by an electrical grinder. The plant extraction carried out by two methods,

Decoction

An aqueous decoction of bay leaves prepared by placing 50 g of a pulverized plant in five hundred milliliters of distilled water and boiled over a hot plate for one hour. The decoction cooled and filtered using layers of guaze, then by Whatmans No.1 filter paper. Lead sub-acetate solution(10%) added to the filtrate and centrifuged for 3 minutes at $18 \text{ rpm} \times 10^3$, clear supernatant was stored at 4°C until use (Dulger and Gonuz, 2004).

Maceration

Fifty grams of powdered material macerated two times in 500 ml distilled water over 48- hour period. The macerates filtered through several layers of guaze, then by Whatmans No. 1 filter paper. Lead sub-acetate solution(10%) added to the filtrate and centrifuged for 3 minutes at $18 \text{ rpm} \times 10^3$, clear supernatant was stored at 4°C until use (Dulger and Gonuz, 2004). Different concentrations of aqueous extracts for each extraction method were prepared (10%, 5%, 2.5%, 1.25%) and sterilized using Millipore filter ($0.22 \mu\text{m}$) to be used for evaluation of antimicrobial activity.

Antimicrobial activity assessment

The *in vitro* antimicrobial properties investigated by the “agar- well diffusion” method. Cultures of bacteria (*Staphylococcus aureus* and *Eschereschia coli*) supplied by the (Research and Production Center for Drugs and Medical Supplies / Ibn Sena Center, Baghdad, Iraq). Reactivation of bacteria done by nutrient agar sub-culturing and 24 hours incubation at 37°C . Suspending inoculum of bacteria into sterile saline to prepare purified bacterial cultures and the suspension standardized to 0.5 McFarland standard. Bacterial strains were seeded on plates containing Muller- Hinton agar. Four holes of 6 mm diameter were punched with a sterile cork borer, and $50 \mu\text{l}$ of extracts of different concentrations were introduced in each well (Jahangirian et al., 2013). The experiment was repeated three times, and the average mean of inhibition zones were calculated. Amoxycillin ($25 \mu\text{g}$) and Gentamicin ($10 \mu\text{g}$) were used as standards for *S. aureus* and *E. coli*, respectively.

Statistical Analysis

To show the effect of different determinants on

study parameters, the “Statistical Analysis System” (SAS, 2012) program was applied. For a significant comparison between means in the study, the” Least Significant Difference (LSD)” test was applied (SAS, 2012).

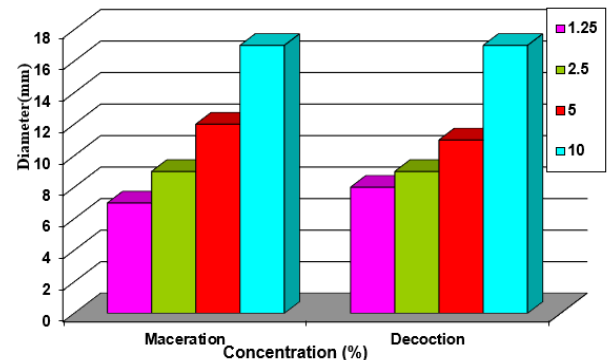


Figure 1: The effect of methods of extraction on the inhibitory activity of bay leaf aqueous extract against *S.aureus*

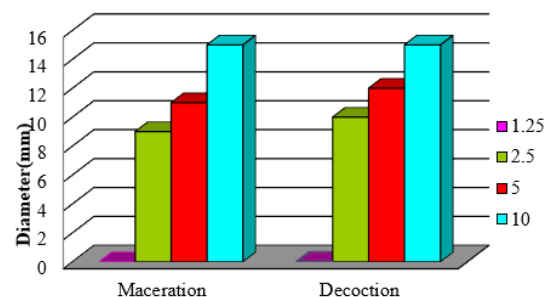


Figure 2: The effect of methods of extraction on the inhibitory activity of Bay leaf extract aqueous against *E. coli*

RESULTS AND DISCUSSION

Different concentrations of water extracts of bay leaf for the two methods of extraction were tested *in vitro* against isolates of *S. aureus* and *E. coli*. The inhibition zones diameter of growth measured in (mm) and registered as the antibacterial property of the aqueous extracts shown in Table 1 and Table 2. The results showed that aqueous extract of both extraction methods exhibited higher antibacterial activity against *S. aureus* for the 1.25% and 10% concentration in comparison to *E. coli* that showed resistance at 1.25% concentration and lower sensitivity for the 10% as demonstrated in Figures 1 and 2 and Figure 3. The results of the current study applying the Least Significant Difference (LSD) test demonstrated insignificant varia-

Table 1: Inhibitory activity (mm) of different concentrations of bay leaf aqueous extracts for both methods of extraction against *Staphylococcus aureus*

Concentration (%)	Mean \pm SE of Diameter		LSD value
	Maceration Method	Decoction Method	
1.25	7 \pm 0.35	8 \pm 0.29	NS
2.5	9 \pm 0.52	9 \pm 0.38	NS
5	12 \pm 0.60	11 \pm 0.46	NS
10	17 \pm 0.67	17 \pm 0.63	NS
Amoxicillin(25 μ g)	30mm	30mm	—
LSD value	2.064 *	2.159 *	—

* (P<0.05) ; NS : Non-significant ; SE : Standard error

Table 2: Inhibitory activity (mm) of different concentrations of bay leaf aqueous extracts

Concentration (%)	Mean \pm SE of Diameter		LSD value
	Maceration Method	Decoction Method	
1.25	-	-	-
2.5	9 \pm 0.41	10 \pm 0.37	NS
5	11 \pm 0.48	12 \pm 0.52	NS
10	15 \pm 0.62	15 \pm 0.66	NS
Gentamicin(10 μ g)	24mm	24mm	—
LSD value	1.883 *	1.796 *	—

* (P<0.05) ; NS : Non-significant ; SE : Standard error

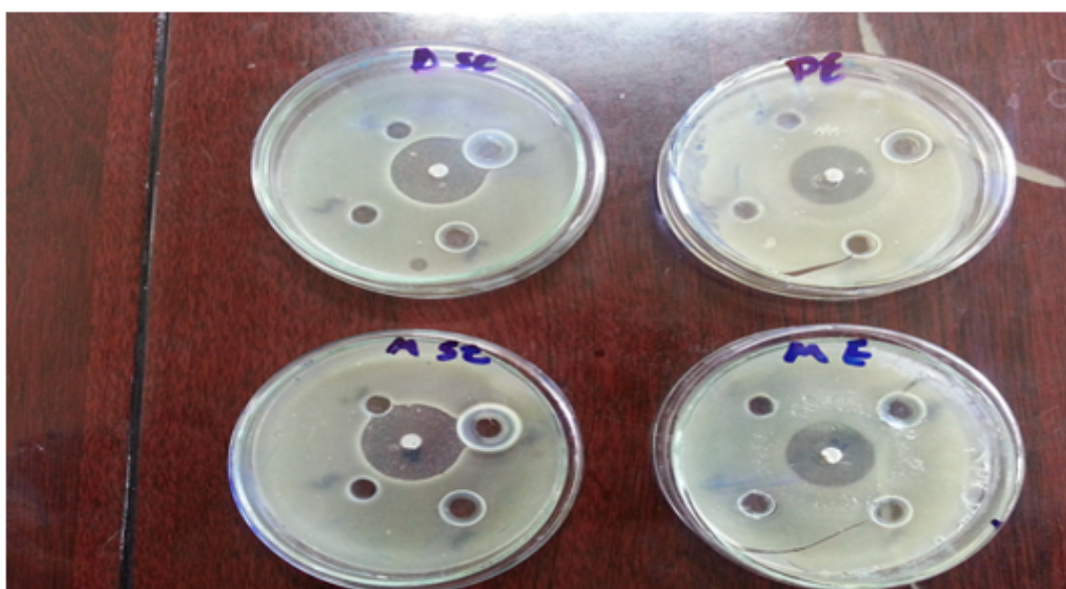


Figure 3: Inhibition zones of test bacterial strains on Muller- Hinton agar plate using the well diffusion method. D-St, D-E (Decoction *S. aureus* and *E. coli*), M-St, M-E (Maceration *S.aureus* and *E.coli*)

tions between the decoction and maceration extraction methods where the $P < 0.05$.

The current study was to investigate the influence of two extraction methods of bay leaf aqueous extract on *in vitro* antibacterial activity. The inhibitory action of the concentrates increased with increasing concentrations. In the contemporary study, the aqueous concentrates of noblis leaf of both techniques of extraction exhibited antibacterial action against *S. aureus*. These results disagree with a research done by (Chaudhry and Tariq, 2006). Another study done by (Al-Hadi, 2011) revealed that the infused aqueous extract showed no inhibitory activity to *S. aureus* at 5%, 10%, and 15%, while the current study showed an inhibitory effect by all of the concentrations of both extraction methods. Methanolic extract of bay leaf in a study conducted by (Ozcan et al., 2010) showed antibacterial activity against *S. aureus* that is consistent with the current study. Despite the moderate antibacterial activity revealed by the present study compared to standard antibiotics (amoxicillin and gentamicin), there was an insignificant distinction between the maceration and decoction techniques of extraction using water as a solvent. Previous studies have shown that the active constituents of bay leaf monoterpenes (1,8-cineol) and flavonoids are best extracted by maceration using 70% methanol and ethyl acetate to obtain a high content of the active constituents that possess antimicrobial activity which may contribute to better inhibitory action (Kaurinovic et al., 2010). The difference in the results between our study and other studies regarding antibacterial effect may be due to the differences in the qualitative and quantitative composition of the extract influenced by the solvent and extraction methods used (Lui and Narkano, 1996).

CONCLUSIONS

The current study concludes that *in vitro* antibacterial efficacy of bay leaves may be affected by the methods and solvent used in the extraction. The efficacy may be potentiated against bacterial strains by considering other methods of extraction and different solvents systems other than water to obtain antibacterial agents to combat the resistance of bacteria to antimicrobials.

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