In vitro Evaluation of the Efficacy of Leaf and its Callus Extracts of Cardiospermum halicacabum L. On Important Human Pathogenic Bacteria

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Abstract: The investigation is aimed to carry out the antimicrobial activities of different solvent extracts of leaf and leaf derived callus extracts of Cardiospermum halicacabum L. (Sapindaceae). The leaf segments of C. halicacabum were cultured on MS medium supplemented with auxins and cytokinins alone. Maximum callus was recorded on medium containing BAP. The antibacterial properties of leaf and its callus extracts were screened against ten human pathogenic bacteria by cup diffusion method. Powdered leaf and leaf derived callus material was subjected to extraction of aqueous and with different organic solvents viz., petroleum ether, chloroform, methanol and ethanol using soxhlet apparatus. All the solvent extracts were evaporated to dryness using rotary flash evaporator. Dry residues was dissolved in methanol, and tested for antibacterial activity. Among all the solvents tested, significant inhibitory activity was observed in ethanol extract of both leaf and leaf derived callus followed by methanol. It also observed that the activity was more pronounced on Gram-positive bacteria than Gram-negative bacteria.

Key words: Antibacterial activity • Cardiospermum halicacabum • Callus

INTRODUCTION

Traditional herbalists in India use a variety of herbal preparations to treat different kinds of ailments including many microbial infections. The uneven distribution of health personnel in rural and urban areas, the rural dwellers are virtually left with no alternative other than to patronize the herbal practitioners. This has been the case even before the introduction of antibiotics and modern drugs. In most cases, the herbal practitioners are ignorant of the pharmacological and toxicological values of their medications [1]. It is a necessity from the scientific point of view, to establish a rational relationship between chemical composition, biological and therapeutic activities. The search for biologically active compounds from natural sources has always been of great interest to scientist looking for new sources from drugs useful in infectious diseases. In recent years a number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plant between chemical composition, biological and therapeutic activities.

Medicinal herbs are moving from fringe to mainstream use with greater number of people seeking remedies and health benefits free from side effects. Recently considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases including microbial infection [2]. Biological studies are very much essential to substantiate the therapeutic properties of medicinal plants and drugs mentioned in Ayurveda on scientific lines. The plant kingdom represents an extraordinary reservoir of novel molecules. The potential of higher plants, as source for new drug is thus still largely unexplored [3].

The use of plants, plant extract provides the foundation to modern therapeutic sciences and thus enabled the man to establish the empirical system of medicine. In view of the commercial importance given to
the secondary metabolites in recent times, efficient production of bioactive compounds by tissue culture technology has gained popularity [4].

Since the secondary metabolites often have a complex stereo structure with many chiral centers, which may be essential for biological activity, many of these cannot be synthesized economically on a commercial basis. Moreover the continuous and non-organized exploitation has resulted in many plants becoming rare and some even became extinct. To overcome this limitation, biotechnologists suggested the “use of cell and tissue culture technology rather than to use the whole plant” for the extraction of certain secondary metabolites [5].

*Cardiospermum halicacabum* Linn. (Sapindaceae) is an herbaceous climber [6], commonly used in the treatment of rheumatism, lumbago, earache, fever [7, 8]. Reports are available on analgesic, anti-inflammatory and vasodepressant activities [9, 10, 11]. Literature survey on this plant revealed efforts have not been made towards the study of antibacterial activity of *C. halicacabum* leaf and its callus on human pathogenic bacteria.

**MATERIALS AND METHODS**

**Explants Preparation:** Healthy, disease free leaves of two-month old *C. halicacabum* were collected from Mysore, Mysore district, Karnataka (India). The leaves were cut into 0.5-1 cm segments. These explants were washed both in liquid detergent Tween 20 for 3-4 minutes and in running tap water for 10 minutes. The washed explants were surface sterilized with 0.1% mercuric chloride solution for 3-4 min. followed by 4 to 5 rinses in cooled sterile distilled water and were inoculated on the culture medium.

**Media Preparation, Callus Initiation and its Proliferation:** Murashige and Skoog [12] medium supplemented with auxins viz., 2,4-D, NAA, IAA and cytokinins viz., BAP and Kn alone at different concentrations was used for callus initiation. The cultures were incubated under cool fluorescent light with 3000 lux for 16 h at a temperature of 25±2°C and 70±10% relative humidity. Each experiment had 10 replicates and repeated at least thrice. The proliferated callus cultures were sub cultured and maintained for 45-50 days on the same medium supplemented with the same growth regulator.

**Preparation of Extracts**

**Aqueous Extract:** Ten grams of both fresh leaf and leaf derived callus were macerated separately with 20 ml of sterile distilled water in a warring blender (Waring international, New Hartford, CT, USA) for 10-15 min. The macerate was first filtered through double layer muslin cloth then centrifuged at 3500 rpm for 30 min. The supernatant was filtered through Whatman No. 1 filter paper and sterilized at 120°C for 30 minutes. The extracts were preserved aseptically at 5°C for further use [13].

**Soxhlet Extraction:** Thoroughly washed leaves and proliferated callus of *C. halicacabum* were dried in shade and then powdered with the help of Waring blender. Ten grams of shade-dried leaf and callus powder was filled in a thimble and extracted with 100 ml of petroleum ether, chloroform, methanol and ethanol successively up to 48 h. Each of the solvent extracts was concentrated separately under reduced pressure [14]. After complete solvent evaporation, one gram of each concentrated solvent extract was dissolved in 9 ml of methanol and used in antibacterial assays.

**Test Microorganisms:** Authentic pure cultures of human pathogenic bacteria like *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella sp.*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sp.*, *Staphylococcus aureus*, and *Yersinia enterocolitica*, were obtained from Central Food Technological Research Institute, Mysore, Karnataka (India).

**Antibacterial Activity Assay:** Antibacterial activity of aqueous and solvent extracts of both leaf and callus extracts was determined by the cup diffusion method on nutrient agar medium [15].

**Aqueous Extract:** The organism to be tested was inoculated into sterile nutrient agar. After incubation period of 24 h at 37°C, a loop of inoculum was transferred into 5 ml of nutrient broth and incubated for 2 h at 37°C served as fresh suspension inoculum. Wells (5 mm diameter) were made in sterile nutrient agar plate using cork borer and inoculum containing 106 CFU ml⁻¹ of test bacteria were spread on the sterile nutrient agar medium in Petridishes was uniformly smeared with test culture. Then 25 µl of aqueous extract of both leaf and callus was introduced into each of the wells. The treatment also includes 25 µl of sterilized distilled water as control. All the plates were incubated for 24 h at 37°C and zone of inhibition if any around the well was measured in millimeter (mm). For each treatment three replicates were maintained. The data was subjected to statistical analysis of variance.
Solvent Extract: One gram of both leaf and its callus concentrated solvent extract of petroleum ether, chloroform, methanol and ethanol were dissolved in 9 ml of methanol. The sterile nutrient agar medium in Petridishes was uniformly smeared with test culture. Wells (5 mm) were made in each Petridish to which 25 µl of solvent extracts dissolved in methanol was introduced. For each treatment three replicates were maintained. Methanol was served as control.

Antibiotics: Streptomycin (10 mcg disc−1) generally used in the management of human pathogenic bacteria was selected for antibacterial activity assay for comparative evaluation.

RESULTS

Antibacterial Activity Assay: The antibacterial activity of aqueous and solvents extracts of C. halicacabum leaf and leaf derived callus against human pathogenic bacteria both Gram-positive and Gram-negative bacteria at 25 µl concentration are presented in Table 1. The results revealed that among ten pathogenic bacteria only Bacillus subtilis and Bacillus cereus belongs to Gram-positive bacteria showed susceptible for leaf aqueous extract when compared to Staphylococcus aureus. In Gram-positive bacteria Bacillus subtilis showed maximum inhibition. Where as Gram-negative bacteria showed least susceptible for leaf aqueous extract.

DISCUSSION

Review of literature reveals lack of information on the antibacterial potential of C. halicacabum leaf and leaf derived callus extracts. Aqueous and different solvent extracts of leaf and leaf derived callus extracts of C. halicacabum evaluated for antibacterial potential, revealed significant activity of ethanol extracts against Gram-positive bacteria followed by methanol extracts. None of the earlier reports have demonstrated the antibacterial potential of this plant leaf callus extracts [8, 10, 11]. In the present investigation the antibacterial activity of this leaf callus extracts has been demonstrated for the first time.

Table 1: Antibacterial activity of aqueous and different solvent extracts of leaf and leaf derived callus of Cardiospermum halicacabum against some human pathogenic bacteria at 25 µl

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Pathogens</th>
<th>Methanol</th>
<th>Control</th>
<th>Petroleum Ether</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Streptomycin (10mcg disc−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacillus cereus</td>
<td>0</td>
<td>7.36</td>
<td>0</td>
<td>9</td>
<td>14.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Bacillus subtilis</td>
<td>0</td>
<td>9.32</td>
<td>0</td>
<td>6.5</td>
<td>7.63</td>
<td>16.43</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Escherichia coli</td>
<td>0</td>
<td>0</td>
<td>7.33</td>
<td>6.83</td>
<td>12.37</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Klebsiella sp.</td>
<td>0</td>
<td>0</td>
<td>7.56</td>
<td>6.5</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Proteus mirabilis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.36</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Pseudomonas aeruginosa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>7.33</td>
<td>10.47</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Salmonella typhi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8.63</td>
<td>8.67</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Shigella sp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6.5</td>
<td>7</td>
<td>8.5</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Staphylococcus aureus</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>9.83</td>
<td>12.83</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Yersinia enterocolitica</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>11.36</td>
<td>0</td>
</tr>
</tbody>
</table>

Zone of inhibition (Mean three replicates±standard error). When subjected to analysis of variance (ANOVA) p<0.05
The ethanol extracts of *C. halicacabum* leaf and leaf-derived callus were most active, and promising results were obtained, which all to a varying extent inhibited the growth of the bacteria. Other tested extracts also inhibited the growth number of test organisms but to a lesser extent and mainly active against Gram-positive bacteria. Since several *Staphylococcus* strains are reported to express drug resistance [16] and natural plants were shown to be good anti-*Staphylococcus* activity [17]. In the study, among extraction assayed, the ethanol extract of leaf and its callus showed significant inhibition against *Staphylococcus aureus*. The result of the present study supported that, plant extract possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by microorganisms.

Successful prediction of botanical compounds from plant materials is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as a solvent, but in our studies we found that plant extracts in organic solvents (ethanol and methanol) provide more consistent antibacterial activity compared to extracted in water. Our finding also supported by several workers [18,19,20,21] has been generally reported that water extracts of plants do not have much activity against bacteria. Eloff [22] reported that methanol and ethanol were the most effective solvent for plant extraction than n-hexane and water. Similarly, in our study ethanol and methanol extracts exhibited higher activity than other solvents in both leaf and leaf derived callus extracts. Our results indicated that ethanol and methanol is ideal solvents to extracts antimicrobial compounds found in leaf and leaf derived callus.

In general, the cell walls of Gram-negative organisms, which are more complex than Gram-positive ones, act as a diffusional barrier and making them less susceptible to the antibacterial agents than the Gram-positive bacteria [23, 24]. In spite of this permeability difference, however, ethanol extracts of leaf and leaf derived callus extracts have still exerted some degree of inhibition against Gram-negative organisms as well.

Plant extracts have great potential as antimicrobial compounds against test bacteria. It also indicated the possible utilization of plant biotechnology towards the development of an effective in cultured derived callus, which were potentially bioactive compounds. However, in the present study the demonstration of bioactivity in *in vitro* derived callus in comparison to that of native plant has been presented. Although, numerous reports have appeared on the antibacterial activity of plants and their secondary metabolites, very few reports are on *in vitro* derived callus. In the current study therefore, the main emphasis was on the potentialities of the use of *in vitro* derived callus of *C. halicacabum* for antibacterial activity have been demonstrated for the first time.

**ACKNOWLEDGMENT**

The authors thank the Department of Studies and Research in Botany, University of Mysore and Central Food Technological Research Institute for providing facilities.

**REFERENCES**