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The Antibacterial, Antiheamolytic, and Antioxidant Activities of *Laurus nobilis* and *Alhagi maurorum* Native to Iraq.

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Abstract:

Ethanolic crude extracts of leaves from *Laurus nobilis* and *Alhagi maurorum*for were screened for alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids, glycosides, and glucosides contents. Biochemical activities, including antibacterial activity, antioxidant, and antihemolytic activity, were investigated. Antibacterial activity against Three types of pathogenic bacteria was detected by disc diffusion analysis and characterized by zone of inhibition (ZOI). Antioxidant properties were determined by a diphenyl-1- picrylhydrazyl (DPPH) method. Results revealed that the inhibitory activity of the plants against G+ve and G-ve bacteria were different, where the greatest ZOI of *Alhagi maurorum* against *Staphylococcus aureus* was 12.66 mm, while its effect against *Pseudomonas aeruginosa* generated an 8.33 mm ZOI, with no did not effects on *Escherichia coli*. Extract of *Laurus nobilis* against *Escherichia coli* with a ZOI reached to 10.33 mm, but did not significantly influence the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. When the plant extracts were mixed in equal quantities, the percentage of the effect were increased, where the greatest effect of the mixed observed in *E. coli* was a 16.66mm ZOI, and in *Staphylococcus aureus* to 15.66mm. In *Pseudomonas aeruginosa* the ZOI reached to a 12.33 mm. This is referred to as synergistic effect between these plants against pathogenic bacteria. The extracts did not have any toxic effects on human red blood cells.

Key words: Antibacterial activity, Antihemolytic activity, Antioxidant activity, Medicinal plants.

Introduction:

Medicinal plants are an abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potent antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries (1). *L. nobilis* leaf belongs to the family Lauraceae, and the pharmacological activity of leaf includes anti-bacterial, anti-fungal, and anti-inflammatory activities. The Lauraceae family has more than 2500 species in subtropical and tropical regions in East Asia and South America, and their roots and fruits are aromatic (2). The chemical composition of leaves has been studied extensively, and many volatile compounds, such as 1, 8-cineole and *R*-terpinyl acetate, have been identified and shown to have antimicrobial efficacy (3) , anticancer (4), and antifungal activity (5).

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The roots and leaves are also sources of sesquiterpene lactones, and two major chemical types are laurenobiolide and costunolide (6). *A. maurorum*, which belongs to the Fabaceae family, reaches a length of 2 m, blooms in July, and grows abundantly in sandy soils(7). *A. maurorum* a large leguminous plant that extends its roots up to 6 feet deep inside the ground, which results in new shoots which can appear at a distance of 20 feet from the main plant, and has a grayish-greenish leaf with long thorns (8). The plants remain as an important source of many compounds that help improve human health (9).As such, these plants, including their raw extracts and chemical components, have been extensively investigated because of their numerous preventive properties, such as antibacterial and antioxidant effectiveness (10). Furthermore, the extract of *A. maurorum* protects liver from damage-induced either by carbon tetrachloride or acetaminophen (11, 12). Research of *Alhagi* sp. exposed the occurrence of numerous compounds, like fatty acids and sterols (13). Fats, alkaloids, flavonoids, anthraquinones, cardiac glycosides, coumarins, saponins, phlobatannins, tannins and terpenoids are contained in the leaves

(14). Many flavonoids were extracted from *A. graecorum* Boiss, and these flavonoids were identified as tamarixtin 3-O-dirhamnoside, isorhamnetin 3-O-glucosylneohesperidoside, isorhamnetine 3-O-robinoside, isorhamnetin 3-Orotinoside, quercetin 3-O rhamnoside, kaempferol 3-O-galactoside, quercetin 3, 7-diglycoside, isorhamnetin 3-rutinoside, daidzein 7,4-dihydroxyisoflavone, calycisin 3-hydroxyformononetin, isorhamnetin and tamarxtinaglycones (15). This study aimed to determine the biological activities for two types of plant in Iraq against some pathogenic bacteria.

Materials and Methods:

Plant Materials:

Leaves of *A. maurorum* were collected from several specimens obtained from Al-Zafranyia, and the plant *L. nobilis* L. were obtained from market in Baghdad, Iraq, in 2017. The plant leaves were washed with tap water to get rid of the dirt, dried at room temperature, and ground to obtain a plant powder.

Preparation of the Extract:

Plant leaves were dried and ground with mortar and the powder of plant leaves were extracted with ethanol by Soxhlet for 7 h, and evaporated by a rotary evaporator under vacuum at 45°C to obtain the crude extract and was stored, protected from light in a refrigerator at 4°C in until use (16).

Phytochemical Screening:

Extracts were analyzed to detect the presence of the active compounds in accordance to (17).

Antibacterial Activity Test:

The bacterial isolates were obtained from the Biotechnology Branch / Applied Science Department at the University of Technology. An agar diffusion method was used to detect antibacterial activity of the plant extracts. After the bacteria were diffused with a spreader on Mueller–Hinton agar, ethanolic plant extracts were melted in 10% DMSO, and wells that were 6mm in diameter were filled with concentrations of (20,60,80) mg mL^{-1}, with 10% DMSO as a negative control. After incubation in $37c^0$ for 24h, the ZOI was measured around each well and compared with the control and conducted in in triplicate (18).

Evaluation of the Synergistic Effect:

This assay was performed using a mixture of two plant extracts. Each composition in this mixture was tested against pathogenic bacteria and the diameters of the cleared zones were measured and compared with those of the single plant extract (19).

Estimation of the Increase in Fold Area:

This was done by calculating the ZOI that was generated by a combination of the plant extracts and compared with the effect of single plant extracts (20). The increase in the fold area was calculated using the following equation: fold increase (%) = [(B − A) / A] × 100, where A refers to the inhibition zone of one plant extract, and B denotes the inhibition zone of the combination of the two plant extracts.

Radical scavenging assay:

Free radical scavenging activity of the fractions was calculated *in vitro* via diphenyl-1-picrylhydrazyl (DPPH) assay (21). This activity was assessed on the basis of the percentage of DPPH. Reactive Oxygen Species (ROS) effects were evaluated by the following equation:

Scavenging effect % =Control absorbance − Sample absorbance/Control absorbance × 100

Hemolytic activity:

The antihemolytic activity of the extracts was detected according to (22) with some modifications. Human red blood cells were concentrated in phosphate buffer saline to obtain a 4% suspension. Plant extracts were prepared with PBS buffer at 100, 80, 60, 40, and 20 mg/mL^{-1}. Afterward, 2 mL from each suspension was added to 1 mL of the plants extract at each concentration, and sufficient amount of PBS was added to obtain the final volume of 5 mL. The sample was incubated for 5 minutes at room temperature; 0.5 mL of 0.3% H_2O_2 was added to stimulate the oxidative degradation of membrane lipids. The mixture was incubated at 37 °C for 4 hours, and then samples were centrifuged at 1500 × *g* for 10 min. Finally, the resulting supernatant was transferred to another tube and used to assess their hemolytic activity by using a spectrophotometer at an absorbance wavelength of 540 nm. RBC lysis with H_2O_2 and without plant extract (positive control) exhibited 100% hemolytic activity. Antihemolysis was calculated as follows:

% Antihemolysis = [(*A*o − *A*1) / *A*o] × 100,

*A*o was referred to as the absorbance of the control (H_2O_2 + RBC), and *A*1 was referred to as the absorbance of the extract.

The experiments were done in triplicate.

Statistical Analysis:

The results are reported as mean ± SD of three independent replicates. Statistical analysis for the data was carried out by SPSS version 11.5 software. Level of significance was evaluated by Analysis of Variance (ANOVA) test. The level of significance was exposed by the least significant difference (LSD) test.

Results and Discussion:

Phytochemical screening showed the phytochemical profile of the leaf extracts of the plants (Table 1). Phytochemical components, like tannins, flavonoids, alkaloids, glycosides, cyanogenetic glycosides, reducing sugar, and a number of secondary metabolites were found in the two plants (10, 23**)**.

Table 1. The screening of active compound for two alcoholic plants extract

Test name	*L.nobolis*	*A.maurorum*
Phenolic coumpound	+	+
Tannins	-	+
Flavonoids	+	+
Terpenes	+	+
Coumarins	-	+
Anthraquinones	+	+
Glycosides	+	+
Saponins	+	-
Alkaloid	+	+
Terpenoides	+	+

Evaluation of antimicrobial activity:

The ethanolic extracts of the tested plants exhibited varying degrees of antibacterial activity. 100 µg mL^{-1} was significantly better than the other concentrations for the two investigated extracts. The highest activities of *A. maurorum* against *S. aureus* and *P. aeruginosa* by ZOI reached to 12.66±0.58 and 8.33±0.58 mm, respectively (Fig. 1A, B), but had not effect on the growth of *E. coli* (Fig. 1C).While we find that the alcoholic extract of the *L. nobilis* has affected on the growth of intestinal bacteria *E. coli* by 10.33± 0.58 mm ZOI (Fig.1F), it did not significantly influence the growth of *S. aureus* and *P. aeruginosa* (Fig. 1D, E). When the plant extracts were mixed in equal quantities, the percentage of the ZOI increased, where the highest effect found in *E. coli* by zone of inhibition reached to 16.66 mm and 15.66 mm on the growth of *S. aureus* and in *P. aeruginosa* by 12.33±0.58mm (Fig.1 G, H, and I). This indicates the presence of a synergistic effect between these plants. Phyto-chemical analysis of the extracts revealed that some of secondary metabolites, such as tannins, coumarins, and anthroquinone, were absent in *L. nobilis*. Saponins were also not detected in *A. maurorum*. Conversely, other metabolites were found in high or moderate concentrations. The antimicrobial, antioxidant, and antihemolytic activities exhibited by the extracts might be attributed to one or more phytochemical constituents present in the plants extract. Flavonoids and glycosides have antibacterial, and antihemolytic and antibacterial activity against G+ve and G-ve bacteria (24). Flavonoids and polyphenolic components have antibacterial activity, which may be attributed to their ability to interact with the cell wall of bacteria, which inhibits the growth of the bacteria (25). Plants extracts are considered a good source of antibiotics, typically have fewer side effects than synthetically produced ones, and are bio-friendly (26). Bioassays are conceded to the simple tools used to test the plants extract activity, and these extracts are subjected to phytochemical studies on the basis of these activities to isolate novel therapeutic agents (5). The presence of terphinoids, fatty acids, carotenes, phenolics, alkaloids, glycosides, flavonoids, and tannins leads to the pharmaceutical activities of plants extract (27). The effect of *A. maurorum* might be attributed to the presence of a high percentage of flavonoid contents (23). The extracts affected the DNA replication and cell reproduction. Phenolic compounds interfere with free radical creation by chelating transition metals and by inhibiting enzymes involved in the initiation reaction (28). Two types of plant extracts showed good antibacterial activity against both G-ve negative and G+ve bacteria when mixed together. Fig.1, and Table 2 shows that there is a synergistic effect which might be attributed to the mixture, which contained phytochemicals that might be active if they were combined with other compounds. These compounds also have a sequential effect on their targets in bacteria and may change the microenvironment of bacteria, thereby disrupting nutrient and water transfer (20, 29). The synergistic effect of plants extract represents the highest percentage of increase in inhibition, which was found against all isolates in this study.

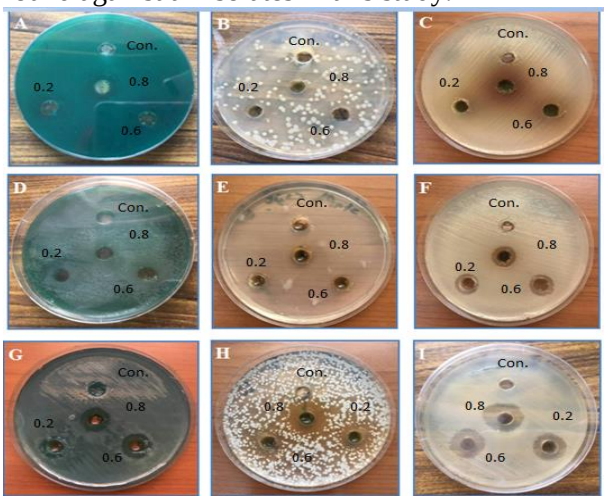

Figure 1: Inhibition zone of the ethanolic extract of *A. maurorum* (A, B, C) and *L. nobilis* (D, E, F). (G, H, I) the combination of two plant extracts: (A, D, G) *P. aeruginosa*, (B, E, H) *S. aureus*, (C, F, I) *E. coli*.

Table 2. The effect of plants extracts on the growth of some pathogenic bacteria. Each value represents the mean ± SD of three experiments.

Microorganism	Inhibition zone of plants extract concentration (mg mL^{-1})				
	A	F.I % (A+C.)	B	F.I % (B+C.)	C.
P.aeruginosa	6.00±1.15	105.5	8.33±0.58	48.01	12.33±0.58
S. aureus	6.00±1.15	161	12.66±0.58	23.69	15.66±1.15
E. coli	10.33±0.58	61.27	6.00±1.00	177.66	16.66±1.15

A= *L. nobilis*, A+C= *L. nobilis* + Combination, B= *A. maurorum*, B+C= *A. maurorum* + combination, C. = combination of Two plants extract. F.I%-Fold Increase = [(B-A)/A] *100 In case of the absence the effect of the plant extract, the diameter of the well, 6 mm, was used to calculate the fold increase (F.I%).

Antioxidant and radical scavenging activities:

The antioxidant and radical scavenging activities of different alcoholic extracts were evaluated in vitro through the assessment of the DPPH radical scavenging activity. The results showed that DPPH was displaced instantly, proportional to the increase in the concentration. The scavenging effect is mostly used, which is a free stable dark purple tulip. To the color changed to yellow when it is replaced by antioxidant compounds through the release of an electron and a proton (30). In all of the tested concentrations (20, 40, 60, and 80 µg/mL), the absorbance of *A. maurorum* aqueous extract was significantly higher ($P \leq 0.001$) than that of Vitamin C, suggesting that the plant extracts were more effective than Vitamin C in terms of reductive ability, which was concentration dependent. Furthermore, 60 and 80 µg/mL of *A. maurorum* have radical scavenging activities (84.00% ± 2.00% and 72.66% ± 1.17%, respectively), but that at concentrations of 20 and 40 µg/mL (61.65% ± 5.76% and 58.66% ± 3.50%, respectively). The antioxidant activities may be attributed for the whole phenolic contents of the leaves (8). The aqueous extracts of *L. nobilis*, with concentrations of 60 and 80 µg/mL shared radical scavenging activities of 74.33% ± 2.00% and 63.66% ± 1.15%, respectively. These values were significantly higher ($P \leq 0.05$) than those with concentrations of 20 and 40 µg/mL (61.66% ± 5.76% and 58.65% ± 3.51%, respectively (Fig. 2,3).

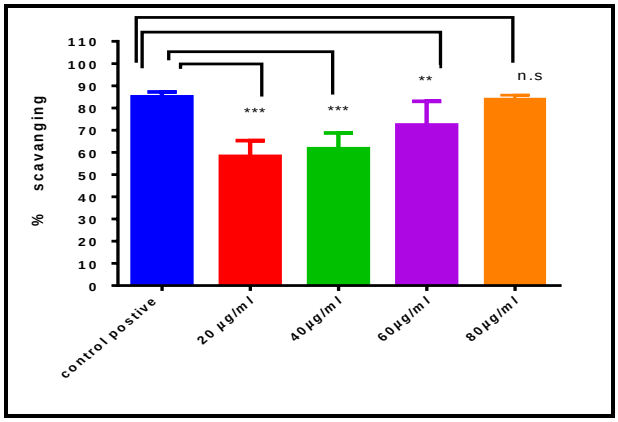

Figure 2: Scavenging activity of *A. maurorum*; each value represents the mean ± SD of three experiments

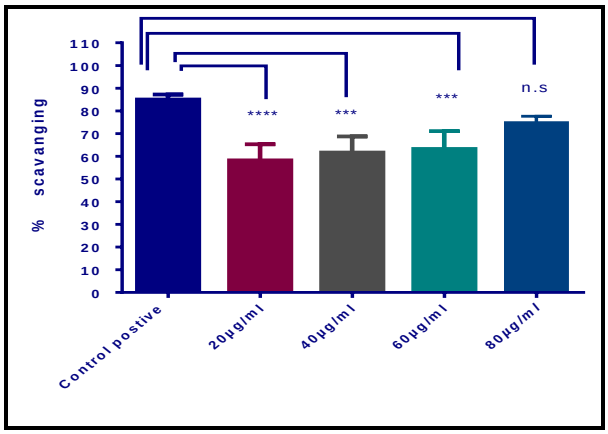

Figure 3: Scavenging activity of *L. nobilis*; each value represents the mean ± SD of three experiments

In the antioxidant tests of the crude extracts, the leaf extracts of the plants showed ROS potential, was possibly due to the presence of phenolic compounds (Table 1). Flavonoids have antibacterial, anti-inflammatory, antiallergic, anti-neoplastic, antiviral, antithrombotic, antioxidant, and vasodilatory activities. Tannins have shown potential antiviral, antibacterial, and antioxidant activities (31). The role of antioxidants is to eliminate free radicals. One important mechanism involves the donation of hydrogen to free radicals in its reduction to an unreactive species. Addition of hydrogen would remove the strange electron feature which is accountable for radical reactivity. The hydrogen-donating activity, measured using DPPH radicals as hydrogen acceptor, showed that there was a significant association could be found between the concentration of extracts and percentage of inhibition (32).

Determination of antihemolytic activity:

Anti-hemolytic activity was determined at (20, 40, 60, 80, and 100) mg/mL. The results were summarized in Table 3, which demonstrated a significant antiheamolysis is a concentration dependent manner. Our results could serve as easily accessible sources of natural antioxidants for the pharmaceutical industry.

Table 3. Antihemolytic activity of plants at different concentrations on blood human

Plant names	Concentration (mg mL^{-1})				
	100	80	60	40	20
A	19.66±0.5	38.33±1.53	56.66±1.53	77.66±1.53	97.66±1.15
B	36.66±1.53	44.66±1.53	58.66±1.53	75.66±1.53	86±1.00

A= *L. nobilis*
B= *A. maurorum*

The relative haemolysis of the control was 100 %. Data are presented as %. Each experiment was performed in triplicate. Lower values indicate higher antihaemolytic activity.

Conclusion:

This study supported the use of the ethanolic extracts of *A. maurorum* and *L. nobilus* as a strong disinfectant in modern medicine. Their antioxidant activity could support uses as a source of natural oxidant to keep humans from harmful oxidative processes and hemolysis.

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Conflicts of Interest: None.

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دراسة الفعاليات المضادة للبكتريا،المضادة للتحلل، والمضادة للاكسدة لنباتي الغار والعاقول في العراق

نهاية نعمة حسين [1] ذرية ردام مرزوك[1] اسماء عزت سليم[2]

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الخلاصة:

تم الكشف عن المركبات الفعالة (القلويدات،السابونين،التانينات،anthraquinones، الستيرويدات، الفلافونويد، جلايكوسيدات، ومشتقات الجلاكوسيدات) في الاجزاء الهوائية لنوعين من النباتات في العراق هما نبات الغار والعاقول ،وكذلك الفعالية البايوكيميائية بما في ذلك الفعالية المضادة للبكتريا المرضية ،والفعالية المضادة للاكسدة والفعالية المضادة لتحليل كريات دم الانسان . استخدمت المستخلصات الكحولية ضد ثلاثة انواع من البكتريا المرضية بطريقة الانتشار بالحفر وقياس مناطق التثبيط .كذلك تم تحديد خاصية مضادات الاكسدة باستخدام طريقة (DPPH). اظهرت النتائج اختلافا واضحا في تاثيرها على نمو الاحياء المجهرية السالبة والموجبة لملون غرام ,بالنسبة الى نبات العاقول سجل اعلى تاثير له ضد بكتريا المكورات العنقودية الذهبية بقطر منطقة تثبيط بلغ 12.66mm، و 8.33mm ضد نمو بكتريا الزوائف الزنجارية ،في حين لم يظهر اي تاثير يذكر له في نمو البكتريا المعوية .اما بالنسبة الى المستخلص الكحولي لاوراق نبات الغار فلقد اظهر تاثير تثبيطي واضح ضد البكتريا المعوية بقطر منطقة تثبيط بلغت 10.33mmولم يؤثر على نمو باقي انواع البكتريا المرضية .ولكن عند مزج المستخلصات الكحولية لكلا النباتين معا لوحظت زيادة في التاثير التثبيطي لهما من خلال ازدياد في قطر منطقة التثبيط الناتجة، اعلى تاثير للمزيج كان في نمو البكتريا المعوية بقطر منطقة تثبيط بلغت 16.66mm،تليها بكتريا المكورات العنقودية الذهبية بقطر منطقة تثبيط بلغ 15.66mmواخيرا في نمو بكتريا الزوائف الزنجارية بقطر منطقة تثبيط بلغ 12.33mmوهذ يدل على حصول تاثير تازري بين المستخلصات .كما اظهرت النباتات فعالية قوية مضادة للاكسدة وفعالية مضادة لتحلل كريات الدم الحمراء للانسان من خلال عدم اظهارها لاي تاثير سام ضد كريات الدم الحمراء.

الكلمات المفتاحية :ضد البكتريا،ضد التحلل ،ضد الاكسدة ،النباتات الطبية.