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Original Article

A Comprehensive Study on *Asparagus officinalis*: Its Antimicrobial, Antioxidant and Phytochemical Characteristics

Amna Mahmood[1*], Malaika Ajaz[2], Waleed Rasool[2], Lahraseb Khan[3], Nida Naeem[2], Ali Hassan[2]

[1]Department of Basic and Applied Chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
[2]Department of Biotechnology, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
[3]Department of Biochemistry, The University of Azad Jamu and Kashmir, Azad Kashmir, Pakistan

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***Corresponding Author:**
Amna Mahmood
Department of Basic and Applied Chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
amnaqureshi10@hotmail.com

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ABSTRACT

The Asparagus plant is considered to be a palatable chemical source against treating infectious diseases and flavorings. Its prevalent distribution is well-known in Asian and sub-Asian regions. **Objective:** To understand different activities that have been functional in the stem and leaf extracts *of Asparagus officinalis* including antioxidant and antibacterial activities. Further, phytochemical constituents of asparagus are also discussed. **Methods:** The antibacterial assay of extracts for the variety of bacteria, indicated a maximum inhibition zone against *Enterococcus faecalis* (ATCC 29212) (24 mm) followed by *Staphylococcus aureus* (ATCC 25923) (34 mm), whereas *Bacillus subtilis* (ATCC 6633) (14 mm) at their respective temperature a minimum inhibition zone after 24 hours and 48 hours of incubation (37 °C for bacteria). **Results:** As a robust antioxidant reference standard, these antioxidant activities resulted in the stable radical 1-diphenyl-2-picrylhydrazyl (DPPH). It can be reduced to yellow-coloured DPPH-H, reaching 75.81% of the DPPH scavenging impact at its 100% concentration in contrast to ascorbic acid. Various experiments have been carried out, including the Molisch test, Ninhydrin test, Wagner's test, Alkaline reagent test, Froth test, Ferric reagent test, and Salkowski test for the phytochemical analysis. **Conclusion:** To sum that up, carbohydrates, saponins, and flavonoids are present in these extracts. These extracts were found to perform satisfactory activities in all tests.

INTRODUCTION

Asparagus officinalis (Green Asparagus) is of utmost importance and has worldwide consumption – mainly because of its rich bioactive and nutritional composition. These bioactive contents include inosine, caffeic acid, ferulic acid, rutin and quercetin [1]. Due to their benefits in different ecosystems, like the provision of fuel, medicine, shelter, food, condiments, perfumes, or aromas, it plays an integral role in global sustainability [2]. Plants constitute the primary life-sustaining system by forming soft green protective layers around the earth. As hydrological preservation units and animal food sources are essential for maintaining atmospheric temperature and equilibrium. The average cultivation life-cycle of *A. officinalis* is up to 40 years, as long as their productivity and quality persist. The growth of asparagus deteriorates as time passes due to different diseases invasion by microorganisms, such as *Phytophthora*, *Fusarium*, *Phomopsis asparagi*, *Stemphylium*, and *Cercospora asparagi Sacc* species. Not to mention that asparagus breed auto toxins and indulge in self-harming, thereby affecting the growth of new asparagus plants [3]. Consequently, it becomes indispensable to regenerate the rootstock to overcome the growth barrier. Moreover, in many countries, including New Zealand, the mature asparagus roots are discarded or flung away in barren lands – which is unproductive practice [4]. Because, the debris of asparagus is reportedly claimed as a

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potential source for bioactive compounds such as polyphenols, saponins, and flavonoids. The Agar dilution method was deployed to determine the antibacterial activity of extracts. At the same time, antioxidant properties were assessed using the '2,2-diphenyl-1-picrylhydrazyl' (DPPH) assay. The results found that the minimum inhibitory concentration (MIC) of the leaf is at 0.125 mg/mL concentration against *S. saprophyticus and E.cloacae*, and 1 mg/mL against *S. aureus* and *B.subtilis* [5]. However, no MIC was found in the stem extract at any given concentration. The stem extract activity has shown very low free radical scavenging activity, though the leaf extract accrued effective activity (72.1%). Additionally, qualitative phytochemical scrutiny of these plant extracts confirmed the presence of various chemicals including saponins, tannins, flavonoids, and phlobatannins [6]. Since applying one antimicrobial and antioxidant technique may not provide efficient results, using antimicrobial analysis techniques in more than one concentration is recommended (25mg, 50mg, 75mg, and 100mg). Besides, the results of the antioxidant analysis demonstrate that in-vivo antioxidant capacity is higher than A. *officinalis*, is a callus tissue, and grows in-vitro. Furthermore, antioxidant numbers in vivo *A. officinalis* are less than in vitro-grown ones. The 'antimutagenic agents' that provide protection against mutations and impede the production of cancerous cells (that often lead to the early stage of the disease) are *Beltsville Area*. The removal of xenobiotic and carcinogenic compounds is facilitated by detoxifying enzymes of category cellular phase II – they are also supporting factors of many liver functions [7]. Enhanced antioxidant activity such as cyclooxygenase-2 suppression (less chronic inflammation) can significantly promote a strong immune system and healthier digestion.

METHODS

The materials (plants) used in this analysis were collected from different areas of Lahore and Karachi. The asparagus plant (leaves) was dried and dissolved in ethanol – filtered afterwards. A rotary evaporator was used to separate solvent and extract; then using falcon tubes, it was dried in a water bath. Later, the dried extract was left to freeze for further analysis [8]. A total of five bacteria were selected to conduct the research study. Gram-positive *Staphylococcus aureus* (ATCC 25923), and five Gram-negative *Pseudomonas Aeruginosa* (ATCC 27853), *B. subtilis* (ATCC 6633), *Enterococcus Faecalis* (ATCC 29212), and *Klebsiella pneumoniae* (ATCC 33152) [9] were obtained from the microbiology department of the University of Lahore. Mueller-Hinton Agar (MHA) was enacted for the growth of bacterial colonies. While the DMSO was used as a preservative for extraction purposes. It was preferred as a negative control and ciprofloxacin as a positive control [10]. Bacterial inoculation was performed in a saline solution (normal pH). Six test tubes were filled with 5ml solution each. One of the methods is Disc Diffusion Assay for testing microorganisms; Muller Hinton Agar (MHA) medium was taken in Petri dishes [11]. The channel paper circles of 5 mm width were set on the agar plates and then loaded with 20µl of plant extract. The plates were placed at a temperature of 37 C for 24 hours. After placing the development restraint zone was measured in millimeters (mm). Another method that was deployed for the detection of microbial activity was the Well diffusion Assay. To assess contamination in Petri dishes, 20 microliters (µl) of Mueller-Hinton Agar (MHA) were discharged into the dishes and left for the growth of microorganisms in an incubator for 24 hours [12]. Plastic straws were used to avoid contamination, first, they were dipped into an ethanol solution and the opening gap between Petri dishes was set at 6mm. Plant extract of 20 µl was taken out in a petri dish, and placed in an incubator at 370 C for 24 hours. The "inhibition zones" (region of zero growth) were measured in millimeters (mm). Phytochemical analysis of *Asparagus officinalis* includes tests for carbohydrates, proteins, alkaloids, saponins and flavonoid compounds. First, the prepared solution was mixed with 2 drops of alcoholic-naphthol solution. Sulfuric acid of 2 ml was added into the prior test tube [13]. The formation of violet rings indicates the presence of carbohydrates. (Molisch's test). Ninhydrin reagent (25%) was added to the extract and heated till boiling. The formation of a Blue-violet color signifies the presence of amino acids or proteins (Ninhydrin test). The filtrate solution was mixed with Wagner's reagent in a test tube. This reagent is prepared by mixing iodine in potassium iodide. The presence of alkaloids is detected by brown or reddish precipitates (Wagner's test). A few drops of sodium hydroxide solution were mixed with the plant extract. An intense yellow color formation was found in the test tube, which becomes colorless, after reacting with dilute acid. This colorless solution confirms flavonoids compounds (Else if the solution does not turn colorless the test result will be negative) [14]. The extract which consists of dry powder is dissolved in distilled water (2ml). Then, it is shaken and allowed to stand for 10 mins. Froth appearance indicates the presence of saponins (Froth test). The test cylinder contained 50 µL of concentrates ranging from 1 to 5 mg/mL. Add 5 mL of 0.1mM DPPH arrangement (4mg/100ml ethanol). It was blended using a vortex mixer and hatched for about 30 minutes at room temperature. It was performed in generally dim spots perused utilizing a spectrophotometer at 517 nm afterwards [15]. An ascorbic corrosive consisting of 10 mg /ml DMSO was used for correlation. The clear was 80% ethanol (v/v). DPPH search

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impact was determined through this technique.

DPPH searching impact (%) = [(AB-AA)/AB] X 100

RESULTS

The ethanolic extract used against *S. aureus*, *K. pneumoniae*, *Enterococcus Faecalis*, *P. aeruginosa* and *B. subtilis* showed no zones of inhibition with ciprofloxacin at 25 concentrations. The extract (ethanolic) detected antimicrobial activity against both types that is, gram-positive and gram-negative bacteria. Inhibition zones which had a dilution of 25% concentration were recorded against bacterial strains. To make dilutions, the ethanolic extract was dissolved in 1ml DMSO which was also used as a negative control in pure form and showed no zone of inhibition. The results showed that the inhibition zone against *S. aureus* was 00mm with ciprofloxacin (20mm), *K. pneumoniae* was 00mm with ciprofloxacin (21mm), *Enterococcus Faecalis* was 00mm with ciprofloxacin (23mm), *P. aeruginosa* was 00mm with ciprofloxacin (25mm) and *B. subtilis* was 00mm with ciprofloxacin (28mm).

Table 1: Antibacterial properties of *Asparagus* (25mg/1ml DMSO)

Bacteria	Plant part	Zone of inhibition	Positive control	Negative control
B. subtilis	Asparagus	0	28	0
P. aeruginosa	Asp	0	25	0
Enterococcus Faecalis	Asp	0	23	0
K. pneumoniae	Asp	0	21	0
S. aureus	Asp	0	20	0

The ethanolic extract used against *S. aureus*, *K. pneumoniae*, *Enterococcus Faecalis*, *P. aeruginosa* and *B. subtilis* showed no zones of inhibition with ciprofloxacin at 50% concentration. The extract found antimicrobial activity against both types of bacteria – gram-positive and gram-negative bacteria. To make dilutions, the ethanolic extract was dissolved in 1ml DMSO which was also used as a negative control in pure form and showed no zone of inhibition. The results showed that the inhibition zone was 00mm with ciprofloxacin (20mm), *K. pneumoniae* was 00mm with ciprofloxacin (21mm), *Enterococcus Faecalis* was 00mm with ciprofloxacin (23mm), *P. aeruginosa* was 00mm with ciprofloxacin (25mm) and *B. subtilis* was 00mm with ciprofloxacin (28mm) against *S. aureus*.

Table 2: Antibacterial properties of *Asparagus* (50mg/1ml DMSO)

Bacteria	Plant part	Zone of inhibition	Positive control	Negative control
B. subtilis	Asp	0	25	0
P. aeruginosa	Asp	0	25	0
Enterococcus Faecalis	Asp	0	23	0
K. pneumoniae	Asp	0	21	0
S. aureus	Asp	0	20	0

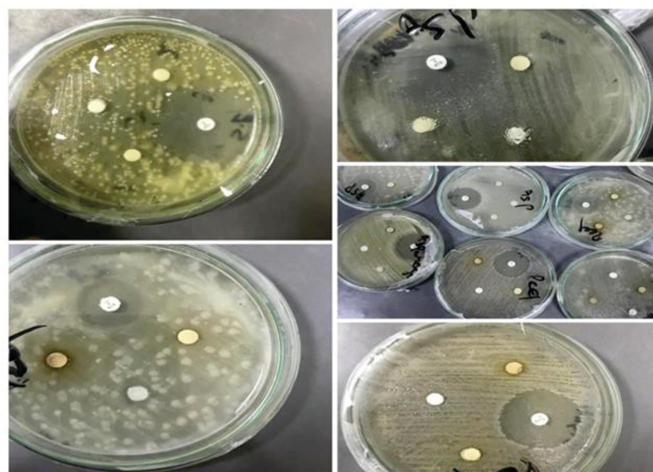

Figure 1: Anti-bacterial properties of *Asparagus* (25mg/1ml DMSO) (50mg/1ml DMSO)

The ethanolic extract used against *S. aureus*, *K. pneumoniae*, *Enterococcus Faecalis*, *P. aeruginosa* and *B. subtilis* showed maximum zones of inhibition with ciprofloxacin at 75% concentration. Antimicrobial activity was found in the extract against both types, i.e., gram-positive and gram-negative bacteria. An inhibition zone of dilution of 75% concentration was recorded against bacterial strains. To make dilutions, the ethanolic extract was dissolved in 1ml DMSO which was also used as a negative control in pure form and showed no zone of inhibition. The result showed that the inhibition zone was 30mm with ciprofloxacin (20mm), *K. pneumoniae* was 13mm with ciprofloxacin (21mm), *Enterococcus Faecalis* was 12mm with ciprofloxacin (23mm), *P. aeruginosa* was 9mm with ciprofloxacin (25mm) and *B. subtilis* was 10mm with ciprofloxacin (28mm) against *S. aureus*.

Table 3: Antibacterial property of *Asparagus* (75mg/1ml DMSO)

Bacteria	Plant part	Zone of inhibition	Positive control	Negative control
B. subtilis	Asp	10	25	0
P. aeruginosa	Asp	9	25	0
Enterococcus Faecalis	Asp	12	23	0
K. pneumoniae	Asp	13	21	0
S. aureus	Asp	30	20	0

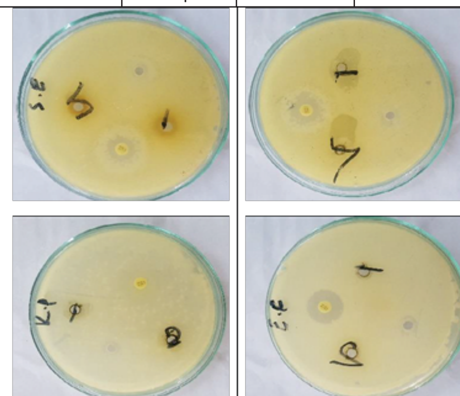

Figure 2: Antibacterial property of *Asparagus* plant (75mg/1ml

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DMSO)

The ethanolic extract used against *S. aureus*, *K. pneumoniae*, *Enterococcus Faecalis*, *P. aeruginosa* and *B. subtilis* showed maximum zones of inhibition with ciprofloxacin at 100% concentration. The extract (ethanolic) detected antimicrobial activity as opposed to both types, that are, gram-positive and gram-negative bacteria. An inhibition zone of dilution of 100% concentration was recorded against bacterial strains. To make dilutions, the ethanolic extract was dissolved in 1ml DMSO which was also used as a negative control in pure form and showed no zone of inhibition. The results showed that the inhibition zone was 40mm with ciprofloxacin (20mm), *K. pneumoniae* was 26mm with ciprofloxacin (21mm), *Enterococcus faecalis* was 29mm with ciprofloxacin (23mm), *P. aeruginosa* was 11mm with ciprofloxacin (25mm) and *B. subtilis* was 14 mm with ciprofloxacin(28mm)against *S. aureus*.

Table 4: Antibacterial property of *Asparagus*(100mg/1ml DMSO)

Bacteria	Plant part	Zone of inhibition	Positive control	Negative control
B. subtilis	Asp	14	20	0
P. aeruginosa	Asp	11	25	0
K. pneumoniae	Asp	29	23	0
Enterococcus Faecalis	Asp	26	21	0
S. aureus	Asp	40	20	0

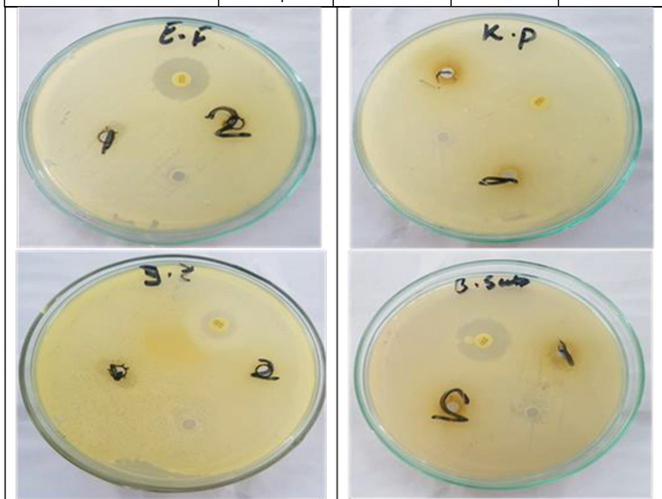

Figure 3: Antibacterial properties of *Asparagus* plant (100mg/1ml DMSO)

Table 5: Results of phytochemical analysis of *Asparagus officinalis*

Phytochemicals	Tests	Result
Carbohydrates	Molisch	Positive
Proteins	Ninhydrin	Negative
Alkaloids	Wagner	Negative
Saponins	Froth	Positive
Proteins	Biuret	Negative
Phenols	Ferric chloride	Positive

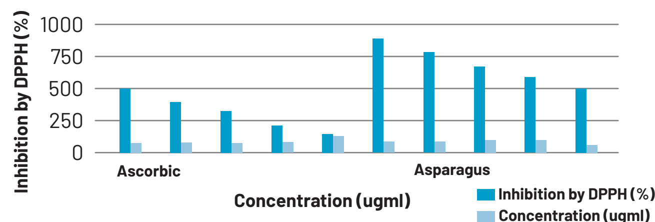

Figure 4: Graph showing antioxidant activity of *Asparagus* ethanolic extract by comparing with ascorbic acid DPPH inhibition

DISCUSSION

The proposed study was based on the medicinal activities of *Asparagus officinalis*. Antibacterial activity of *Asparagus* plant extract demonstrated zones of inhibition against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 33152). A group of study conducted by Amare in 2015 they found that *S. aureus* exhibited larger zones of inhibition when treated with ethanolic extracts of the *Asparagus* plant. Our results are in line with the previously work done [18]. In a study presented by Sriyab *et al.*, observed that only the alcoholic extract of turnip, rather than the extract of *Brassica napus L.*, exhibited zones of inhibition against *Pseudomonas aeruginosa* [19]. Inhibition zones of 2, 8, and 6 mm were observed on three consecutive days, respectively. There was no significant effect in the well diffusion assay. In comparison, this study found that a 25 mg/1 ml DMSO solution produced fewer zones of inhibition than a 50 mg solution. *Proteus* exhibited larger zones of inhibition in the 25 mg solution than in the 50 mg solution, while *P. aeruginosa* showed more zones of inhibition in the 50 mg solution than in the 25 mg solution. A similar study was elaborated by Olivier *et al* and showed that *S. aureus* was more sensitive to the plant extract [20]. Our results showed the potent activity of *Asparagus officinalis* as the plant was enriched with phytochemicals. Similar results were presented in 2019 by a group of scientists who elaborated the phytochemical contents [21]. Our results in line with another study which was conducted by Linka *et al.*, and Jianu *et al.*, they screened vitamin C, phytic acid, fiber content and tocopherol [22, 23]. Similar results were presented by Pandey in 2013 they confirmed the potent antioxidant activity of the plant [24]. No experiment was conducted on ethanolic leaves extract of *Asparagus officinalis* that depicts the novelty of the proposed study. For human consumption, Asparagus has been an admirable vegetable since its early domestication, especially by virtue of its medicinal properties. Nowadays, its cultivation has been transmitted across the globe in all continents including Europe, Africa and beyond. Considering the escalating demand of consumers for

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fresh, frozen and canned asparagus more and more area has been acquired for its cultivation. In recent study work, different experiments have been performed to identify and characterize the specific phytochemicals. By concluding all of the above research, it has been confirmed that Asparagus leaves have excellent antimicrobial activity. The business community has also shown interest in manufacturing new drugs for the therapy of distinct diseases in both research institutes and pharmaceutical industries. Leaves extract is a promising candidate for the use of human health as an antioxidant based on natural products. Lastly, it is suggested that there are many emerging possibilities and strategies that can allow the effective growth of Asparagus leaf system extracts to be applied as a natural element of food preservation, cosmetics and medicinal products such as they have strong antioxidant, antimicrobial and phytochemical effects.

CONCLUSIONS

By concluding all of the above research, it has been confirmed that Asparagus leaves have excellent antimicrobial activity. The business community has also shown interest in manufacturing new drugs for the therapy of distinct diseases in both research institutes and pharmaceutical industries. Leaves extract is a promising candidate for the use of human health as an antioxidant based on natural products. Lastly, it is suggested that there are many emerging possibilities and strategies that can allow the effective growth of Asparagus leaf system extracts to be applied as a natural element of food preservation, cosmetics and medicinal products such as they have strong antioxidant, antimicrobial and phytochemical effects.

Conflicts of Interest

The authors declare no conflict of interest.

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