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Research Article

# PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *ASPARAGUS RACEMOSUS* LEAF EXTRACT ON FOOD BORNE ENTEROBACTERIACEAE

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## ABSTRACT

Enterobacteria are often responsible for various gastrointestinal foods borne infection in humans. But due to profuse use of commercial antibiotic and synthetic pesticides for human and crop protection growing incidences of drug-resistant pathogens of both clinical and agricultural importance have increased. This has aggravated the search for antimicrobials from plants sources. Medicinal plants have intrinsic ability to resist pathogenic microorganisms and this has led the researchers to investigate and explore them for the treatment of food borne infections of humans by developing new antimicrobial agents. The aim of this study was to evaluate the antimicrobial properties of hydroalcoholic leaf extracts of *Asparagus racemosus* against foodborne bacterial isolates isolated from fresh raw vegetables. Primary qualitative analysis showed presence of different phytochemicals in the leaf extracts. The leaf extracts were screened *in vitro* in the laboratory for their antibacterial activity against the isolated enteric bacteria, and the results showed that hydroalcoholic extracts of *Asparagus racemosus* can be optimized clinically for chemotherapeutic control of these food-borne enteric infections. In conclusion, *Asparagus racemosus* extract exhibits antimicrobial activities and thus might be developed as natural sanitizer for washing raw vegetables.

**Keywords:** Foodborne pathogens, Enterobacteria, Phytochemicals, Antimicrobial activity.

## 1. INTRODUCTION

Food-borne illness resulting from consumption of food contaminated with pathogenic bacteria has been of vital concern to public health. While food borne diseases remain an important public health threat worldwide, one of the most important food safety hazard is associated with raw and ready to eat food products and are supposed to be responsible for significant amount of human illness because of the relatively high frequency of contamination with pathogens. Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources. Antimicrobial agents are mostly synthetic chemicals and some agents are limited to use since they may cause adverse effects on public health and reluctance by consumers. Therefore to reduce the health hazards and economic losses due to food-borne microorganisms, the use of natural products as antimicrobial compounds seem to be an interesting way to control the presence of pathogen in food. Traditionally, the crude extracts of different parts of medical plants, including root, stem, flower, fruit, and twigs, were widely used for treatments of some human diseases [1]. Medicinal plants contain several phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids, which possess antimicrobial and antioxidant properties [2]. *Asparagus*

*racemosus* (Family Asparagaceae; Liliaceae), is commonly called as Shatavari. Literature survey studies claimed some therapeutic attributes for the leaves of *A. racemosus* and has been used in treatment of boils, as antiseptic [3] and pesticide [4]. *A. racemosus* leaves are also used for various traditional uses in India like tonic [4], heel cracks [5], scabies [6], stomachache [7], urinary disorders [7] and high blood pressure [8], as galactogogue and aphrodisiac [9]. Based on the traditional uses of *A. racemosus*, the present study was undertaken to evaluate antimicrobial effect of hydroalcoholic leaf extracts of *Asparagus racemosus* against foodborne pathogens isolated from fresh raw vegetables.

## 2. MATERIAL AND METHODS

### 2.1. Collection of Plant Material

*Asparagus racemosus* plants were purchased from Vindhya Herbals (MFPPARC) Bhopal, India. Dirt was removed from the plant parts by rinsing in clean water. The leaves were shade dried at room temperature. All dried material was reduced into fine powder followed by defatting with petroleum ether.

### 2.2. Preparation of Plant Extracts by Soxhlet Apparatus

The dried powder of leaves was successively extracted with hydroalcoholic mixture of pure ethanol and water (80:20) in soxhlet apparatus. [3] The extracts obtained were concentrated, then percentage yield of each extract and organoleptic properties were evaluated.

### 2.3. Qualitative analysis of Phyto-chemicals

The extracts of different plants were analysed for the presence of alkaloids, flavonoids, tannins, glycosides, terpenoids and saponins according to standard methods [4].

### 2.4. TLC analysis

The hydroalcoholic extract was subjected to TLC analysis with specific reference to standard flavonoid quercetin to mark the presence of quercetin like flavonoids. Thin layer silica gel 60G F254 DC Kieselgel (5×7 cm) plate was used as stationary phase. The spots were made with capillary tube, and Toluene: Ethyl acetate: Formic acid (ratio= 5:4:0.2) was used as solvent system. The spots were observed under UV light and Rf values of the extract were calculated [5].

Rf Value = Distance travelled by the sample/ Distance travelled by the solvent

### 2.5. Test microorganisms

The test microorganisms used for the antimicrobial activity screening are *Serratia entomophila, Proteus vulgaris, Pragia fontium, Enterobacter aerogenes*. These food borne microorganisms were isolated from the fresh raw vegetables in my previous studies and the identity of each organism was confirmed by using standard culture, morphological and biochemical techniques.

### 2.6. Antimicrobial activity

To carry out antibacterial activity of leaves extract, agar well diffusion method was performed [6]. The gentamycin (25µg/ml) were taken as positive control. The experiment was carried out in triplicates and average ZOI (zone of inhibition) was recorded.

## 3. RESULTS AND DISCUSSION

On the basis of extraction capacity of solvents, compounds from powdered form of leaves of *A. racemosus* were extracted in the hydroalcoholic mixture of pure distilled water and ethanol (20:80). The amount of extracts (% yield) and the organoleptic properties of extracts are mentioned in Table 1.

**Table 1: Organoleptic properties of Extract**

| Samples Used | % Yield | Organoleptic Properties | | |
|---|---|---|---|---|
| | | Colour | Texture | Smell |
| A. *Racemosus* leaves extract | 14.2% | Dark Brown to Black | Hard Crystalline appearance | Organic, Aromatic Pleasant |

The results of the preliminary phytochemical screening of hydroalcoholic extracts of *A. racemosus* are presented in Table 2. The preliminary phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, tannins, glycosides, terpenoids and saponins. The results of the thin layer chromatography (TLC) of hydroalcoholic extracts of *A. racemosus* are presented in Table 3. The plates when placed under UV light showed fluorescent spots. $R_f$ values of the spots were as 0.2, 0.35, 0.65, 0.77 and 0.98. The results of TLC revealed the presence of flavonoids in the extracts (Fig .2). Antibacterial activity was tested by agar well diffusion method and the findings have been indicated in Table 4. The solvent extracts of various concentrations showed

good activity against the test bacteria ranging from 3-15 mm. The maximum zone of inhibition was 15 mm for *Proteus vulgaries*, 13mm for *Enterobacter aerogenes*, 12 mm for *Serratia entomophila* and 11 mm for *Pragia fontium*. The broad-spectrum antibacterial activity exhibited by the extract could be related with the concentrations of alkaloids, flavonoids, tannins, glycol-sides, terpenoids and saponins in these extracts. These classes of compounds are known to show curative activity against several pathogens and may explain some of its antimicrobial actions since antimicrobial actions of most of these phytochemical substances have been documented [7-8]. The literature survey of plant reported the presence of flavonoids [9-11] and preliminary phytochemical screening as well as thin layer chromatography of the extracts revealed that the leaves of *A. racemosus* contain flavonoids. Several flavonoids isolated from the medicinal plants have been discovered to possess significant antimicrobial activity.

**Table 2: Preliminary phytochemical analysis of Extract**

| S. No. | Tests Conducted | Results |
|---|---|---|
| **1.** | Alkaloid | + |
| **2.** | Flavonoid | + |
| **3.** | Tannins | – |
| **4.** | Glycoside | – |
| **5.** | Terpenoid | + |
| **6.** | Saponins | + |

**Table 3: TLC analysis of *A. racemosus* leaf extract**

| Extract | No. of spots | Distance travelled by Solvent (cm) | Distance travelled by solute (cm) | Rf Values |
|---|---|---|---|---|
| Quercetin (standard) | 1 | 6 | 2.2 | 0.367 |
| Hydroalcoholic Extracts | 2a | 6 | 1.2 | 0.2 |
| | 2b | 6 | 2.1 | 0.35 |
| | 2c | 6 | 3.9 | 0.65 |
| | 2d | 6 | 4.6 | 0.77 |
| | 2e | 6 | 5.9 | 0.99 |

**Table 4: Susceptibility study of *Asparagus racemosus* leaf extracts against test microorganisms**

| Extract/ Standards | Conc | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|
| | (µg/ml) | *S. entomophila* | *P. vulgaris* | *P. fontium* | *E. aerogenes* |
| Hydroalcoholic Leaves extract of *Asparagus racemosus* | 5 | 5 | 6 | 3 | 5 |
| | 10 | 8 | 11 | 7 | 8 |
| | 15 | 9 | 12 | 8 | 10 |
| | 20 | 12 | 15 | 11 | 13 |
| Gentamycin | 25 | 18 | 14 | 22 | 21 |

## 4. CONCLUSION

The plant extractive studied could be an answer to the people seeking for better therapeutic agents from natural sources which is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. In present study, hydroalcoholic extract leaves of *Asparagus racemosus showed* significant inhibitory action against all the tested food borne bacteria, it is concluded that hydroalcoholic extract of the *Asparagus racemosus*, can be effectively used for curing the foodborne diseases. Hence this study confirmed that the hydroalcoholic extract of leaves of *A. racemosus* exhibit antimicrobial activity and the effects observed are attributable due to the presence of flavonoids and should be screened further for active constituents

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