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Evaluation of the Antibacterial activity of *Cocculus hirsutus*

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Abstract

Herbal products prepared either from single or multiple botanical ingredients are usually complex and variable in nature. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered. For this reason, Cocculus hirsutus (L.) a medicinal plant belonging to family Menispermaceae, the perennial climber was selected for the present study. The study was formulated with the objective to assess the antibacterial activity of Cocculus hirsutus. The leaves stem and the root of Cocculus hirsutus were extracted with solvents namely petroleum ether, chloroform, benzene, acetone, methanol and water for the determination of antibacterial activity. The chance to find antibacterial activity was more apparent in methanol and acetone extracts than the other extracts. The benzene and methanol extracts of Cocculus hirsutus leaves have shown strong antibacterial activity against all the organisms tested. The zone of inhibition ranged from 8-16 mm. The petroleum ether, acetone and methanol extracts of Cocculus hirsutus stem have shown pronounced antibacterial activity against all the microorganisms tested. Zones diameter ranged from 9-21 mm. Based on the results, it can be concluded that the Cocculus hirsutus plant extracts have great potential as antimicrobial components against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms.

Key words: Medicinal plants, Antibacterial activity, well diffusion method, *Cocculus hirsutus*, MIC, MBC.

Introduction

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents. The cost of production of synthetic drugs is also high and they produce adverse effects compared to plant derived drugs. Hence much attention has been paid recently to the biologically active compounds derived from plants used in herbal medicine[1, 2].
For these reasons, medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents [3].

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Therefore in recent years, considerable attention has been directed towards the identification of plants with antimicrobial activity [4].

The plant chosen for the present study is *Cocculus hirsutus* (L.) belonging to the family *Menispermaceae* which is a perennial climber mainly found in tropical and subtropical climatic condition [5]. The *Cocculus hirsutus* shows diuretic, laxative activity [6], hypolipidemic activity [7] and spermatogenic activity [8]. Traditionally the plant was patronized for its unique property of healing all type of cuts, wounds and boils in very less time and with less pain. It is also used in the treatment of gonorrhea, spermatorrhoea, urinary troubles, diarrhea and hyperglycemia [9]. Literature survey revealed that the leaves of the plant have been evaluated for anti hyperglycemic [10] and diuretic effects. Folk medicine claims that it may be used in jaundice. The hepatoprotective effects of *Cocculus hirsutus* could be due to the presence of phytochemicals like β-sitosterol, trilobine, isotrilobine, syringaresional, protoquercitol, ginnol and glycosides [11].

Materials and Methods

Collection of the plant samples

Cocculus hirsutus plants were procured from local markets in Coimbatore and duely authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Coimbatore. The leaves, stem and the root of the plant were separated out and they were washed, air dried in the shade at room temperature for five days. Dried leaves stem and roots of *Cocculus hirsutus* were powdered and stored until use.

Preparation of the plant extracts

Five gram powdered sample of leaves, stem and root of *Cocculus hirsutus* were sequentially extracted with solvents namely petroleum ether, benzene, chloroform, acetone, methanol and also with water by Soxhlet apparatus for 48 hrs. Then it was filtered through Whatman.No.1 filter paper and their crude extracts were evaporated in a water bath to give gummy solid residue. The residue was dissolved in dimethyl sulphoxide. These crude extracts were stored in refrigerator and screened for antibacterial activity. 0.001 g of extracts per 20µl were taken for the assay.

Determination of the antibacterial activity

Selection of the microorganisms

The bacterial strains used for this study include *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli, Shigella dysentriae* and *Klebsiella pneumoniae.* All the bacterial strains were grown and maintained in nutrient agar.

Well Diffusion method

The method of Rios *et al* [12] was used in this study to determine the antibacterial activity of the various solvent extracts selected. The results are tabulated in Table: 1.

Minimum Inhibitory Concentration (MIC)

The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The minimum inhibitory concentration (MIC) values were determined by broth dilution assay. The cultures were prepared at 24hr broth cultures of *B. subtilis, S. aureus, E. coli, S. dysentriae* and *K. pneumoniae* respectively [13].

Minimum Bactericidal Concentration (MBC)

MBC was determined for each set of wells. After incubation, the concentration at which no visible growth was noted as minimum bactericidal concentration [14].

Results and discussion

There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross infection. However, the development of new antibiotics should be continued as they are of prime importance to maintain the effectiveness of antimicrobial treatment. In developing countries, the WHO estimates that about three quarters of the population relies on plant based preparations used in their traditional medicinal system and as the basic need for human primary health care [15].

Table 1: Antibacterial activity of *Cocculus hirsutus* leaf extract by well diffusion method (Diameter of zone in mm)

Bacterial strains	Solvent extracts						Control
	Petroleum ether	Chloroform	Benzene	Acetone	Methanol	Aqueous	
B. subtilis	17.0±1.0	13.0±1.2	12.0±0.5	8.0±1.0	15.0±0.3	7.0±0.5	20
E. coli	16.0±0.6	8.0±0.5	15.0±0.0	12.0±0.5	14.0±0.5	8.0±0.7	20
S. aureus	9.0±0.0	9.0±0.3	15.0±0.7	9.0±0.6	12.0±0.0	14.0±1.0	24
S. dysentriae	9.0±0.5	7.0±1.2	16.0±0.0	8.0±0.0	15.0±0.5	7.0±0.6	21
K. pneumoniae	20.0±0.8	7.0±0.5	8.0±1.0	9.0±1.0	8.0±0.5	7.0±0.5	23

Values are mean±SD in triplicates

Table 2: Antibacterial activity of *Cocculus hirsutus* stem extract by well diffusion method (diameter of zone in mm)

Bacterial strains	Solvent extracts						Control
	Petroleum ether	Chloroform	Benzene	Acetone	Methanol	Aqueous	
B. subtilis	18.0±0.0	20.0±0.7	10.0±0.5	17.0±0.7	16.0±0.1	9.0±0.0	23.0
E. coli	9.0±0.0	7.0±0.0	7.0±0.5	13.0±0.7	14.0±0.5	9.0±0.0	24.0
S. aureus	20.0±0.5	12.0±0.7	8.0±0.0	9.0±1.0	21.0±1.0	7.0±1.0	20.0
S. dysentriae	12.0±0.7	6.0±0.5	7.0±0.0	13.0±0.7	11.0±1.0	9.0±1.0	21.0
K. pneumoniae	17.0±1.0	19.0±0.0	15.0±0.6	13.0±0.5	12.0±1.2	10.0±0.7	20.0

Values are mean±SD in triplicates

The various extracts such as petroleum ether, chloroform, benzene, acetone, methanol and aqueous extracts of *Cocculus hirsutus* leaves, stem and root were tested against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Shigella dysentriae* and *Klebiella pneumoniae* for its antibacterial activity. The results are presented in Tables I-III.

In our study, all the extracts of the leaves of *Cocculus hirsutus* showed low activity against *S. aureus* except the benzene and aqueous extracts which showed pronounced inhibition (15 mm) and (14 mm) respectively against *S. aureus.*

Table 3: Antibacterial activity of *Cocculus hirsutus* root extract by Well diffusion method (diameter of zone in mm)

Bacterial strains	Solvent extracts						Control
	Petroleum ether	Chloroform	Benzene	Acetone	Methanol	Aqueous	
B. subtilis	9.0±1.0	7.0±0.5	15.0±0.0	14.0±0.6	13.0±0.7	7.0±0.5	25.0
E. coli	7.0±0.7	12.0±0.5	7.0±0.0	15.0±0.5	14.0±0.7	10.0±0.5	23.0
S. aureus	13.0±1.0	7.0±0.7	8.0±0.5	20.0±0.5	18.0±0.0	9.0±0.5	24.0
S. dysentriae	20.0±0.0	8.0±0.0	9.0±0.7	11.0±0.5	19.0±0.0	8.0±0.7	20.0
K. pneumoniae	17.0±0.0	14.0±0.0	7.0±1.0	8.0±0.5	21.0±0.0	9.0±0.7	21.0

Values are mean±SD in triplicates

Therefore, the potency of the benzene and aqueous extracts of the leaves against *S. aureus* largely could support their traditional use in the treatment of respiratory tract infections. The petroleum ether extracts of leaves of *Cocculus hirsutus* were found to be active against *K. pneumoniae* and *B. subtilis* and showed low acitivity against the rest of the tested organism. The methanol extracts of the leaves of *Cocculus hirsutus* were found to be active against all the tested organism.

The growth of *E. coli* was controlled by the methanol extract of *Cocculus hirsutus* stem which indicated that they could inhibit the bacterial activity, which can cause diarrhoea and dysentry. Similarly the presence of steroids and aminoacids in *Cocculus hirsutus* could correspond to its high antimicrobial activity exhibited against *E. coli.* The petroleum ether extract of the stem of *Cocculus hirsutus* was found to be highly active (20 mm) against *S. aureus.* Steroids and anthocyanins of *Cocculus hirsutus* stem could be responsible for their antimicrobial activity against *S. aureus.*

All the extracts of the root of *Cocculus hirsutus* exhibited pronounced activity against all the tested organism.

However, petroleum ether and benzene extracts against *E.coli* and chloroform and benzene extracts against *S. aureus* showed low activity. These findings support the traditional knowledge of local users and it is a preliminary scientific validation for the use of this plant for antibacterial activity.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was done for selected organisms using acetone and methanol extracts which gave maximum zone in well diffusion method. The results are presented in Tables IV-VI.

Table 4: Antibacterial activity of C*occulus hirsutus* leaf extract against *Bacillus subtilis* and S*taphylococcus aureus* by (MIC)

Extracts	*B. subtilis*					*S. aureus*				
Con. (mg/100 ml)	0	4	2	1	0.5	0	4	2	1	0.5
Acetone	-	+	+	-	-	-	+	+	-	-
Methanol	-	+	+	+	+	-	+	+	-	-

+: indicates positive activity - : indicates negative activity

Table 5: Antibacterial activity of *Cocculus hirsutus* stems extract against *Bacillus subtilis* and *Staphylococcus aureus* by (MIC)

Extracts	*B. subtilis*					*S. aureus*				
Con. (mg/100 ml)	0	4	2	1	0.5	0	4	2	1	0.5
Acetone	-	+	+	+	+	-	+	-	-	-
Methanol	-	+	+	+	+	-	+	+	-	-

+: indicates positive activity - : indicates negative activity

Table 6: Antibacterial activity of *Cocculus hirsutus* root extract against *Bacillus subtilis* and *Staphylococcus aureus* by (MIC)

Extracts	*B. subtilis*					*S. aureus*				
Con. (mg/100 ml)	0	4	2	1	0.5	0	4	2	1	0.5
Acetone	-	+	+	+	-	-	+	+	-	-
Methanol	-	+	+	-		-	+	-	-	-

+: indicates positive activity - : indicates negative activity

Acetone and methanol extracts of leaves, stem and root of *Cocculus hirsutus* were inoculated against *Bacillus subtilis* and *Staphylococcus aureus* in the concentration of 4-0.5 mg/100 µl. The growth of *Bacillus subtilis* and *Staphylococcus aureus* were inhibited by *Cocculus hirsutus.* 4-1 mg/100 µl of acetone and methanol extracts were found to be more effective against all the microorganisms analysed.

Minimum Bactericidal Concentration

Based on the minimum inhibitory concentration results, the minimum bactericidal concentration (MBC) was performed against *B. subtilis, E. coli, S. aureus, S. dysentriae* and *K. pneumoniae.* There was no visible growth of any of these microorganisms.

Hence *Cocculus hirsutus* was found to have significant antibacterial activity against the microorganisms tested. The results of the present study support the traditional usage of *Cocculus hirsutus* and it can be recommended for usage as antimicrobial agents in new drugs for the therapy of infectious disease caused by pathogens.

Conclusion

Based on the results, it can be concluded that the *Cocculus hirsutus* plant extracts have great potential as antimicrobial components against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antibacterial activity and the underlying mechanisms.

References

1. Shai LJ, McGawa MA, Aderogbaa LK, Eloff JN, Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm. f) leaves, J. Ethnopharmacol, 119, 2008, 238-244.
2. Firas A, Bayati A, Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts, J. Ethnopharmacol., 116, 2008, 403-406.
3. Ushimaru PI, silva M, Stasi L, Barbosa L, Junior AF, Antibacterial activity of medicinal plant extracts, Brazilian Journal of Microbiology, 38, 2007, 717-719.
4. Patel VR, Patel PR, Kajal SS, Antioxidant activity of some selected medicinal plants in western region of India, Advances in Biological Research, 4(1), 2010, 23-26.
5. Panda BK, Mishra US, Antibacterial activity of the leaves of *Cocculus hirsutus*, Indian Drugs, 44, 2007, 108-110.
6. Ganapathy S, Dash GK, Suresh P, Diuretic, laxative and toxicity studies of *Cocculus hirsutus* aerial parts, Fitoterapia, 73, 2002, 28-31.
7. Palsamy P, Malathy R, Evaluation of hypoglycemic and hypolipidemic activity of methanol extract of *Cocculus hirsutus* (L) diels leaves in sreptozotocin-induced diabetic mellitus rats, Int. J. Biol. Chem., 1, 2007, 205-12.
8. Jayakar B, Sangameswaran B, Antidiabetic and spermatogenic activity of *Cocculus hirsutus* (L) Diels, African. J. Biotech., 6, 2007, 1212-16.
9. Kritikar KR, Basu BD, Indian Medicinal Plants, Sri Satguru Publication, New Delhi, 3rd revised enlarged edition, 1, 2002, 120.
10. Badole S, Patel N, Badhankar S, Jain B, Bharadwaj S, Antihyperglycemic activity of aqueous extract of leaves of *Cocculus hirsutus* (L) Diels in alloxan induced diabetic mice, Ind. J. of Pharmacol., 38(1), 2006, 49-53.
11. Thakare SP, Jain HN, Patil SD, Upadhyay UM, Hepatoprotective effect of *Cocculus hirsutus* on bile duct ligation-induced liver fibrosis in Albino Wistar rats, Bangladesh. J. Pharmacol., 4, 2009, 126-130.
12. Rios JL, Recio MC, Villar A, Screening methods for natural products with antibacterial activity, A review of literature, J. Ethnopharmacol., 23, 1988, 127-149.
13. Eloff JN, A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria, Planta Med., 64, 1998, 711-713.
14. NCCLS, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard Fifth edition, 2000, NCCLS dominant M1745; NCCLS, Wayne, PA. USA.
15. Kaneria M, Baravalia Y, Vaghasiya Y, Chanda S, Determination of antibacterial and antioxidant potential of some medicinal plants from Saurashtra region, India, Indian. J. Pharm. Sci., 71(4), 2009, 406-412.