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Extracting alkaloids from medicinal herbs and studying their biological effectiveness against *Staphylococcus aureus*

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ABSTRACT

Readily available and less expensive medications are more widespread than contemporary medications, and medicinal herbs are used extensively worldwide. Numerous phytochemicals with potential biological activity have been identified, but many chemical substances in a single plant reduces the effectiveness of these phytochemicals. Thus, extensive research into the effects of these substances, which are present in a broad range of plants, are needed to ascertain their safety and efficacy. The objective of this study was to quantify alkaloids in the aqueous extracts of 10 varieties of medicinal plants found in local markets and examine their biological efficacy in combating *Staphylococcus aureus* infection. Results indicated that the aqueous extract of a chamomile plant (*Matricaria chamomilla*) had the highest concentration of alkaloids (124.9 µg/gm), followed the extract of a fenugreek plant (*Trigonella foenum-graecum*; 94.5 µg/gm). The lowest concentration was found in the ginger (*Zingiber officinale*) extract (5.7 µg/gm). Biological efficacy experiments showed that the *Cucurbita* extract did not show any inhibitory effect against bacteria at all concentrations in contrast to the extracts of *Piper nigrum*, *Nigella sativa*, and *T. foenum-graecum*. As for the rest of the extracts, their effects varied by concentration, and the bacteria showed resistance as dilution increased.

Introduction

Various infectious diseases have been historically and even currently treated with herbs, which remain as the essential components of traditional medical systems in underdeveloped nations [1]. Among the most diverse classes of secondary metabolites in living things are alkaloids, which have various structural types, biosynthesis pathways, and pharmacological effects [2]. They belong to a family of secondary metabolites found in plants and categorized as basic compounds that are derivatives of amino acids [3]. Phenylalanine, tryptophan, lysine, and tyrosine are the primary amino acids converted into alkaloids and generated by 300 plant families [4]. The most bioactive phytochemicals are alkaloids, which can regulate biochemical reactivity, gene expression, and protein inhibition [5]. *N*-Heterocyclic compounds, often referred to as true alkaloids, constitute the majority of alkaloids [6].

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Plant-derived alkaloids have emerged as major subjects of interest in modern medicine because they are consistent sources of medications for long-term conditions, such as diabetes, cancer, and neurological disorders. Their application in medicine and other disciplines has increased because they not only shield plants from herbivores but also reduce fungal and bacterial infections [7]. Moreover, alkaloids derived from plants exert anti-inflammatory effects that inhibit the activity of various pro-inflammatory protein complexes connected to inflammatory signaling pathways. Butyrylcholinesterase, acetylcholinesterase, and monoamine oxidase inhibitors; *N*-methyl-D-aspartate, muscarinic, and adenosine receptor agonists, and alkaloids inhibit the pathogenesis of NDDS [8].

Alkaloids' antibacterial and antiviral properties have long been documented. Research into these properties is crucial given that pathogenic bacteria are becoming increasingly resistant to drugs. Casciaro et al. [9] revealed that nigritanine, which an alkaloid derived from *Strychnos nigritana* (a flowering plant in Loganiaceae family), exhibits strong antibacterial

activity against *Staphylococcus aureus*. This bacterium is one of the most important pathogenic bacteria worldwide; the authors examined the link between monomer or dimer structure and antibacterial activity, providing crucial insights into the mechanism of action of the alkaloid against *S. aureus*. Alkaloids in *Chelidonium majus* organs have been explored by numerous studies.

The combined antimicrobial effects of extracts and individual metabolites against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Candida albicans,* and *Klebsiella pneumonia* have been investigated. *Tecomella undulate* flowers contain a number of biologically effective alkaloids [10]. Natural bioactive substances called alkaloids can be abundantly found in herbal-based beverages, including wine, tea, and beer [11]. Simple chemical processes involving amino acids can be used in elucidating the biosynthesis of various alkaloids' structural components. Essential general reactions involve the transamination of amino acids for aldehyde or amine production. These reactions generate a Schiff base, which might then react in a Mannich-type condensation with a carbanion [12].

Materials and Methods

Plant collection

Plant materials, including seeds *Nigella sativa* (NS), *Piper nigrum* (PN), *Foeniculum vulgare* (FV), *Trigonella foenum-graecum* (TF), *Pimpinella anisum* (PA), *Cucurbita* (CB), *Coffea Arabica L.* (CF), flowers of *Matricaria chamomilla* (MC), rhizome stems of *Zingiber officinale* (ZO), and herbaceous stems of *Syzygium aromaticum* (SA), were collected from a local market in Fallujah City, Iraq, in August 2023.

Chemical compounds

The chemicals used in this study were of analytical grade and obtained from Sigma-Aldrich and BDH Chemicals.

Determination of total alkaloid content

Preparation of solutions

The method of Shamsa [13] was used in determining total alkaloid content. A bromocresol green (BCG) solution was prepared by completely dissolving 69.8 mg of BCG in 3 ml of NaOH 2N and 5 ml of distilled water through heating. Distilled water was used to dilute the solution to 1000 ml. The pH of 2 M sodium phosphate (71.6 g of Na2HPO4 in 1000 ml of distilled water) was adjusted to 4.70 with citric acid (42.02 g of citric acid in 1000 ml) for the preparation of a phosphate buffer solution (pH 4.70). A standard atropine solution was prepared by dissolving 50 mg of pure atropine (Sigma Chemical, USA) in 100 ml of distilled water (stock 1), and 2 ml of stock 1 was mixed with 10 ml of distilled water for the preparation of stock 2 at a concentration of 0.1 mg/ml.

Preparation of the standard curve

The aliquots of the atropine standard solution (stock 2) were measured precisely (0.1, 0.5, 1, 1.5, and 2 ml), and then each aliquot was transferred to a separator funnel. Next, 2.0 ml of BCG solution and 2.0 ml of pH 4.7 phosphate buffer. Finally, the mixture was agitated with 4 ml of chloroform. The extracts were mixed in a 10 ml volumetric flask, and chloroform was added to a specific volume. Using a spectrophotometer made by Regul Technologies (China), we evaluated the complex's absorbance in chloroform at 470 nm in comparison with a blank prepared similarly but without atropine [14]. Three times at 40 °C for an hour, 5 g of the dry ground material was extracted using 100 ml of chloroform. The chloroform extracts were dried using a rotary evaporator (SHIMADZU QR 2005-V, Japan) at 60 °C and low pressure [14].

Determination of alkaloid content

Chloroform (10 ml) was used to dissolve the crude extracts. A constant volume of chloroform was used after 1 ml of the liquid was added to a 10 ml volumetric flask. NaOH-$KHC_8H_4O_4$ was used as the buffer solution. A sample solution (0.5 ml), buffer solution (pH 4; 0.5 ml), BGC agent (2.0 ml), and chloroform (9 ml) were added to a separator funnel and heated for 1 h. Lastly, the solution from the chloroform layer was mixed with Na_2SO_4 for dehydration, and absorbance values were determined. Distilled water (0.5 cc) was used in preparing the control with the same procedures. At 470 nm, the complex's absorbance in chloroform was calculated [15].

Antibacterial activity using broth microdilution test Mueller–Hinton broth and nutrient agar. The medium powder was weighed with a sensitive balance (Sartorius, Germany) according to the manufacturer's instruction.

Preparation of activated and purified bacterial colonies

The identified and pure colonies of *S. aureus* were tested for the antibacterial experiment. The colonies were

transferred to a new nonselective medium of nutrient agar with a loop in streaking manner to yield pure bacterial colonies and incubated in an incubator for 24 h at 37 °C.

Inoculum preparation for dilution tests

To attain the turbidity required (0.5 McFarland criterion), the suspension was modified. The prepared suspension had (1–2) × 10^8 colony-forming units per milliliter.

Broth microdilution method

Each well contained 0.1 ml of the broth. The process provided in ISO 20776-1.1 is identical to the broth microdilution technique described in M07. Intermediate twofold dilutions of the antimicrobial agent were prepared volumetrically in the broth. Microdilution trays were prepared after 100 µl of MHB was added to each well in the microtiter plate. A pipette was used in measuring each diluent before the first tube was filled with the stock antimicrobial solution. The pipette was replaced after every dilution step, and a sterility (uninoculated) well and a growth control well (positive control) were placed on each tray (negative control).

Incubation

Inoculated macrodilution tubes or microdilution trays were incubated at 35 ± 2 °C for 16–20 h in an ambient air incubator within 15 min after inoculum addition.

Determining minimal inhibitory concentration end points

The minimum inhibitory concentration is the lowest concentration of an antimicrobial agent at which the growth of an organism in tubes or microdilution wells is prevented. To determine the growth end points for each set of tests, we compared the degree of growth in antimicrobial agent–containing wells or tubes with that in growth-control wells or tubes (no antimicrobial agent) in each test set. Acceptable growth (≥2 mm button or definite turbidity) in the growth-control well is required for the test to be considered as valid. [16]

Results and Discussion

Alkaloid content

At pH 4.7, alkaloid compounds may be fully extracted from medicinal plants by using chloroform (Table 1). Salt forms when alkaloids with hydrogen ions interact under specific pH conditions. Interactions between salt and acid dyes generate nonferrous complexes, which can be removed using an organic solvent (chloroform) [15]. For different atropine concentrations, a calibration curve was plotted (Fig. 1). The range was 1–20 µg of atropine per 1 ml of chloroform. Given that the plant components were extracted using a technique that only yielded alkaloids as the final residues, the resultant solution did not contain other organic compounds that would have reacted with BCG [17]. Some studies used caffeine as a measurement standard in estimating the total alkaloid content with the BGC method, as in the study of John et al. [18]. In another study, aconitine was used as a standard of measurement [19].

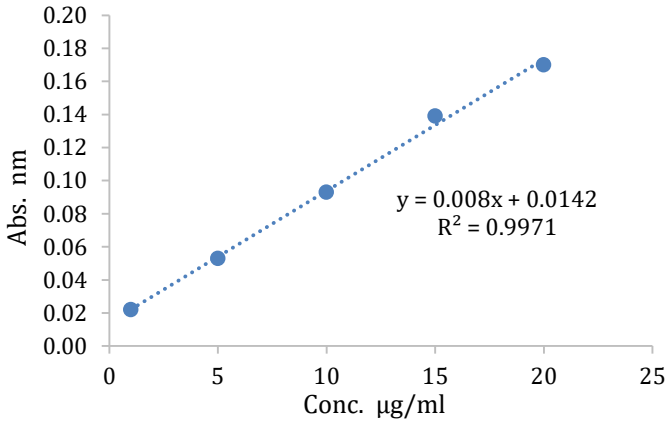

Figure 1. Calibration curve for various concentrations of atropine at 270 nm

The total alkaloid content in the stems, roots, leaves, and fruit of the medicinal plants was determined using BCG as a chromogenic agent and NaOH-$KHC_8H_4O_4$ as a buffer solution with a pH of 4.7 [20]. The pH value for this study (4.7) was also used by John et al. [18]. Other studies used nearly the same pH, such as the study of Liu et al. [19]. The overall alkaloid content of the examined plant materials, as assessed by the BCG–complex production method, is displayed in Table 1. *M. chamomilla* had the highest alkaloid concentration (124.9 µg/gm), followed by *T. foenum-graecum* (94.5 µg/gm). *Z. officinale* had the lowest (5.7 µg/gm). This study demonstrated the basis for these plants' therapeutic effects, establishing the groundwork for the thorough development and application of these plants. Several techniques with varying sensitivity, such as titrimetric and gravimetric approaches, have been developed to detect alkaloids in plant materials.

However, these techniques have several issues and are not sensitive enough. Given that TLC reveals many spots, the residues obtained, as in most gravimetric methods, had impurities. One drawback of the titrimetric assay is that an extract's color can obscure the endpoint. Moreover, no universal technique for all alkaloids has been established. Highly sensitive techniques, including HPLC, are not commonly used for determining total alkaloid content because they are expensive and need specialized equipment. The BGC method allows for the spectrophotometric determination of total alkaloid content and does not require specialized equipment. Another benefit of the method is that it is not time-consuming [21].

Table 1. Total alkaloid content in the tested medical plant (1 g) with BCG–complex formation

Plant name	Part used	Alkaloid content µg/gm
Nigella sativa	**seeds**	**8.0**
Piper nigrum	**seeds**	**17.6**
Foeniculum vulgare	**seeds**	**28.5**
Trigonella foenum-graecum	**seeds**	**94.5**
Matricaria chamomilla	**flowers**	**124.9**
Pimpinella anisum	**seeds**	**31.4**
Zingiber officinale	**rhizome stems**	**5.7**
Cucurbita	**seeds**	**64.3**
Syzygium aromaticum	**herbaceous stems**	**23.5**
Coffea	**seeds**	**65.3**

Antibacterial activity

Using the disk diffusion method (Table 2), we investigated 10 plant species to determine their antibacterial activity against *S. aureus* (Fig. 2). The findings showed that CB had no antibacterial activity at any concentration, and nine plant extracts were efficient in reducing the microbiological development of *S. aureus*. The FV, NS, TF, and PN extracts exerted inhibitory effects against bacteria at all concentrations, whereas the SA extract exerted inhibitory effects except at a concentration of 0.0171 mg/ml. The MC extract exerted inhibitory effects in the first three dilutions only. The CF, PA, and ZO extracts had the lowest inhibitory effects. The bacteria showed resistance in the last six dilutions. Table 2 shows that the ZO extract, despite containing a small percentage of alkaloids, showed resistance to bacteria at a concentration of 0.015. Active substances, including alkaloids, inhibit the activity of necessary enzymes in microorganisms, thus preventing the growth of these organisms, especially bacteria [22]. Alkaloids are commonly known to have a considerable influence on the treatment of various infections [23, 24]. Indoquinoline alkaloids exhibit activity against gram-negative bacteria and yeast [25], whereas quinine exerts antiprotozoal effects against malarial parasites [26]. Bioactive plant extracts often contain complex mixtures of ingredients, and their combined action can result in an enhanced effect. These compounds can affect microbial cells in a variety of ways, and the cytoplasmic membrane is their primary target site. Their effects can lead to changes in the membrane's structure, integrity, permeability, or functionality [27].

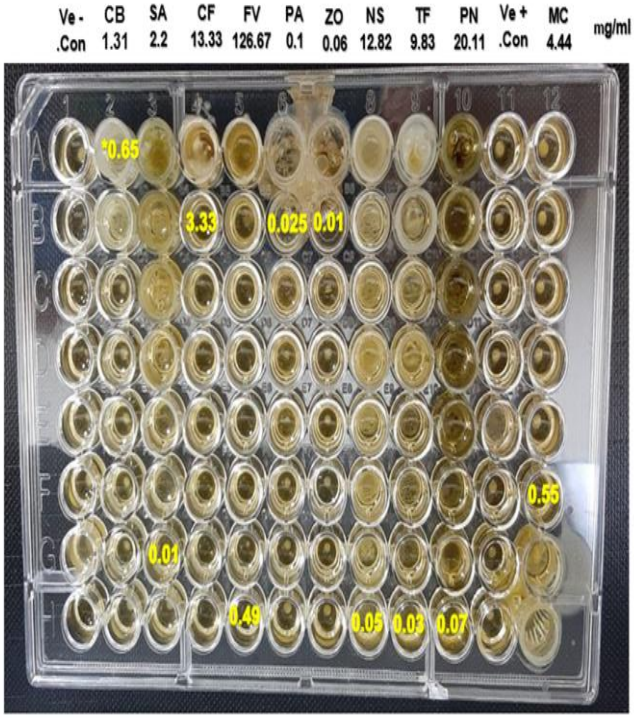

Figure 2. Illustrated antibacterial activity of plant extracts at variable concentrations

Table 2. Minimum inhibitory concentration (mg/ml) of plant extract against *Staphylococcus aureus*

Dilution	Plant Extract									
	CB	**SA**	**CF**	**FV**	**PA**	**ZO**	**NS**	**TF**	**PN**	**MC**
1/1	0.655	1.1	6.665	63.335	0.05	0.03	6.41	4.915	10.055	2.22
1/2	0.3275	0.55	3.3325	31.6675	0.025	0.015	3.205	2.4575	5.0275	1.11
1/4	0.1637	0.275	1.6662	15.8338	0.0125	0.0075	1.6025	1.2288	2.5138	0.555

1/8	0.0818	0.1375	0.8331	7.9169	0.0063	0.0038	0.8013	0.6144	1.2569	0.2775
1/16	0.0409	0.0688	0.4166	3.9584	0.0031	0.0019	0.4006	0.3072	0.6284	0.1388
1/32	0.0204	0.0344	0.2083	1.9792	0.0016	0.0009	0.2003	0.1536	0.3142	0.0694
1/64	0.0102	0.0172	0.1041	0.9896	0.0008	0.00045	0.10016	0.0768	0.1571	0.0347
1/128	0.0051	0.0171	0.0521	0.4948	0.0004	0.00023	0.0500	0.0384	0.0786	0.0173

Conclusions

The results of the current study demonstrated the inhibitory activity of most plant extracts against *S. aureus*. The highest levels of activity were observed in the FV, NS, TF, and PN extracts. The CB extract did not show any inhibitory effect. The results also showed difference in alkaloid content among the plants, and the MC extract had the highest concentration, followed by the TF extract.

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القلويدات من الاعشاب الطبية ودراسة فعاليتها الحيوية ضد بكتيريا المكورات العنقودية الذهبية *Staphylococcus aureus*

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الخلاصة:

تستخدم النباتات الطبية على نحو واسع في المجتمعات كونها أكثر توافرًا وأرخص سعرًا من الأدوية الحديثة. عُرفت العديد من المواد الكيميائية النباتية ذات النشاط الحيوي المثبت أو المحتمل، لكن امتلاك النبات الواحدة لعدد كبير من المواد الكيميائية المتنوعة جعل تأثير استخدام النبتة كاملةً قليل الفعالية، ومنع تقييم الفعاليات المتعلقة بهذه المواد الموجودة في الكثير من النباتات في الأبحاث العلمية الدقيقة الهادفة لتحديد فعاليتها وأمانها. كان الهدف من هذا البحث هو تحديد كمية القلويدات الموجودة في المستخلص المائي لعشرة أصناف من النباتات الطبية الموجودة في الأسواق المحلية وفحص كفاءتها البيولوجية في مكافحة بكتيريا المكورات العنقودية الذهبية *Staphylococcus aureus*. بينت النتائج ان المستخلص المائي لنبات البابونج *Matricaria chamomilla* احتوي اعلى كمية من القلويدات بلغت 124.9 ميكروجرام/جرام، يليه مستخلص نبات الحلبة *Trigonella foenum-graecum* بمقدار 94.5 ميكروجرام/جرام. وكان أدني تركيز للقلويدات في مستخلص نبات الزنجبيل *Zingiber officinale* بمقدار 5.7 ميكروجرام/جرام. أظهرت تجارب الفعالية البايولوجية ان مستخلص نبات القرع Cucurbita لم يظهر تثبيطا ضد البكتريا في جميع التركيزات، في حين أظهرت مستخلصات الفلفل الاسود *Piper nigrum* والحبة السوداء *Nigella sativa* والحلبة *Trigonella foenum-graecum* تثبيطا ضد البكتريا في جميع التركيزات، أما باقي المستخلصات فكان تاثيرها متفاوتا حسب التركيزات، إذ ابدت البكتريا مقاومة مع زيادة التخفيف.

الكلمات المفتاحية: القلويدات ، الفعالية الحيوية ، الاعشاب الطبية ، بكتيريا المكورات العنقودية.