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Research Article

Investigation of Phytoconstituents, TLC Profile and Antimicrobial Activity of Methanol Extract of *Asparagus racemosus* willd. Roots

*Rajeshwar Y, Sreekanth T, Shyamsunder A, Tejaswi Divya B

Department of Pharmaceutical Chemistry, S. R. College of Pharmacy, Ananthasagar, Hasanparthy, Warangal - 506 371, Andhra Pradesh, India.

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ABSTRACT

The present study is aimed at the development of the phytoconstituents and to investigate the medicinally active substances present in methanol extract of *Asparagus racemosus* (MEAR) Willd. roots. The roots of the plant material was successively extracted with petroleum ether, benzene, chloroform, ethyl acetate and methanol. Preliminary phytochemical screening of the extracts revealed the presence of steroids/triterpenoids, saponins, alkaloids, tannins, carbohydrates, flavonoids, lactones, phenolic compounds and mucilages. Thin Layer Chromatography (TLC) profile was performed for MEAR in order to identify the bioactive compounds. In the present study, the most suitable TLC system for analysis was shown to be chloroform:methanol with the largest discriminating power. Derivatization of TLC plates was done by UV light at 254nm. Different bands were observed and corresponding R_f values were determined. R_f values of each spot was calculated as R_f = Distance travelled by the solute (in cm)/Distance travelled by the solvent front (in cm).

The effect of MEAR roots was evaluated for antimicrobial activity against various gram positive and gram negative organisms. It was found that MEAR at a concentration 200µg/ml exhibited significant antimicrobial activity against all the tested microorganisms. The extract showed strong antibacterial activity against *Staphylococcus aureus*, *Staphylococcus pneumonia*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella dysenteria*, and *Vibrio cholera*. However, their activity against *Micrococcus luteus*, *Pseudomonas auruginosa*, and *Solmonella typhi* was found to be significantly less. The antimicrobial activity of MEAR was compared with the standard Chloramphenicol at a concentration 10µg/ml.

Key words: *Asparagus racemosus*, methanol extract, phytoconstituents, TLC, and antimicrobial activity.

INTRODUCTION

Medicinal plants are the nature's gift to human being to make disease free healthy life. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because, better compatibility with the human body and fewer side effects[1]. In India, thousands of species are known to have medicinal values and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times[2]. From over 3,00,000 species of higher plants to occur in nature, only about 2% have been screening so for. Extract of plants from 157 families have been reported to be active against microorganisms[3-4].

Asparagus racemosus Willd. is belonging to both Liliaceae and Asparagaceae plant families[5]. This is a woody climbing plant growing to 1-2m in height that grows in low forest areas throughout India[6]. The roots are cylindrical, fleshy and tuberous. The roots are 30-100cm in length, 1-2cm in thickness and creamy-yellow in colour. The roots contain long needle shaped structure known as pith which is meant for the conduction of water[7].

Traditionally, the plant has been used for its phytoestrogenic properties. It has been considered to be a lactogogue in lactational inadequacy[8] and useful to decrease post-operative adhesions and it also have anticandidal activity[9]. The other common uses of the plant are for the treatment of diarrhea, dysentery, rheumatism, nervous breakdown, and is thought to be an aphrodisiac[10]. From the traditional usage of the plant, it is very clear that the plant roots of AR have the antimicrobial activity. Therefore, the present investigation has been designed to study the possible mechanism of MEAR roots on the different microorganisms. The isolation of individual phytoconstituents responsible for the antimicrobial property and other possible biological activities are under progress in our laboratory.

MATERIALS AND METHODS

Plant Material: The roots of *Asparagus racemosus* were collected in the month of July, 2013 from Warangal District of Andhra Pradesh State, India. The plant was botanically identified and authenticated by the Head, Department of Botony,

Kakatiya University, Warangal (AP), India. A specimen was deposited in the Department of Pharmaceutical Chemistry, S. R. College of Pharmacy, Warangal (AP), India for future reference.

The collected roots were cleaned with water and dried

*Author for correspondence: Email: yerrarajeshwar@gmail.com

Table 1: % yield of the *Asparagus racemosus* roots

Sl. No.	Extract	% yield
1	Petroleum ether	1.61
2	Benzene	1.24
3	Chloroform	1.73
4	Ethyl acetate	2.10
5	Methanol	9.30
6	Water	9.44

Table 2: Phytoconstituents present in different extracts of *Asparagus racemosus* roots

Sl. No.	Phytoconstituents	Petroleum ether extract	Benzene extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1	Carbohydrates	-ve	-ve	-ve	+ve	+ve	+ve
2	Amino acids and proteins	-ve	-ve	-ve	-ve	-ve	-ve
3	Steroids	+ve	+ve	+ve	+ve	+ve	-ve
4	Alkaloids	-ve	-ve	+ve	-ve	+ve	-ve
5	Flavonoids	-ve	-ve	-ve	-ve	+ve	-ve
6	Tannins and phenolics	-ve	-ve	+ve	ve	+ve	+ve
7	Saponins	-ve	-ve	-ve	+ve	+ve	+ve
8	Triterpenoids	+ve	+ve	+ve	+ve	+ve	-ve
9	Glycosides	-ve	-ve	-ve	-ve	-ve	-ve
10	Mucilages	+ve	-ve	-ve	-ve	+ve	+ve
11	Lactones	+ve	-ve	+ve	-ve	+ve	-ve

(+ve) - indicates the presence of phytoconstituent; (-ve) - indicates the absence of phytoconstituent.

Table 3: Effect of MEAR on antibacterial activity against various gram positive and gram negative bacteria.

Microorganism	Diameter of zone of inhibition (in mm) MEAR (50µg/ml/disc)	MEAR (100µg/ml/disc)	MEAR (200µg/ml/disc)	Chloramphenicol (10µg/ml/disc)
Gram positive				
Staphylococcus aureus	9	14	21	26
Staphylococcus pneumonia	8	15	22	27
Bacillus subtilis	8	14	19	24
Bacillus sphericus	6	11	17	25
Micrococcus luteus	7	10	14	26
Staphyloccus epidermidis	7	12	19	26
Gram negative				
Escherichia coli	9	16	23	25
Pseudomonas aeruginosa	8	11	16	24
Klebsiella pneumonia	8	13	19	23
Solmonella typhi	7	13	17	26
Shigella dysenteriae	8	14	20	25
Vibrio cholera	9	12	19	25

All the values were the average of three experiments. The values given are the diameter of zone of inhibition (in mm) including disk diameter of 6mm. 6≤10 – poor activity; 11-17 – moderate activity ≥18 – strong activity.

under shade for about 15 days. The dried pieces of roots were cut into small pieces and made powder with a mechanical grinder. The powdered plant material was used for the extraction, phytochemical analysis, and antimicrobial activity.

Preparation of extract and fractions: The powdered plant material (roots) was extracted with methanol in a stainless steel extraction tank for approximately 4 days at room temperature by changing methanol daily. The combined extract was filtered and evaporated to dryness under reduced pressure to yield dry crude extract. The dry sample was stored in a vacuum desiccator for further use. The methanol extract was subsequently partitioned into petroleum ether, benzene, chloroform, and ethyl acetate. They were evaporated to dryness to get petroleum ether, benzene, chloroform, and ethyl acetate-soluble materials (crude extracts). These crude extracts were used for phytochemical and for biological screening.

Phytochemical analysis: The petroleum ether, benzene, chloroform, ethyl acetate and methanol extracts were subjected to different qualitative tests for the detection of phytoconstituents[11-14].

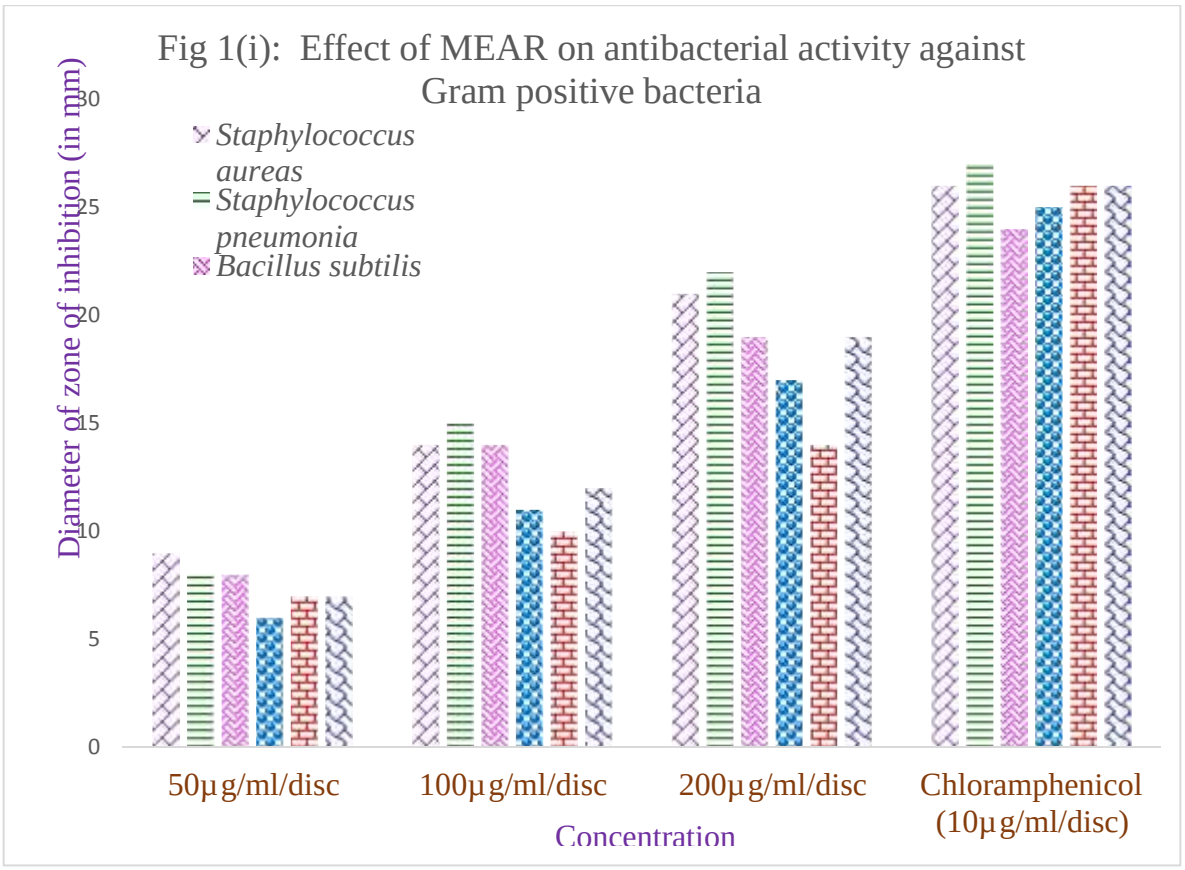
Fig 1(i): Effect of MEAR on antibacterial activity against Gram positive bacteria
Diameter of zone of inhibition (in mm)
0
5
10
15
20
25
30
Staphylococcus aureas
Staphylococcus pneumonia
Bacillus subtilis
50µg/ml/disc
100µg/ml/disc
200µg/ml/disc
Chloramphenicol (10µg/ml/disc)
Concentration

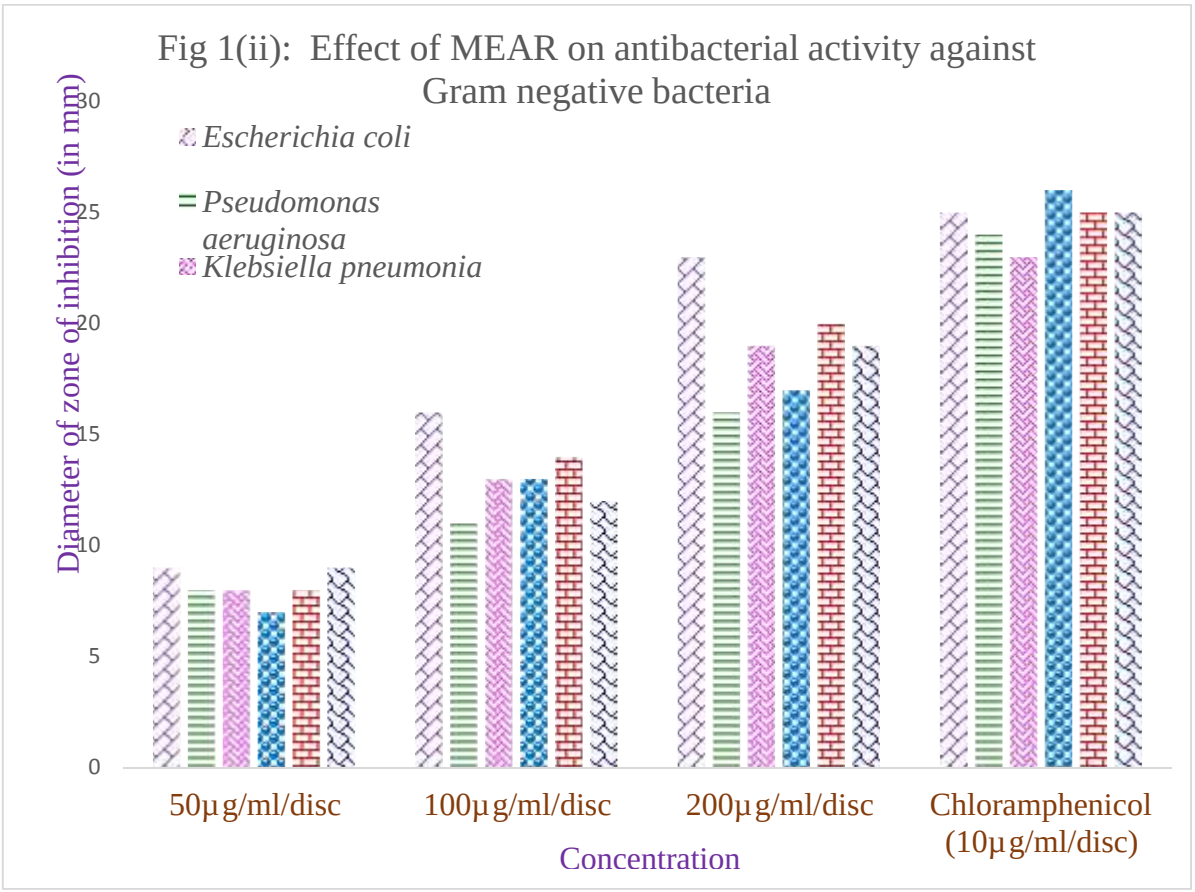
Fig 1(ii): Effect of MEAR on antibacterial activity against Gram negative bacteria
Diameter of zone of inhibition (in mm)
0
5
10
15
20
25
30
Escherichia coli
Pseudomonas aeruginosa
Klebsiella pneumonia
50µg/ml/disc
100µg/ml/disc
200µg/ml/disc
Chloramphenicol (10µg/ml/disc)
Concentration

TLC profile: TLC is one of several techniques useful for the identification of phytochemical compounds[15-16].

In this study, the TLC was performed on precoated 20x20cm and 0.25mm thick plates. The plates were prepared by using silica gel-G for TLC, were left overnight for air drying. These plates were activated by hot air oven at 100ºC for 1 hour. Cold alcoholic extract (MEAR) was plotted on TLC plates[17]. The plates were dried and developed in suitable solvents for rapid screening chloroform/methanol in the ratio 3:7. The plates were run in the above solvent systems and dried at room temperature. Derivatisation of TLC plates was done by UV light at 254nm. Different bands were observed and corresponding R_f values are determined. R_f value of each spot was calculated as –

R_f = Distance travelled by the solute (in cm)/Distance travelled by the solvent front (in cm).

The plates were visualized under white and UV light for the identification of the spots/bands. This experiment was performed in triplicate.

Antimicrobial Activity

Preparation of test microorganisms: Bacteria were obtained from the stock cultures of the Department of Microbiology, Kakatiya University, Warangal, Andhra Pradesh, India. The bacterial stock cultures were maintained on Muller Hinton Agar slants, respectively, which were stored at 4ºC. For the purpose of antimicrobial investigation, twelve different microorganisms of gram positive and gram negative were used.

Determination of Antimicrobial activity: Agar cultures of the test microorganisms were prepared as described by Mackeen et al., 1997[18]. Three to five similar colonies were selected and transferred with loop into 5ml of Tryptone soya broth. Tryptone soya broth is a highly nutritious versatile medium, which is recommended for general laboratory use and used for the cultivation of aerobes and facultative anaerobes, including some fungi. The broth cultures were incubated for 24 h at 37ºC. For screening, sterile, 6mm diameter filter paper disc were impregnated with 50-200µg/ml of the MEAR. They were dissolved in sterile water for the assay by magnetic stirrer. Then the paper discs placed onto Mueller Hinton agar. The inoculum for each organism was prepared from broth cultures. The concentration of cultures was to 10^8 colony forming units ($1x10^8$ cfu/ml). The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate the presence of antimicrobial activity. All data on microbial activity are the average of triplicate analyses. Chloramphenicol (10µg/ml/disc) was used as reference standard.

STATISTICAL ANALYSIS

The experimental results were performed in triplicate and each data point in the results is the average of three replicate tests.

RESULTS AND DISCUSSION

Percentage yield: The yield of the petroleum ether, benzene, chloroform, ethyl acetate, methanol and water extracts of the roots of *Asparagus racemosus* was found to be 1.61, 1.24, 1.73, 2.10, 9.30, and 9.44, respectively (Table 1).

Qualitative phytochemical analysis: The petroleum ether, benzene, chloroform, ethyl acetate and methanol crude extracts of *Asparagus racemosus* roots were subjected to the qualitative phytochemical analysis for the presence of various phytoconstituents. The results are given in Table 2.

TLC of MEAR: Methanolic extract (MEAR) was subjected to TLC in order to identify the bioactive compounds. In the present study, the most suitable TLC system for analysis was shown to be chloroform:methanol with the largest discriminating power. Different bands were found with R_f values of 0.4, 0.45 and 0.48. Further studies are going on in our laboratory for the identification of the phytoconstituents.

Antimicrobial activity: Disc diffusion methods are extensively used to evaluate the antibacterial activity of natural products and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of substances to be examined. In the case of solutions with a low activity, however, a large concentration or volume is required. The limited capacity of discs means that holes or cylinders are preferably used[19]. Most of the bacterial species were inhibited antimicrobial activity as shown in Table 3, Fig 1(i) and (ii). In this investigation, twelve different microbial species were used to screen the possible antimicrobial activities of MEAR. The MEAR showed broad spectrum of activity against all the bacterial strains at the tested concentrations (50-200µg/disc). Chloramphenicol (10µg/disc) was used as positive control for bacteria.

The data shown in the Table 3 and Figures 1(i) and 1(ii), clearly indicate that MEAR inhibit the growth of some of the tested microorganisms (Gram positive and Gram negative) to various degrees. The MEAR at a concentration 200µg/ml exhibited significant antimicrobial effect against all the tested microorganisms. The extract showed strong antibacterial activity against *Staphylococcus aureus*, *Staphylococcus pneumonia*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella dysenteria*, and *Vibrio cholera*. However, their activity against *Micrococcus luteus*, *Pseudomonas auruginosa*, and *Solmonella typhi* was found to be significantly less. The antimicrobial activity of MEAR was compared with the standard Chloramphenicol at a concentration 10µg/ml.

Thus, the results of antimicrobial activity of MEAR roots supports the folk medicinal utilization of the plant. Further work is in progress to identify the possible mechanism of action and to identify the lead molecules responsible for the antimicrobial activity.

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