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Antimicrobial and Minimum Inhibitory Concentration activity of leaf extract of *Asparagus aphyllus*

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Abstract: **The aim of the present study was to explore the leaf extract of *Asparagus aphyllus* belonging to family Liliaceae for its antimicrobial activity. The *in vitro* antimicrobial activity of the leaf methanol extract of *Asparagus aphyllus* and others extarct (ethanol, aqueous, ethyl acetate and chloroform) were assayed using the agar plate diffusion. Test microorganisms studied were *Escherichia coli*, *Kiebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Methanol extract (100µg/ml) inhibited the growth of all the test organisms and the maximum zone of inhibition against gram positive organism is *Kiebsiella pneumonia* (2.27 cm); against *Staphylococcus aureus* is (2.24 cm) and *Pseudomonas aeruginosa* (2.14 cm). Methanol, ethanol and aqueous extract showed minimum inhibitory concentration (MIC) of less than 6.25µg/ml against all test microorganisms. The minimum inhibitory concentration (MIC), above 12.5µg/ml showed by ethyl acetate extract for *Staphylococcus aureus* and less than 12.5µg/ml for *Klebsiella pneumonia* and *Pseudomonas aeruginosa.* The methanol extract exhibited antimicrobial activities followed by ethanol and aqueous. Ethyl acetate exhibited least antimicrobial activity.**

Keywords: ***Asparagus aphyllus*, Antimicrobial, Minimum Inhibitory Concentration, *Staphylococcus aureus.***

1. INTRODUCTION

Medicinal plants have been used for centuries in traditional health care systems and numerous cultures around the world still rely on plants for their primary health care. With the recent advancements in plant sciences, there has been a tremendous increase in the use of plant based health products in developing as well as developed countries. About 70-80% people around the globe rely on medicinal plants for primary health care [1, 2].

Traditional plant-based medicines still exert a great deal of importance to the people living in developing countries and also lead to discovery of new drug candidates for a variety of diseases that threaten human health. *Asparagus* is the name of a genus of plants, a member of the family Asparagaceae (formerly placed in the Liliaceae). The *Asparagus* genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant [3]. *Asparagus* is the Greek word for "stalk" or "shoot". About 300 species of *Asparagus* are known to occur in the world in many countries in both hemispheres and throughout temperate and tropical regions.

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [4]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include leaf, root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries [5]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated [6].

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2. MATERIAL AND METHODS

Plant Extraction: The plant leaves of *Asparagus aphyllus* was collected and thoroughly washed it. The plant material were dried in shade for five days and then powdered with the help of Waring blender. 25 g of shade-dried powder was filled in the thimble and extracted successively with methanol, ethanol, chloroform, ethyl acetate and aqueous in Soxhlet extractor for 48h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

Microorganism: The test microorganisms used for the antimicrobial activity screening were *Escherichia coli* (*E. coli*), Kiebsiella pneumonia, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (*S. aureus*).

Antimicrobial Tests: Antimicrobial activity of the methanol, ethanol, chloroform, ethyl acetate and aqueous was determined using agar plate diffusion technique [7, 8]. The dried plant extract and fractions were dissolved in 1% aqueous dimethylsulfoxide (DMSO) to a final concentration of 100 µg/ml. The microorganisms were maintained on agar slants. The inocula were prepared by inoculating the test organisms i.e. bacteria in nutrient broth and incubating them for 24 hours at 37°C. One milliliter of the diluted cultures was inoculated into sterile molten nutrient agar (48°C) and poured into sterile petri dishes. These were gently swirled and allowed to solidify. Afterwards, 6 mm wells were bored into the solidified and inoculated agar plates using sterile borer. The wells were filled with 100 µl of 100 µg/ml of each extract. At the end of the incubation period, inhibition zones were recorded in cm as the diameter of growth free zones around the bored holes using a transparent metre rule. Each extract and standard antibiotics were independently tested in triplicate.

Minimum Inhibitory Concentration: MIC was determined to prepare at a different concentration 6.25, 12.5, 25, 50 µg/ml [9, 10]. The minimum inhibitory concentration value was determined for the microorganisms that were sensitive to the extracts/fractions under study fraction i.e. Methanol, ethanol, chloroform, ethyl acetate and aqueous

3. RESULTS AND DISCUSSION

Results of MIC and antimicrobial activity of different extract of *Asparagus aphyllus* leaves are graphically presented in figure 1. The methanol extract showed higher inhibitory effect against *Klebsiella pneumoniae* (2.27 cm), *Staphylococcus aureus* (2.24 cm) and *Pseudomonas aeruginosa* (2.14 cm). Also it showed high to intermediate inhibitory effect against *E.coli* (2.08cm). The leaves ethanol and aqueous extract showed intermediate inhibitory effects against almost all test microorganisms. The diameter of inhibition zone of leaves ethanol extract was recorded as *Staphylococcus aureus* (2.54 cm), *E.coli* (1.47cm), *Proteus vulgaris* (1.36cm), *Pseudomonas aeruginosa* (2.09cm) *and Klebsiella pneumonia* (2.14cm). The diameter of inhibition zone of leaves aqueous extract was recorded as *Staphylococcus aureus* (1.57cm), *E.coli* (1.77cm), *Proteus vulgaris* (1.16 cm), *Pseudomonas aeruginosa* (2.46cm) *and Klebsiella pneumonia* (2.41cm). The leaves chloroform and ethyl acetate extract showed low inhibitory effect against almost all test microorganisms. The microorganisms against ethyl acetate extract showed a low inhibitory effect for *Klebsiella pneumonia* (1.46cm) and ethyl acetate extract showed a low inhibitory effect for *E.coli* (1.54cm).

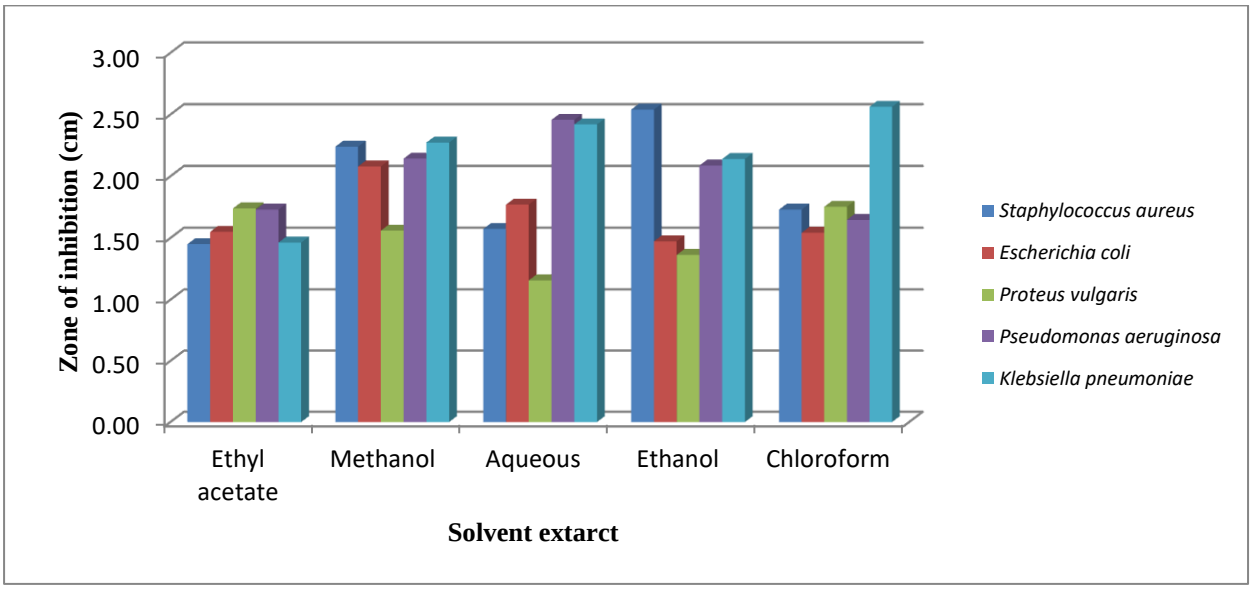

Figure 1: Antimicrobial activity of different solvent extracts of *Asparagus aphyllus* leaves.

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The different concentrations (50, 25, 12.5, and 6.25µg/ml) of different extracts were investigated to determine their MICs against test microorganisms and are presented in table 1 and illustrated in figure 2. The *Asparagus aphyllus* extracts of methanolic, aqueous and ethanol showed MIC less than 6.25µg/ml against all test microorganisms. Chloroform extract showed MIC less than 6.25 µg/ml against *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* microorganisms except *E.coli* and *Staphylococcus aureus* which exhibit MIC of 12.5 µg/ml or less but more than 6.25 µg/ml. Ethyl acetate extract showed minimum inhibitory concentration (MIC) above 12.5µg/ml for *Staphylococcus aureus* and less than 12.5µg/ml for *Klebsiella pneumonia* and *Pseudomonas aeruginosa.* Interaction effect between microorganism and different concentration was found to be significant at all extract of *Asparagus aphyllus* leaves but interaction effect of aqueous extract was non significant. Based on the antimicrobial activity, methanol and ethanol appeared the best solvents for extraction in this investigation, whereas was least chloroform and ethyl acetate suitable. Aqueous was also seen as the optimum extraction solvents for the plants.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [11]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [12, 13, 14, 15, 16, 17]. The methanol extract of *Asparagus* exhibited antimicrobial activities followed by ethanol extract fraction. Ethyl acetate exhibited least antimicrobial activity. Methanol extract, ethanol extract and aqueous extract exhibited appreciable activity against *Klebsiellia pneumoniae* bacterium [18]. It also showed appreciable activity against *E. coli.* However, a limited data is available so far regarding its efficacy. The broad-spectrum antibacterial activity exhibited by the methanol extract could be related with the concentrations of sterols, flavonoids, saponins, phenolic compounds and carbohydrates in these extracts. These classes of compounds are known to show curative activity against several pathogens [19] and may explain some of its antimicrobial actions since antimicrobial actions of most of these phytochemical substances have been documented [20, 21, 22].

Table 1: Minimum inhibitory concentration study of the extracts of *Asparagus aphyllus* against test microorganisms

S. No	Extract	Microorganisms	Conc. 6.25 µg/ml	Conc. 12.5 µg/ml	Conc. 25 µg/ml	Conc. 50 µg/ml
1	Chloroform Extract	*Staphylococcus aureus*	0.00	1.83	2.27	1.67
		Escherichia coli	0.00	1.20	1.17	1.40
		Proteus vulgaris	0.83	1.43	1.43	1.67
		Pseudomonas aeruginosa	1.13	1.37	1.43	1.53
		Klebsiella pneumoniae	1.87	2.03	2.13	2.57
2	Aqueous Extract	*Staphylococcus aureus*	1.03	1.43	1.47	2.53
		Escherichia coli	1.17	1.33	1.37	1.40
		Proteus vulgaris	0.73	1.03	1.13	1.20
		Pseudomonas aeruginosa	1.10	1.37	1.43	1.87
		Klebsiella pneumoniae	1.60	1.87	2.00	1.97
3	Ethanol Extract	*Staphylococcus aureus*	1.03	1.13	1.37	1.47
		Escherichia coli	1.17	1.10	1.33	1.67
		Proteus vulgaris	0.67	0.73	1.03	1.03
		Pseudomonas aeruginosa	1.37	2.03	2.13	2.33
		Klebsiella pneumoniae	1.70	1.97	2.30	2.33
4	Methanol Extract	*Staphylococcus aureus*	0.53	0.97	1.33	2.17
		Escherichia coli	0.60	1.13	1.40	1.97
		Proteus vulgaris	0.93	1.33	1.27	1.43
		Pseudomonas aeruginosa	1.33	1.33	1.83	2.03
		Klebsiella pneumoniae	1.23	1.37	1.67	2.03
5	Ethyl acetate Extract	*Staphylococcus aureus*	0.00	0.00	2.03	1.37
		Escherichia coli	0.00	1.37	1.67	1.47
		Proteus vulgaris	1.27	1.33	1.47	1.63
		Pseudomonas aeruginosa	0.00	1.27	1.53	1.60
		Klebsiella pneumoniae	0.00	1.13	1.27	1.37

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Figure 2: Antimicrobial activity of different solvent extracts of *Asparagus aphyllus.*

A-Chloroform extract, B-Aqueous extract, C- Ethanol extract, D- methanol extract, E- Ethyl acetate extract

4. CONCLUSION

In conclusion, the fact that the methanol and ethanol extract produced inhibitory activities but against almost all the test bacteria and fungi provides some scientific basis for some of the uses in traditional medicine. The present study justified the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. We therefore, suggest the isolation and characterization of the antibacterial active constituents from the extracts of this plant species have been initiated.

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