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Screening Antibacterial Activity of Root Extract of *Cocculus hirsutus* (L.) Diels. used in Folklore Remedies in South India

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Abstract: It has been well known since ancient times that plants and spices have antimicrobial activity. There has been a considerable interest to use plants and spices for the elimination of microorganisms because of increasing antibiotic resistance of microorganisms. Petroleum ether, chloroform, ethyl acetate, acetone, methanol and aqueous root extracts of *Cocculus hirsutus* were evaluated for antimicrobial activity against clinically important bacteria viz., *Escherichia coli* (MTCC 1195), *Enterobacter aerogens* (MTCC 2823), *Klebsiella pneumoniae* (MTCC 2405), *Salmonella typhi* (MTCC 733), *Proteus vulgaris* (MTCC 1771) and *Pseudomonas aeruginosa* (MTCC 2642) (gram-negative), *Staphylococcus aureus* (MTCC 1430) and *Bacillus cereus* (MTCC 1272) (gram-positive). The *in vitro* antimicrobial activity was performed by agar disc diffusion method. The results showed the chloroform root extract of *Cocculus hirsutus* highly affected the activity of *Pseudomonas aeruginosa* and *Bacillus cereus*. The inhibition against *Staphylococcus aureus* and *Enterobacter aerogens* was moderate and the remaining bacterial strains had no activity. This study suggests that the root of *Cocculus hirsutus* obtained by infusion can be used in the treatment various bacterial diseases.

Key words: Antibacterial activity, *Cocculus hirsutus*, disc diffusion assay, menispermaceae

INTRODUCTION

During the past decade, traditional systems of medicine have become increasingly important in view of their safety. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs (Farnsworth and Soejarto, 1991).

The increasing antimicrobial resistance of pathogens isolated from humans and animals, combined with the increasing awareness of the consumers on chemical substances used as food preservatives, necessitates research for more efficient antimicrobial with fewer side-effects on human health. Recently, the antimicrobial effects of various plant extracts against certain pathogens have been reported by a number of researchers (Renzo *et al*., 2007; Al-Bakri and Afifi, 2007; Abere *et al*., 2007; Akinpelu and Onakoya, 2006; Durmaz *et al*., 2006; Sanjay and Ashok, 2006).

In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to

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the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens (Cragg *et al*., 1997; Recio, 1989).

A scandent shrub, *Cocculus hirsutus* (L.) Diels (Menispermaceae family) occurring almost throughout India, its commonly known as sirungattukodi. The juice of the leaves contains a mucilage. When mixed with water it forms a jelly which is taken as a cooling medicine for gonorrhoea and used externally for eczema, prurigo and impetigo (Chadha, 1950).

The roots of *Cocculus hirsutus* (L.) Diels have been mentioned as bitter, acrid, alterative, laxative, demulcent and antiperiodic in fever, tonic and diuretic. The juice of leaves coagulates in water and forms mucilage, which is used externally as a cooling and soothing agent in prurigo, eczema and impetigo (Nadkarni, 1982). In this study, we investigated the antibacterial activity of Petroleum ether, chloroform, ethyl acetate, acetone, methanol and aqueous root extracts of *Cocculus hirsutus* against a panel of Gram positive and Gram negative bacteria. To our knowledge, antibacterial activities of these plants against a wide range of other microorganisms have not been studied in the literature.

MATERIALS AND METHODS

The medicinal plant *Cocculus hirsutus* (L.) Diels root, used in this study, were collected around Tiruchirappalli district, (India) in the month of December 2006. The collected plant materials were identified at Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, South India. The roots were shade-dried at room temperature for 10 days.

Extraction Procedure

The dried and powdered plant materials (100 g) were extracted successively with 600 mL of Petroleum ether, chloroform, ethyl acetate, acetone, methanol and aqueous (1:6 w/v) by using soxhlet extractor for 48 h at a temperature not exceeding the boiling point of the solvent (Lin *et al*., 1999). The extracts were filtered using Whatman No. 1 filter paper and then concentrated in vacuum at 40°C using a Rotary evaporator. Each extracts transferred to glass vials and kept at 4°C before use.

Bacterial Strains

Eight different laboratory bacterial strains were used namely, *Escherichia coli* (MTCC 1195), *Enterobacter aerogens* (MTCC 2823), *Klebsiella pneumoniae* (MTCC 2405), *Salmonella typhi* (MTCC 733), *Proteus vulgaris* (MTCC 1771) and *Pseudomonas aeruginosa* (MTCC 2642) (gram-negative), *Staphylococcus aureus* (MTCC 1430) and *Bacillus cereus* (MTCC 1272) (gram-positive). The bacterial strains were supplied by Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

Preparation of Inoculum

The bacterial strains preserved in the nutrient agar at 4°C were revived in nutrient broth (liquid medium) and incubated at 37±1°C for overnight and the suspensions were checked to provide approximately 10^5 cfu mL^{-1}.

Microbiological Tests of Plant Extracts

The disc diffusion assay methods of Iennette (1985) as described by Rosoanaivo and Ratsimanaga-Urverge (1993), Rabe and Van Staden (1997), were used with modification to determine the growth inhibition of bacteria by plant extracts. The diluted bacterial culture (200 µL) was spread over nutrient agar plates using sterile glass L-rod. Fifty microliter of the each extracts was applied per

filter paper disc (Whatman No. 1, 6 mm dia) and was allowed to dry before being placed on the layer of the agar plate. Each extracts was tested in triplicate (3 discs plate^{-1}) and the plates were inoculated at 37±1°C for 24 h. After incubation, the diameter of inhibition zones and the sensitivity were measured with a caliper. Standard antibiotic of streptomycin (10 mg disc^{-1}) was used as reference of positive control.

Statistical Analysis

Random sampling was used for the entire test in triplicates. Calculations were carried out in triplicate with their mean values and standard error by using the formula given by Gupta (1977). Positivity index was calculated by comparing the zone of inhibition of bark extracts with standard antibiotics.

$$\text{Activity index} = \frac{\text{Inhibition area of test sample}}{\text{Inhibition area of standard antibiotic}}$$

RESULTS AND DISCUSSION

The present study was designed to obtain preliminary information on the antibacterial activity of *Cocculus hirsutus* traditionally used in India. The disc diffusion method was preferred to be used in this study. Present study showed a remarkable antibacterial activity. Pure organic solvents did not show any antibacterial activity. Petroleum ether, chloroform, ethyl acetate, acetone, methanol and aqueous root extracts of *Cocculus hirsutus* were tested against *Escherichia coli*, *Enterobacter aerogens, Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris* and *Pseudomonas aeruginosa* (gram-negative), *Staphylococcus aureus* and *Bacillus cereus* (gram-positive) pathogenic bacteria. The disc diffusion assay showed that the chloroform root extract of *Cocculus hirsutus* inhibits the activity of *Pseudomonas aeruginosa* and *Bacillus cereus*. The inhibition against *Staphylococcus aureus* and *Enterobacter aerogens* was moderate and the remaining strains *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Proteus vulgaris* had no activity. Ethyl acetate and acetone extract exhibit very poor activity associated with *Enterobacter aerogens*, *Salmonella typhi*, *Bacillus cereus* and *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, respectively, rest of the extract like Petroleum ether, methanol and aqueous showed no activity against above the bacterial strains. These results were compared with standard antibiotic, streptomycin as a standard (Table 1).

Table 1: Evaluation of antibacterial activity, indicated by Diameter of Inhibition Zones (DIZ, cm), of root extracts of *Cocculus hirsutus* by disc diffusion method

Bacterial strains	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Aqueous	Streptomycin
Escherichia coli (MTCC 1195)	-	-	-	0.27±0.02	-	-	1.25±0.76
Enterobacter aerogens (MTCC 2823)	-	0.17±0.09	0.1±0.60	-	0.11±0.90	-	0.30±0.58
Klebsiella pneumoniae (MTCC 2405)	-	-	-	-	-	-	1.05±0.87
Salmonella typhi (MTCC 733)	-	-	0.2±0.20	0.22±0.08	-	-	0.55±0.27
Proteus vulgaris (MTCC 1771)	-	-	-	0.15±0.70	-	-	1.20±0,98
Pseudomonas aeruginosa (MTCC 2642)	-	0.63±0.09	-	-	0.16±0.78	-	1.90±0.68
Staphylococcus aureus (MTCC 1430)	-	0.2±0.08	-	-	-	-	0.45±0.87
Bacillus cereus (MTCC 1272)	-	0.43±0.12	0.1±0.09	-	0.05±0.84	-	1.20±0.53

Values are shown in mean±SE

Most of the drug resistant pathogenic bacteria were spreading hazards in the world. An alternative to combat the problem of microbial resistance alternative to combat the problem of microbial resistance ineffective ones. Accordingly, medicinal plants and microorganisms are the proper candidates and should receive continuous research attention. The use of higher plants to treat infections is an age-old practice in a large part of the world population. Furthermore, because of the side effects and the resistance that pathogenic micro organisms build against the common antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plants used in herbal medicine (Essawi, 2000; Cos *et al*., 2002; Kokoska *et al*., 2002; Shahidi *et al*., 2002).

Smith *et al.* (1999) express that The emergence of bacterial resistance threatens to return us to the era before the development of antibiotics and I like to express that "For not loosing the battle in the war against antimicrobial-resistant bacteria, one major way is to extend man's knowledge about the new antimicrobial sources". The results of such studies form the avenue for further investigations to find new drugs for therapeutic usages.

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