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RESEARCH ARTICLE

MORPHO-PHYTOCHEMICAL AND ANTIBACTERIAL STUDIES OF IMMUNITY BOOSTING MEDICINAL PLANT ASPARGUS RACEMOSUS UNDER VARIOUS SOIL CONDITION

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ABSTRACT

Asparagus racemosus, commonly known as Shatavari, is highly valued in Ayurveda, often referred to as the "Queen of Herbs" due to its rejuvenating properties. This study seeks to explore the morphological, phytochemical, and antibacterial characteristics of *Asparagus racemosus*, with a specific emphasis on how different soil types and climatic conditions in Agra affect its immune-enhancing properties. Morphological observations indicated improvements in plant height, flower, fruit, and seed development when cultivated in vermicompost, with notable enhancement in the shape and size of roots in the vermicompost mixture. Phytochemical analysis of methanol extracts from the roots of *Asparagus racemosus*, grown under varied soil conditions, identified the presence of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, and tannins. Antibacterial testing of these methanolic root extracts demonstrated effectiveness against pathogens such as *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. These findings validate the conventional use of *Asparagus racemosus* in treating bacterial infections, emphasizing the potential of plant-derived products as sources of antimicrobial agents.

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INTRODUCTION

Morpho-phytochemical studies of medicinal plants involve analyzing both the physical characteristics and chemical composition of the plants to gain a deeper understanding of their therapeutic potential. This research is crucial for identifying the plant's morphological and anatomical features, as well as its chemical constituents, which are essential for ensuring quality, purity, and accurate identification. Such studies are fundamental for recognizing and utilizing the medicinal properties of plants in modern applications. Many researchers are actively exploring this area. There is considerable variation in the morphological traits of medicinal plants, and these different morphotypes often exhibit variability in their bioactive compounds (Nandini *et al.* 2015) emphasized that plant morphology plays a key role in the visual identification of plants. Phenotypic and genetic variations within and between species contribute to differences in bioactive compounds. These bioactive compounds are primarily secondary metabolites. Phytochemistry is the study of the chemical compounds produced by plants, focusing on their structure, biosynthesis, functions, and how they interact within living systems (Chukwuebuka *et al.*, 2019). These plant-derived chemicals, known as phytochemicals, can be divided into two main categories based on their chemical structures and synthesis processes: primary and secondary metabolites (Gnanavel *et al.*, 2018). Primary metabolites include proteins, carbohydrates, lipids, and nucleic acids. These compounds are essential for the plant's metabolism, growth, development, and survival.

They have a uniform structure across different plant species. On the other hand, secondary metabolites, such as polyphenols, flavonoids, and alkaloids, are not essential for the plant's survival but play a vital role in the plant's interaction with its environment. They help protect plants from pests and diseases, reduce damage caused by extreme conditions, and contribute to the plant's ecological niche (Saurabh *et al.*, 2015). Phytochemical methods are primarily employed for quality control in herbal, Ayurvedic, and allopathic medicine formulations. These formulations typically consist of various chemical components, including alkaloids, volatiles, saponins, flavonoids, anthraquinones, phenols, tannins, and terpenoids (Okwu, 2004). Many medicinal plants, particularly those used in Ayurveda and Unani systems, are recognized for their antimicrobial properties and therapeutic applications. Their leaves serve multiple purposes, including antibacterial, anti-helminthic, astringent, emetic, febrifuge, sedative, and stimulant effects. Natural products, whether as isolated compounds or standardized plant extracts, present considerable promise for the development of novel pharmaceuticals. Antibiotics, in particular, remain the primary treatment for bacterial infections (Tambekar and Khante, 2010). Plant-based medicines, widely used around the world for preventing and treating infections, contain various secondary metabolites such as tannins, alkaloids, terpenoids, and flavonoids. These compounds have demonstrated antimicrobial activity in laboratory studies and may provide an alternative, effective, affordable, and safe solution for combating microbial infections (Cowan, 1999). *Asparagus racemosus*, commonly known as Shatavari, is a spiny under-shrub native to tropical and subtropical regions of India. It thrives at elevations of up to 1500 meters and is characterized by tuberous roots, which are used in traditional

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medicine. Shatavari holds significant importance in Ayurveda, often referred to as the "Queen of Herbs" due to its rejuvenating effects. The plant contains steroidal saponins and saponinins, which contribute to its therapeutic properties. Shatavari is recognized for a variety of health benefits, including antispasmodic, anti-inflammatory, anti-diabetic, antioxidant, immune-boosting, and hepatoprotective properties, among others. It is commonly used to promote vitality and support overall health. The present study explores the morphological, phytochemical, and antibacterial characteristics of *Asparagus racemosus*, with a focus on how different soil types and climatic conditions in Agra influence its immune-enhancing properties.

MATERIAL AND METHODS

The current study was carried out at the Botanical Garden of R.B.S. College in Agra to examine the impact of different soil conditions

1. **Ordinary Soil:** Garden soil was collected from a 25 cm thick layer of a cultivated field, then finely sieved before use.
2. **Farm Yard Manure (F.Y.M.):** F.Y.M. was combined with the cultivated field soil in a 1:1 ratio, and this mixture was used to fill the pots.
3. **Vermicompost:** Freshly produced vermicompost was sourced from a local supplier and was mixed in small quantities with garden soil.

For the experiment, *Asparagus racemosus* tubers were planted in earthen containers. The pots, freshly taken from the water tank and allowed to dry, were filled with the potting mixture. Each container was precisely filled with 4-4.50 kg of the mixture, leaving about 3-4.00 cm of space at the top to accommodate water application.

Morphological Studies: Ten plants were selected and tagged for a year-long morphological study, during which data on plant, leaf, flower, fruit, and seed morphology was collected.

Plant Collection and Extract Preparation: Tubers of *Asparagus racemosus* were collected from the Earthen Pots Botanical Garden at R.B.S. College, Agra, U.P. After washing the tubers under running tap water and drying them on paper towels, the aerial parts were blended. The mixture was then extracted with methanol by macerating at room temperature (30°C) for 72 hours. The macerated product was filtered under vacuum, and the resulting filtrate was evaporated under reduced pressure to calculate the percentage yield of the root extract.

Phytochemical studies: The powdered root was analyzed to determine the qualitative presence of the following key phytochemicals.

Test for Alkaloid

Mayer's Test: To test for the presence of alkaloids, 2 ml of plant extract was mixed with 5 ml of 1% aqueous HCl. Then, 100 µl of freshly prepared Mayer's reagent was added. The formation of a buff-colored precipitate confirms the presence of alkaloids (Sofowara, 1993).

Test for Glycosides: Legal Test: A small quantity of pyridine was introduced to the sample in a test tube, followed by a few drops of alkaline sodium nitroprusside solution. The appearance of a blood-red color indicates the presence of glycosides.

Test for Steroids: Salkowski Test: The test involves mixing 0.5-1 ml of the test solution with chloroform in a test tube. After adding a few drops of concentrated sulfuric acid and shaking, the appearance of a red color in the lower layer indicates the presence of steroids.

Test for Flavonoids: 2 ml of 10% sodium hydroxide was added to 2 ml of the filtrate, resulting in a yellow color that turned colorless upon

the addition of dilute hydrochloric acid, indicating the presence of flavonoids (Trease and Evans, 1998).

Test for Phenols: A few drops of a 10% ferric chloride solution were added to 2 mL of the extract. The development of a bluish-green or black coloration confirms the presence of phenolic compounds (Sofawara 1993).

Test for Saponins: The experiment involved mixing 2 ml of plant extract with 2 ml of distilled water in a test tube and shaking it vigorously. The formation of a frothy foam suggests the presence of saponins in the plant extract (Sofawara, 1993).

Tannins: FeCl₃ Test: When 2 mL of plant extract is mixed with a few drops of 0.1% FeCl₃ (ferric chloride) solution, the appearance of a blue-green or blackish-green color or precipitate suggests the presence of tannins in the plant extract. This is a positive test for tannins, as they react with FeCl₃ to form these characteristic colours. (Trease and Evans, 1998).

Anti-Bacterial studies: The antibacterial properties of the samples were evaluated using the agar well-diffusion technique, as outlined by (Perez, et al., 1990). Mueller-Hinton Agar (MHA, Hi Media, India, no. 2) was employed as the bacterial growth medium. Extracts were prepared in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml. A standardized bacterial inoculum, diluted in sterile 0.9% saline, was used to inoculate the agar plates. Wells, 6 mm in diameter, were created on the MHA plates, and 40 µl of each extract at varying concentrations was introduced into the wells. The plates were then incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zones surrounding each well. Ciprofloxacin (40 µl) served as a control antibiotic. The experiment was performed in triplicate to minimize experimental errors, and the mean values were calculated. The four human pathogenic bacteria used in the assay were *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The antibacterial activities of methanol extracts (40 µl) derived from the roots of *Asparagus racemosus*, cultivated under various soil conditions, were tested.

RESULTS AND DISCUSSION

Morphological Observations of *Asparagus racemosus* in Various Soil Conditions

Plant Morphology: *Asparagus racemosus* is a small to medium-sized, erect annual herb. The plant has a branched stem, The stems is straight, smooth, and shiny yellow with the branches being that reaches a height upto 90-100 cm. in ordinary soil 110-115cm in farm yard manure. whereas those grown in vermicompost can attain up to 120cm.

Leaf Morphology: The leaves are simple, small reduced to minute needle like scale. The leaf size is fairly consistent across different soil conditions.

Flower Morphology: *Asparagus racemosus* produces small, white flowers on short, spiky stems. Table 1 indicates that the largest flower size, measuring 0.40 cm, was observed in plants grown in vermicompost. Similarly, plants grown in farmyard manure also exhibited flowers of 0.40 cm, while those cultivated in ordinary soil showed no significant difference in flower size. The length and width of the tepals remained consistent across all soil conditions. The flowers possess a perianth consisting of six obovate tepals, ranging from 2.5 to 3.00 mm in length and 1 mm in width. The androecium consists of six stamens with white filaments and dark brown anthers. The style is short, approximately 0.5 mm long, with three recurved stigmas. Both the stigma and the ovary are well-developed.

Fruit Morphology: Fruiting primarily occurs between September and October. The fruits are small, round, and measure 0.5–0.7 cm in

diameter. They range in colour from light orange to dark orange, with a glossy outer skin. In the early stages, the fruits are globular or faintly three-lobed, green in color, and become red upon maturing. They are typically 8–10 mm in diameter, sometimes reaching up to 13 mm, and generally contain one seed, though occasionally there may be two to three seeds.

Seed Morphology: The seeds are small and black in color, with each fruit containing one or two seeds. The seeds have a dry texture and, in September, as they ripen, they turn purplish-black. When fully mature, the seeds become hard and brittle.

Root Morphology: The study on root morphology in *Asparagus racemosus* plants examined root length, width, and color under different soil conditions. The longest roots (12.00 cm) were found in plants grown in vermicompost, followed by those in farmyard manure (10.00 cm) and ordinary soil (11.00 cm). The roots are fleshy, fibrous, cylindrical, and white, with a tuberous, radish-like shape, tapering at the ends. They typically grow in clusters, with 10-12 roots per plant across all soil conditions. The morphological characteristics of the *Asparagus racemosus* plant, including its unique stem structure, flowers, and fruit, provide important clues for botanists and medicinal researchers in identifying and understanding its medicinal properties. The consistent findings from various studies over time, including those from Hooker (1894); (Polunin and Stainton, 1984), and others, confirm the plant's morphological features and its relevance in different regions. The clustering of roots and the presence of needle-like cladodes offer valuable insights into its growth patterns and ecological adaptations. The fruit is a small berry (Ellison, 1986). In addition, *Asparagus* plants have tuberous roots that grow in clusters at the base of the stem (Chen, et. al. 2000) noted that these tuberous roots are clustered at the stem's base. Other studies on the morphology of *Asparagus* species, including those by (Irshad et al. 2019) and (Chen, et. al. 2020), have confirmed these findings, providing additional support for the observations in our study. The fruit is a small berry (Ellison, 1986). In addition, *Asparagus* plants have tuberous roots that grow in clusters at the base of the stem (Chen et al. 2000) noted that these tuberous roots are clustered at the stem's base. Other studies on the morphology of *Asparagus* species, including those by (Irshad et al. 2019) and (Chen et al. 2020), have confirmed these findings, providing additional support for the observations in our study.

Phytochemical Analysis: Table 2 presents the findings from the phytochemical analysis of methanol extracts from the tubers of *Asparagus racemosus*, cultivated under different soil conditions. The results reveal that these methanol extracts are particularly rich in a variety of phytochemical compounds. Notably, the extracts consistently contain high levels of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, and tannins across all soil types. Various researchers have consistently reported similar findings regarding *Asparagus racemosus*. These findings show slight similarities from previous work of (Wani et al., 2011). The root extract of *Asparagus racemosus* was evaluated for its phytochemical components to identify the presence of alkaloids, flavonoids, tannins, phytosterols, and glycosides. The ethanolic root extract of *A. racemosus* was found to contain alkaloids (Roy et al., 1971; Dalvi et al., 1990), flavonoids, tannins, phytosterols, glycosides, carbohydrates, proteins, and fats. Additionally, trace elements such as copper, zinc, manganese, cobalt, potassium, calcium, and selenium were detected (Mohanta et al., 2003). The fruits and flowers of *Asparagus racemosus* also contain flavonoids, specifically glycosides of quercetin, rutin, and hyperosides (Kamat et al., 2000).

Antimicrobial Studies: The antibacterial properties of methanol extracts (40 µl) from the roots of *Asparagus racemosus*, cultivated under different soil conditions, were evaluated against four human pathogenic bacteria: *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The findings are presented in Table 3. Table 3 clearly indicates that the strongest antibacterial activity was observed against *Pseudomonas aeruginosa*, with the largest zone of inhibition measuring 18.00 mm when plant roots were cultivated in vermicompost. In contrast, the weakest activity was observed against *Escherichia coli*, with a maximum inhibition zone of 12.00 mm in roots grown in ordinary soil. The disc diffusion method revealed that the root extract possesses notable antibacterial properties. For comparison, Ciprofloxacin (40 µl) was utilized as a control antibiotic. This study evaluated the antimicrobial properties of *Asparagus racemosus* root extracts, finding that methanolic extracts exhibit significant antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. These results support the traditional use of the plant in treating microbial infections and highlight its potential as a source of antimicrobial agents.

Table 1. Morphological characters of *Asparagus racemosus* in various soil conditions

Characters		Soil conditions		
		Ordinary Soil	Farm yard Manure	Vermicompos
1.	Plant height	90-100 cm	100-105 cm	105-110 cm
2.	Flower (Length)	0.40 cm	0.40 cm	0.40 cm
3.	Fruit(Diameter)	0.5-0.6 cm	0.5-0.6 cm	0.5-0.6 cm
4.	Number of Seed/Fruit	2-3	2-3	2-3
5.	Root	10.00cm	11.00cm	12.00cm

Table 2. Phytochemical studies of root extract of *Asparagus racemosus* in various soil conditions

S. No.	Soil conditions	Ordinary Soil	Farm yard Manure	Vermicompost
1.	Phytochemicals			
	Alkaloids	+	+	+
2.	Glycoceroids	+	+	+
3.	Flavonoid	+		
4.	Phenols	+	+	+
5.	Saponin	+	+	+
6.	Steroids			
7.	Tanins	+	+	+

Table 3. Antibacterial activity of methanol Root extract of *Asparagus racemosus* against some human pathogenic Bacteria in various soil conditions

Sample		Inhibition zone (mm)				
	Name of Bacteria	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>	Standard
	Concentration	40 µl	40 µl	40 µl	40 µl	(40 µl)
1	Ordinary Soil	12.00	14.50	14.00	15.00	37
2	Farm yard	12.00	14.00	15.00	15.50	37
3	Vermicompost	15.00	18.00	17.00	17.00	37

The study found that methanolic root extracts of *Asparagus racemosus* exhibited significant antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. This supports the potential of the plant as a herbal medicine for treating infections caused by these bacteria, as compared to synthetic antibiotics. The results align with (Sanker et al., 2011), who noted that ethanolic extracts of *A. racemosus* contain bioactive secondary metabolites, including flavonoids, particularly in acetone extracts. Medicinal plant extracts provide natural, safe, and cost-effective alternatives to synthetic antimicrobials. They are widely accessible, easy to use, and offer significant therapeutic benefits, making them an affordable treatment option (Ghosh et al., 2008 and Pandey and Kumar 2012). Also, Medicinal plant extracts could serve as a valuable alternative therapy for addressing various side effects and overcoming drug resistance (Pandey and Tripathi 2014; Ingle et al 2018).

CONCLUSION

The study explored the cultivation of *Asparagus racemosus* under varying soil conditions, focusing on morphological studies with its root development when treated with farmyard manure and vermicompost. Phytochemical analysis of methanol extracts from the roots identified several bioactive compounds, including alkaloids, glycosides, flavonoids, phenols, saponins, steroids, and tannins. Additionally, the root extracts demonstrated antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. The results indicate that methanol is an efficient solvent for extracting these bioactive compounds, which could potentially contribute to the advancement of traditional medicine and offer a promising alternative in addressing bacterial infections and combating drug resistance.

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