

ANTIBACTERIAL ACTIVITY OF *CISSAMPELOS PAREIRA* LINN AGAINST CERTAIN BACTERIAL STRAINS

Muthuselvam D.*

Department of Botany, Bishop Heber College, Puthur, Tiruchirappalli, Tamilnadu,
India-620 017.

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***Correspondence for
Author**

Muthuselvam D.

Department of Botany,
Bishop Heber College,
Puthur, Tiruchirappalli,
Tamilnadu, India- 620017.

ABSTRACT

Cissampelos pareira is slender tomentose climber, is known for its medicinal properties to cure various ailments. The antibacterial properties of leaves (acetone, aqueous and ethanol) extracts screened against seven bacterial strains such as *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens* by adopting disc diffusion and agar well diffusion methods. Maximum 18 mm inhibition zone in the disc diffusion method and 19 mm in that of agar well diffusion method is observed when tested against *Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella typhi*. In rest of the test organisms growth inhibition is affected and is attributed to the

phytochemical present in it. This study supports the traditional medicine to cure many disease like diarrhea, intestinal tract, throat, ear infections, cough, ulcers, fever and skin diseases.

KEYWORDS: medicinal plant, antibacterial, phytochemical and traditional medicine.

INTRODUCTION

Human infections particularly those living microorganisms i.e. bacteria, fungi, viruses, nematodes, they cause serious infections in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic organisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases. The increasing interest on traditional ethno medicine may lead to discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals, nutraceuticals, cosmetics and food supplements (Kokate 1994; Perumalsamy and Ignacimuthu 2000). The medicinal properties of several herbal plants have been documented

in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases (Handa *et.al.*, 1996). The active principle of plant drugs are commonly more concentrated in storage organs. Roots, seeds, barks and leaves are much represented in *Materia Medica*. Flowers, herbaceous stems are usually relatively inert. The medicinal substances such as alkaloids, glycosides, resins, volatile oils, gums, tannin and flavonoids etc., plants are known to produce large amount of secondary metabolites which are biosynthesized in plants for different purposes including growth regulation inter and interaction and defense against predators and infections. Medicinal properties of plants are attributed to those of secondary metabolites only (Cragg *et.al.*, 1999).

Cissampelos pareira Linn (family: Menispermaceae) is a perennial climbing herb/shrub with small greenish-yellow flower. It belongs to the genus *Cissampelos*, of which 30-40 species are distributed in the tropical and subtropical world. One species is found in India. It has medicinal properties to cure various ailments such as Velvet leaf or Abuta is antibacterial, anti-inflammatory, antihistamine, antioxidant, antispasmodic, diuretic, hypotensive, muscle relaxant, uterine relaxant, antiseptic, analgesic, antihemorrhagic, cardiogenic, diaphoretic, expectorant, febrifuge, hepatoprotective stimulant and tonic. It is known as Midwife's herb as mainly used in women ailments. It is used for menstrual problems, hormonal imbalance, ease childbirth, postpartum pain, prevent threatened miscarriage, and control uterine hemorrhages, hormonal acne and premenstrual syndrome. It is used for heart problems, kidney stones, kidney infections and pains, asthma, arthritis, muscle cramps and stomach pains. It is also used to kill bacteria, prevents convulsions, ulcers, indigestion, skin irritations, cough, fever, intestinal worms, wounds and in snake bite. It is used against poisonous snake bites and is very effective in skin related ailments. All parts of the plant is known for its medicinal value (Kritikar and Basu 1975). Hence, there is an urgent need to study the screening of antimicrobial properties of climber, which will be helpful in the treatment of several disease caused by microorganisms. During the present study, efforts are being made to investigate the active principle of therapeutic value by testing in against selected bacterial strains *in vitro*.

MATERIALS AND METHODS

Collection of plant materials

The aerial part of leaves *Cissampelos pareira* Linn. were collected from the natural habitat in presterilized polythene bags and were brought to the lab. The botanical nomenclature of the plants was duly identifies by using standard floras and also cross checked with Herbarium

records (Rapinat Herbarium Herbarium, Tiruchirappalli (RHT), India. They were washed under tap water, blot dried and kept in hot air oven for 72 hours for 50°C.

Preparation of crude extracts

The dried plant material was crushed and powdered in homogenizer to obtain coarse dry material, 50 grams of which were added to 300 ml of ethanol, acetone and aqueous left in soxhlet apparatus for 24 hours to obtain extracts.

Preparation of basal medium

Nutrient agar medium with following composition was prepared and was used as basal medium such as dextrose- 5g, peptone- 5g, beef extract -3g, yeast extract – 1.5g, sodium chloride – 1.5 g, agar- 15g and distilled water -1000 ml. The are taken in presterilized 1000 ml conical flask and autoclaved at 121°C for 15 minutes and on cooling it is poured into sets of presterilized petriplates under aseptic conditions.

Experimental organisms

Seven bacterial strains viz. *Bacillus subtilis*, *Escherichia coli*, *Klepsiella pneumoniae* *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, and *Serratia marcescens* as pure cultures are obtained from Sea Horse Hospital, Tiruchirappalli, Tamil Nadu which are used as experimental organisms. They are maintained in Nutrient broth (without addition of agar) medium in order to obtained bacterial suspensions.

Setting up of the experiments

Disc diffusion method (Nene and Thapliyal 1979)

2 ml each bacterial suspensions are inoculated into separated sets of petriplates containing basal medium, with the help of sterilized pipetted and spread over the medium using 'L' shaped glass rod to ensure equal distribution under aseptic conditions. 6mm each of Whatmann's number 1 filter paper discs are cut with the help of disc cutter and are sterilized by autoclaving at 15 lbs pressure for 15 minutes under aseptic conditions. Three discs dipped in extract are placed over the medium at three different places, while the fourth disc without treatment with extract is placed separated as control.

Agar well diffusion (Kudi *et.al.*, 1999)

2 ml each bacterial suspensions are inoculated into separate sets of presterilized petriplates containing basal medium under aseptic conditions. With the help of sterilized pipette and spread over the medium using 'L' shaped glass rod to ensure equal distribution under aseptic conditions. With the help of presterilized 6 mm cork borer at three places agar wells bored. In two of them 0.2 ml of extracts are added, while the third without treatment with extract as that control. The above sets of petriplates are incubated at 37°C for 24 hours. At the end of said period, inhibition zone due to treatment with alkaloid extracts in above experiments are observed and measured with the help of meter scale and values are represented in the Table 1.

RESULTS AND DISCUSSION

Inhibition zone in growth of experimental organisms in response to crude extracts present in *Cissampelos pareira* is represented in Table-1. Maximum 19 mm of inhibition in disc diffusion method and 19 mm in agar well diffusion method in *Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella typhi* is being observed due to the presence of phytochemical compounds. Accordingly the phytochemical present in entire plant are proven to be effective ingredients in checking the growth of all experimental organisms *in vitro*. Various medicinal parts of this herb are known to possess medicinal value and are being used in treating diseases such as liver, gastric disorders, chest pain, cough and piles. Thus the main active components in *Cissampelos pareira* with biological activity is found to alkaloids which exhibited antibacterial effects when tested against experimental organism. The phytochemical compounds are found in nature to protect them against spoilage due to microorganisms. These substance save human beings and other animals from diseases (Dodia 2000; Samanta and Bhattacharya 2012). The aim of various medicinal system was, to determine the effective medicinal properties of hither to unexplored plants which could be brought as cheaper substitutes for pharmacological and extra pharmacological drugs (Amresh 2007; Clark 1996; Grewal 2000; Nirmal 2009; Nishanthi, 2012; Verma 2001). One of the major problems with many of the traditional medicines is that the active ingredients are not well defined. It is important to have molecular and chemical fingerprints of the components of active extracts from medicinal plants. This will not help analysis therapeutic efficacy of the product, but also help standardize the product in terms of active principles as markers (Brain and Turner 1975; Harborne, 1998; Verma, 2002;). Different chemical compounds such as triterpene, flavonoids, glycosides, alkaloids, and carbohydrates were detected in the plant, which could make the plant useful for treating different ailments and having potential of providing useful drugs of human use. Traditionally and experimentally, it has been found that

the *Cissampelos pareira* leaves is a potential herbal medicinal agent. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of cultivars in the country.

Table: 1. Inhibition zone in growth of experimental organisms in response to phytochemical present in *Cissampelos pareira*.

Method	Solvent used	Diameter of inhibition zone (mm)						
		<i>Proteus vulgaris</i>	<i>Salmonella typhi</i>	<i>Styphyllococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Serratia marcescens</i>
Disc diffusion	Aqueous	13	15	11	13	14	12	11
	Acetone	17	18	15	18	16	17	15
	Ethanol	18	18	15	18	16	18	16
Agar diffusion	Aqueous	11	16	11	12	14	13	11
	Acetone	15	19	16	14	17	19	14
	Ethanol	19	19	15	18	16	19	13
Standard antibiotic		15	18	19	19	20	23	17
Control		-	-	-	-	-	-	-

Control: Sterile disc soaked in solvents.

Antibiotics – Streptomycin.

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