

## ANTIMICROBIAL AND WOUND HEALING STUDIES ON THE EXTRACTS OF THE MEDICINAL PLANT *COCCULUS HIRSUTUS* (LINN).

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**ABSTRACT:** The antimicrobial activity of methanol and aqueous extracts of the medicinal plant *Cocculus hirsutus* was studied using *E.coli*, *Vibrio cholerae*, *Pseudomonas fluorescense*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, *Candida gillermontii*, *Tricophyton rubrum*, *Tricophyton tansuans*, *Aspergillus ochraceus*, *Candida albicans* and *Rodatrula sp* as test microorganisms. The methanol extract of the plant was found to be more effective against *Vibrio cholera* (18) and *Staphylococcus aureus* (17mm) and in the mean time the aqueous extract was found to be more effective against *Vibrio cholera* (13) and *Klebsiella pneumonia* (14mm). Both methanol and aqueous extract of this plant was found with no fungal activity against fungal pathogens. Phytochemical screening of the methanol extract of *Cocculus hirsutus* revealed the presence of alkaloids, steroids, tannins and saponins, which suggests may be responsible for antimicrobial activity. The wound healing potential of this plant was found to be comparably better than that of the commercial antibiotic Neomycin.

**Keywords:** Antimicrobial activity, Leaves, Well cut method, Clinical pathogens, Phytoconstituents.

### INTRODUCTION

Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Venkatesh et al., 2003). Therefore investigation on some active principles from traditional medicinal plants has become more important (Suba et al., 2004). The world health organization (WHO, 1980) has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs (Upathaya and Pandey, 1984). *Cocculus hirsutus*, known well as Broom creeper, is found in moderately cool and hot regions of India particularly Tamilnadu, Bihar and Punjab. *Cocculus hirsutus* Linn (Menispermaceae) is commonly known as Jal-Jammi (Chopra et al., 1958). In Tamil this plant is known as Kattukkodi. Indian tribes use various plant parts of this plant for a wide range of ailments including constipation, kidney problems (Caius, 1986). In Tamilnadu Kaani tribes of Karaiyar using this plant for the treatment of skin disease, sexual debility and wound healing. It belongs to the family Menispermaceae. In traditional medicine, the roots are used for the treatment of rheumatism, tuberculosis, leprosy, skin diseases, dyspepsia, pruritis, and flatulence, laxative, aphrodisiac, antipyretic and leaves are used in biliousness, eczema, gonorrhoea, ophthalmia, sexual debility and neuralgia (Warrier et al., 2005). The juice of leaves coagulates in water and forms mucilage, which is used externally as a cooling and soothing agent in eczema and impetigo (Nadkarani, 1982). The leaf juice of this plant is used in the treatment of eczema (Masilamani, 1981). The roots of *Cocculus hirsutus* have been mentioned as bitter, acrid, laxative, tonic and diuretic. The plant has been reported to contain essential oil,  $\beta$ -sitosterol, ginnol (Merchant et al., 1962). The anti-inflammatory and analgesic activities of the roots were also reported (Nayak and Singhai, 1993). Since there is no report on the antimicrobial, phytochemical and wound healing potential of this plant, an attempt was made to provide much information for the same.

## MATERIAL AND METHODS

### Plant Collection:

*Cocculus hirsutus* was collected from Karaiyar during the month of March 2010, which is located in Tirunelveli district, Tamilnadu. This plant was then identified by the book "The flora of Presidency of Madras" (Gamble, 1958). The voucher specimen of this plant was maintained in Dept of Zoology, Sri Paramakalyani College, Alwarkurichi. The collected leaves of this plant were brought about to the laboratory, where the leaves were shade dried well for a week.

### Crude extract preparation:

#### 1. Preparation of Methanol Extract:

Then the shade dried leaves of *Cocculus hirsutus* were ground well in a mixer grinder and made into powder form of about 30 g. The powdered leaves of about 20g were extracted with methanol in a soxhlet apparatus. Then, the extract was evaporated in a rotary vacuum evaporator at 40°C under reduced pressure. The crude extract of about 3 g was obtained which is equivalent to about 20% of total extraction.

#### 2. Preparation of aqueous extract:

The aqueous extract for wound healing assay was prepared by mixing 10 g of powdered leaves of this plant with sterile distilled water and boiled to slow heat for 2h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the procedure was repeated twice. The extracted supernatant was concentrated to make the final volume one-fourth of the original volume (Harbone, 1983). It was then autoclaved at 121°C and at 15 lbs pressure and stored at 4°C.

### Microbes and Media:

The clinical bacterial isolates such as *E.coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Staphylococcus aureus* and clinical fungal isolates such as *Candida albicans*, *T.rubrum*, *T.tansuans*, *C.gillermontii*, *A.ochraceus* and *Rodatrula sp.* were used. The bacterial and fungal isolates were obtained from Asan memorial college, Chennai and the slants were maintained in nutrient agar and potato dextrose agar respectively for bacterial and fungal isolates, which were stored at 4°C.

### Antimicrobial activity:

The extracts were screened for their antimicrobial activity invitro by agar well cut method using *E.coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Staphylococcus aureus*, and *Candida albicans*, *T.rubrum*, *T.tansuans*, *C.gillermontii*, *A.ochraceus* and *Rodatrula sp* as test organisms. Three to five similar colonies were selected and transferred to 5 ml broth with a loop and the broth cultures were incubated at 37°C for 24 h and in the same time six similar fungal colonies were selected and transferred to 5 ml potato dextrose broth and kept for incubation at 37°C for 48 h in an incubator. After incubation the suspension was checked for 0.7 McFarland value and was used as the seeding culture. For screening Muller-Hinton agar and potato dextrose agar was prepared and seeded with respective bacterial and fungal pathogens respectively. Then the wells were made by using a sterile cork borer and is added with different volumes (25µl, 50µl, 75µl and 100µl) of the crude extract of *Cocculus hirsutus* (1g /10ml D.W) and kept for incubation at 37°C for 24h and 37°C for 48 h respectively for the bacterial and fungal isolates. After incubation at 24 h and 48h the results were recorded for the zone of inhibition. Streptomycin (10µg/5ml) and gentamycin 10µg/5ml were used as standards for bacteria and fungi respectively (Kudi et al., 1999)

### Preliminary phytochemical screening:

The coarse powdered leaves of *Cocculus hirsutus* was extracted with methanol in soxhlet apparatus and was subjected to various chemical tests to detect the presence of different phytoconstituents (Elmahmood et al., 2007).

**Wound healing assay:****Acclimatization of Rabbits:**

The aqueous extracts of this plant *Cocculus hirsutus* was tested for its wound healing potential. The wound healing assay was performed in *Orchtolacus sp.* of rabbits. Three rabbits were procured from the rabbit form in Tirunelveli and brought to the laboratory, where the rabbits were reared up in a cage and the temperature is maintained to 27°C and the relative humidity was maintained in the range of about 70-80 % for the acclimatization purpose. The rabbits were fed up with cabbage and carrot and provided with water.

**Excision wound and dressing:**

Out of these three fine rabbits, one was kept as a control, the second one rabbit was treated with the aqueous extract of *Cocculus hirsutus* and one of the rabbit was treated with the standard antibiotic neomycin. The wound of about 2.5cm was made in the thigh region of the test rabbits by using a sterile scissor and the markings were made on the rabbits. One of the rabbit was applied with 1g aqueous extract of *Cocculus hirsutus* and in the due course of time another one rabbit was treated with the commercial antibiotic neomycin of about 0.5 g. The treatment was subsequently followed for about 10 days.

**Measurement of wound:**

The wound healing activity was measured by outlining the wound as accurately as possible on a super imposed sheet of sterile cellophane. This outline was then transferred directly to a sheet of tracing paper and the area within it determined with a radial plantimeter. Subsequent measurements of the wound were made at regularly spaced intervals as healing progressed (Carrel and Hatmann, 1916).

**RESULTS AND DISCUSSION**

The methanol extract of *Cocculus hirsutus* was found to be more effective against *E.coli*, *Vibrio cholerae*, *Staphylococcus aureus* and *A.ochraceus* and in the mean time the aqueous extract of this plant showed the maximal inhibitory activity against *E.coli* and *Staphylococcus aureus* and it did not even show the minimal inhibitory against the fungal pathogens such as *C.gillermontii*, *T.rubrum*, *T.tansuans*, *A.ochraceus*, *Candida albicans* and *Rodatrula sp.* (Fig 1 and 2).

**Table: 1 showing the antimicrobial activity of methanol extracts and aqueous extracts of the plant *Cocculus hirsutus***

S.No	Bacterial culture	Diameter(mm) of Zone of inhibition of different concentration of extracts									
		Methanol				Streptomycin	Aqueous				
		25 µl	50 µl	75 µl	100 µl			25 µl	50 µl	75 µl	100µl
1	<i>Escherichia coli</i>	11.00±0.00	12.33±0.33	13.67±0.33	18.00±0.00	30.67±0.33	11.33±0.33	12.67±0.33	13.00±0.00	14.00±0.00	30.67±0.33
2	<i>Vibrio cholerae</i>	13.00±0.00	16.33±0.33	17.67±0.33	19.67±0.33	18.33±0.33	12.00±0.00	13.00±0.00	13.33±0.33	15.33±0.67	18.33±0.33
3	<i>Pseudomonas fluorescence</i>	12.00±0.00	13.33±0.33	15.33±0.33	16.33±0.33	21.67±0.33	10.00±0.00	12.00±0.00	13.33±0.33	14.00±0.00	21.67±0.33
4	<i>Klebsiella pneumoniae</i>	12.00±0.00	12.67±0.33	14.00±0.00	16.00±0.00	15.33±0.33	12.00±0.00	13.00±0.00	13.00±0.00	13.67±0.33	15.33±0.33
5	<i>Staphylococcus aureus</i>	13.00±0.00	14.33±0.33	15.67±0.33	17.33±0.33	21.00±0.00	12.00±0.00	13.00±0.00	14.33±0.33	15.00±0.00	21.00±0.00

(n=3) ±Standard deviation, p<0.05, when compared with standard streptomycin (Student T-test)

**Table: 2 showing the wound healing potential of the extracts of the plant *Cocculus hirsutus*.**

S.no	Number of Days	Neomycin(mm <sup>2</sup> )	<i>Cocculus hirsutus</i> )
1.	First day	14.00±0.00	14.00±0.00
2.	Fifth day	13.00±0.00	11.33±0.33
3.	Sixth day	12.00±0.00	10.00±0.00
4.	Seventh day	11.33±0.33	9.00±0.00
5.	Eighth day	10.33±0.33	7.67±0.33
6.	Ninth day	10.33±1.45	7.00±0.58
7.	Tenth day	8.33±0.33	5.33±0.33

(n=3) ±Standard deviation, p<0.05, when compared with standard Neomycin (Student T-test)

Phytochemical screening of methanol extract of *Cocculus hirsutus* showed the presence of alkaloids, steroids, saponins and tannins, which suggest may be the quite reason for its antimicrobial activity. (Fig-3). The profound presence of tannins which makes the flavour of the extract of *Cocculus hirsutus* like a tea. This may be the reason for using the powdered leaves of this plant for the preparation of tea by the Kani tribes of Karayar in Tamilnadu. Hence in near future we can use the leaves of *Cocculus hirsutus* an alternative source for the commercial tea.

**Table: 3 Showing the result for phytochemical study**

S.No	Name of the Phytoconstituent	Methanol extract	Aqueous extract
1	Alkaloid	+	+
2	Steroid	+	+
3	Flavanoid	-	-
4	Terpenes	-	-
5	Saponins	+	+
6	Tannins	+	+
7	Xanthoproteins	-	-

+ indicates the presence, - indicates the absence

The wound healing assay showed the aqueous extract of *Cocculus hirsutus* having potential wound healing activity as by healing the wounds in the eighth day of the post wounding. In case of rabbits treated with commercial antibiotic neomycin cured the wound on tenth day of the post wounded days. Since this plant is healing wounds we can rely on the bioresource of this plant for the preparation of commercially valuable wound healing ointments and drugs. Further research is needed to isolate the bioactive compounds from *Cocculus hirsutus* for the preparation of new drugs.

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