MIC VALUES OF INFLORESCENCE AND LEAVES EXTRACTS OF ACYRANTHES 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 flexneri 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 Aparang, found as a weed throughout India and an important medicinal plant used in various diseases like odontologic, rheumatism, bronchitis, skin disease, rabies1, fever, dysentery and diabetes. The entire plant of A. aspera showed antibacterial activity against Staphylococcus aureus, Streptococcus haemolyticus and Bacillus typhosus2. It is used as antiviral, anticoagulant, antihypertensive, diuretic, aphrodisiac, antiinflammatory, antispasmodic and as an antitumor agent3.4.5. It is also used to treat children for ‘colic’ with ‘hydrophobic’, ‘hypoglycemic’, ‘thyroid stimulating brain tonic’4.5.7.8,9, antifungal and antibacterial activity10. Various extracts of the leaves also shows antimicrobial activity11,12. 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 flexneri 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 then 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 flexneri (MTCC NO. 1457) and Pseudomonas aeruginosa (MTCC NO. 1680). 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. flexneri 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. flexneri 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. flexneri. 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.

<table>
<thead>
<tr>
<th>A. aspera INFLORESCENCE EXTRACTS</th>
<th>MINIMUM INHIBITORY CONCENTRATION (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name of microorganisms</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>Pet ether</td>
<td>12.5</td>
</tr>
<tr>
<td>Chloroform</td>
<td>100</td>
</tr>
<tr>
<td>Benzene</td>
<td>100</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>50</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12.5</td>
</tr>
<tr>
<td>Aqueous</td>
<td>25</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>A. aspera LEAVES EXTRACTS</th>
<th>MINIMUM INHIBITORY CONCENTRATION (µg/ML)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name of microorganism</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>Pet ether</td>
<td>12.5</td>
</tr>
<tr>
<td>Chloroform</td>
<td>50</td>
</tr>
<tr>
<td>Benzene</td>
<td>12.5</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>50</td>
</tr>
<tr>
<td>Ethanol</td>
<td>50</td>
</tr>
<tr>
<td>Aqueous</td>
<td>100</td>
</tr>
</tbody>
</table>

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.
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. It was reported that Achyranthes aspera possesses antimicrobial potential against several pathogenic microorganisms. 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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REFERENCES