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Research Article

MIC VALUES OF INFLORESCENCE AND LEAVES EXTRACTS OF *ACHYRANTHES ASPERA* AGAINST USUAL PATHOGENIC BACTERIAL STRAINS

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ABSTRACT

Achyranthes aspera is an important medicinal herb that possesses many therapeutic properties. Present study was carried out to evaluate minimum inhibitory concentration values of inflorescence and leaves extracts of *A. aspera* against various pathogenic strains of bacteria i.e. *Escherichia coli*, *Bacillus cereus*, *Staphylococcus epidermis, Shigella flexineri* and *Pseudomonas aeruginosa*. Increasing the dilution from the stock solution i.e. 100 µg/ml, in order to 2 times i.e. 50 µg/ml, 4 times i.e. 25 µg/ml and 8 times i.e. 12.5 µg/ml, the concentration of active antimicrobial compound was found to be decreased. Decreased absorbance with varying concentrations of plant extracts indicated that minimum concentration of various plant extract is enough to inhibit the growth of bacteria. For both plant parts, all the extracts have MIC values ranges from 12.5-100 µg/ml for various pathogenic strains.

Keywords: *Achyranthes aspera*, Minimum inhibitory concentration (MIC), Medicinal herb, Pathogenic strains.

INTRODUCTION

Achyranthes aspera Linn. (Amaranthaceae family) is an annual, stiff erect herb, commonly known as Apamarg, found as a weed throughout India and an important medicinal plant used in various diseases like odontalogic, rheumatism, bronchitis, skin disease, rabies[1], fever, dysentery and diabetes. The entire plant of *A. aspera* showed antibacterial activity against *Staphylococcal aureus, Streptococcus heamolyticus* and *Bacillus typhosa*[2]. It is used as antiviral, anticoagulant, antihypertensive, diuretic, aphrodisiac, antifertility, antispasmodic and as an antitumor agent[3, 4, 5]. It is also used to treat children for 'colic' with 'hydrophobic' 'hypoglycemic' 'thyroid stimulating brain tonic'[6, 7, 8, 9], antifungal and antibacterial activity[10]. Various extracts of the leaves also shows antimicrobial activity[11]. It is reported that it contain alkaloids, flavonoids, saponins, steroids and terpenoids.

Keeping the above mentioned facts in mind, the present study was carried out to determine the minimum inhibitory concentration values of various sequential extracts i.e. pet ether, benzene, chloroform, ethyl acetate, ethanol and aqueous of leaves and inflorescence parts of *A. aspera* to inhibit the growth of various pathogenic strains of bacteria i.e. *Escherichia coli, Bacillus cereus, Staphylococcus epidermis, Shigella flexineri* and *Pseudomonas aeruginosa.*

MATERIAL AND METHODS

Procurement of Plant Material

Achyranthes aspera was collected from the roadsides of university campus, Banasthali University, Banasthali, District-Tonk, Rajasthan, India, in the month of January-2012. The plant material was taxonomically identified by Botanist of Krishi Vigyan Kendra, Banasthali.

Preparation of plant extracts by sequential extraction method

Leaves and inflorescence parts of the plant were separated, cleaned, dried and powdered with the help of mixer grinder separately. The powdered parts of plants were then extracted with Soxhlet using various sequential solvents that is pet ether, benzene, chloroform, ethyl acetate and ethanol for 16 h. Sequential aqueous extracts of various plant parts were also obtained by soaking plant part powder in double distilled water. The extracts were than concentrated on a rotary evaporator below 50˚C and were stored in air tight containers at 4˚C for further experimental studies.

Microbial strains

The bacterial species used for the test were *Escherichia coli* (MTCC NO. 119), *Bacillus cereus* (MTCC NO. 430), *Staphylococcus epidermis* (MTCC NO. 435), *Shigella flexineri* (MTCC NO. 1457) and *Pseudomonas aeruginosa* (MTCC NO. 1688) . All the strains were obtained from Microbial Type Culture Collection (IMTECH, Chandigarh, India).

Culture media and inoculum preparation

Nutrient broth (Sisco Research laboratory pvt. Ltd., Mumbai) were used as the media for bacterial culture. To 1 ml of mother culture respective bacterial strains were inoculated in nutrient broth in aseptic condition and then incubated at 37˚C for 24 h.

Procedure

Minimum inhibitory concentration (MIC) of different extracts of *A. aspera* was determined from the culture tubes that had the lowest concentrations and prevented the growth of bacterial strain. Firstly, prepared the stock solutions of different extracts at the concentration 0.1 mg/ml and then diluted it to 2 times, 4 times and 8 times to obtained various concentrations. The test containing 2 ml Nutrient agar media and 50 µl extract and 20 µl bacterial suspensions were incubated at 37˚C for 24 h. Bacterial density were then measured at 610 nm to determine minimum inhibitory concentration.

RESULTS

Increasing the dilution from the stock i.e. 100 µg/ml, in order to 2 times i.e. 50 µg/ml, 4 times i.e. 25 µg/ml and 8 times i.e. 12.5 µg/ml, the concentration of active antimicrobial compound was found to be decreased. It has been concluded that increasing the dilution of plant extract, O.D. or absorbance was increased along with turbidity due to microbial growth of bacteria. Decreased absorbance with varying concentrations of plant extracts also indicated that minimum concentration of plant extract is also efficient to inhibit the growth of various pathogenic bacteria. The minimum inhibitory concentration values of inflorescences and leaves extracts of *A. aspera* are given in Table 1 and Graph 1.

(a) Minimum inhibitory concentration values of various *A. aspera* inflorescence extracts for different bacterial population

The results depicted in Table 1 and Graph 1 showed that the minimum inhibitory concentration of pet ether extract was 12.5 µg/ ml for *E. coli, B. cereus* and *S. flexineri* but 50 µg/ ml for both *S. epidermis* and *P. aeruginosa*. Similarly, chloroform and benzene extracts showed same MIC values i.e. 100 µg/ ml for *E. coli* but different for *S. flexineri* which were 50 & 100 µg/ ml. Chloroform extract showed same MIC values for *B. cereus, S. epidermis* and *P. aeruginosa* i.e. 12.5 µg/ ml. The highest variation was showed by aqueous extract for different microbial population, means 25 µg/ ml for *E. coli*, 12.5 µg/ ml for both *B. cereus* and *P. aeruginosa* and then 100 µg/ ml for *S. epidermis* and *S. flexineri*. Ethanolic extract also

showed same MIC values i.e. 50 µg/ ml, for all except *E. coli* which showed 12.5 µg/ ml minimum inhibitory concentration. Similarly, Ethyl acetate showed 12.5 µg/ ml MIC values for *B. cereus*, *S. flexineri* and *S. epidermis* whereas 50 µg/ ml for *E. coli* and *P. aeruginosa*.

Table 1: Shows MIC values of different inflorescence extracts of *A. aspera* for various pathogenic bacterial strains.

A. aspera INFLORESCENCE EXTRACTS	MINIMUM INHIBITORY CONCENTRATION (µg/ml)				
	Name of microorganisms				
	E. coli	*B. cereus*	*S. epidermis*	*P. aeruginosa*	*S. flexineri*
Pet ether	12.5	12.5	50	50	12.5
Chloroform	100	12.5	12.5	12.5	50
Benzene	100	50	25	12.5	100
Ethyl acetate	50	12.5	12.5	50	12.5
Ethanol	12.5	50	50	50	50
Aqueous	25	12.5	100	12.5	100

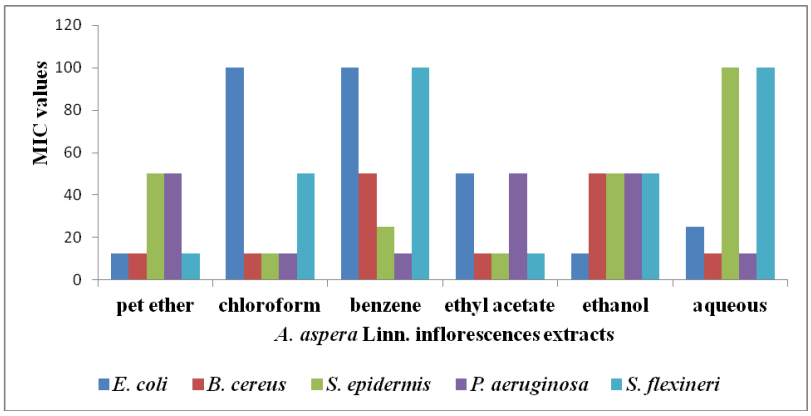

Fig 1: Shows MIC values of different inflorescence extracts of *A. aspera* against various pathogenic bacterial strains.

2. Minimum inhibitory concentration values of various *A. aspera* leaves extracts for different bacterial population

According to Table 2 and Graph 2, the minimum inhibition concentration of pet ether extract were 12.5 µg/ ml for *S. flexineri* and *E. coli,* 100 µg/ ml for both *B. cereus* and *P. aeruginosa* but 50 µg/ ml for *S. epidermis.* Chloroform, benzene, ethyl acetate, ethanol and aqueous showed same MIC values i.e. 12.5 µg/ ml for *B. cereus* whereas benzene, ethyl acetate, ethanol and aqueous showed same MIC values i.e. 12.5 µg/ ml for *P. aeruginosa*. Chloroform exhibited same minimum inhibitory concentration values for *P. aeruginosa, E. coli* and *S. flexineri* which was 50 µg/ ml. Chloroform, ethyl acetate, aqueous and ethanol showed 100 µg/ ml for *B. cereus* whereas ethyl acetate and ethanol showed 50 µg/ ml MIC value for *E. coli*. Ethanol and aqueous extracts showed 12.5 µg/ ml minimum inhibitory concentration for *S. flexineri*.

Table 2: Shows MIC values of different leaves extracts of *A. aspera* against various pathogenic bacterial strains.

A. aspera LEAVES EXTRACTS	MINIMUM INHIBITORY CONCENTRATION (µg/ML)				
	Name of microorganism				
	E. coli	*B. cereus*	*S. epidermis*	*P. aeruginosa*	*S. flexineri*
Pet ether	12.5	100	50	100	12.5
Chloroform	50	100	12.5	50	50
Benzene	12.5	50	12.5	12.5	100
Ethyl acetate	50	100	12.5	12.5	100
Ethanol	50	100	12.5	12.5	12.5
Aqueous	100	100	12.5	12.5	12.5

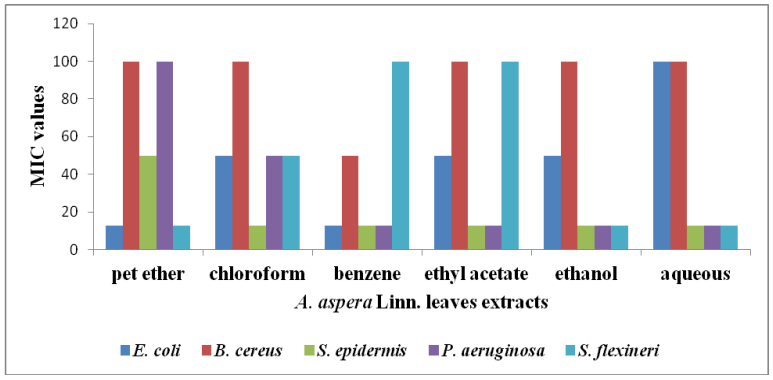

Fig 2: Shows MIC values of different leaves extracts of *A. aspera* against various pathogenic bacterial strains.

DISCUSSION

From the given results, it is suggested that the minimum inhibitory concentration values of pet ether, ethyl acetate and ethanolic inflorescence extracts for gram negative and gram positive bacteria was in the range of 12.5-50 µg/ml whereas chloroform, benzene and aqueous extracts have MIC values ranges from 12.5-100 µg/ml. In case of leaves extracts, all the extracts have MIC values ranges from

12.5-100 µg/ml for both gram positive and gram negative bacteria. The present work clearly indicates that chloroform and benzene extracts of inflorescences part of *A. aspera* extracts at higher concentration (100 µg/ml) effectively control the growth of *E. coli* (both extracts) and *S. flexineri* (only benzene extract) whereas aqueous extract control the growth of *S. epidermis* and *S. flexineri*. In case of leaves, all the extracts were effective at this concentration against respective pathogenic strains. Our results suggested that minimum concentration of *A. aspera* extracts inhibit the growth of various pathogenic strains of bacteria. This activity may be due to different chemical compounds present in extracts including flavonoids, triterpenoids, essential oils (esp. thymol) and natural phenolic compounds or free hydroxyl groups which are classified as active antimicrobial compounds[12]. It was reported that *Achyranthes aspera* possesses antimicrobial potential against several pathogenic microorganism[13, 14]. Our studies also proved these assumptions and showed that *A. aspera* possesses high antibacterial activity. Thus, from the present work, it is concluded that the various extracts of *A. aspera* may be used for the preparation of various pharmacological formulations. Further, the active compounds can be isolated that could be used for the treatment of various infectious diseases caused by bacterial pathogen.

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