

**ANTIMICROBIAL ACTIVITY OF THE ROOTS OF *COCCULUS HIRSUTUS*****Satish Nayak and A.K. Singhai**Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidhyalaya, Sagar (M.P.)  
India

Received : 03.10.2002

Accepted : 25.12.2002

**ABSTRACT:** *Cocculus hirsutus* Linn was studied for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* using agar disc-diffusion method. Petroleum ether extract, Ethanolic extract and Crude alkaloidal fraction were screened for the activity in various concentrations and zone of inhibitions were recorded. Results suggest that the Ethanolic extract and Crude alkaloidal fraction have significant antimicrobial activity against test microorganisms and the activity is found to be concentration dependent. Present findings justify the claimed uses of *Cocculus hirsutus* in the indigenous systems of medicine to treat various infectious diseases.

**Key words :** Antimicrobial activity, *Cocculus hirsutus*, Menispermaceae.

**INTRODUCTION**

*Cocculus hirsutus*<sup>1</sup>, Linn., Diels, belongs to the family Menispermaceae commonly known as Jaljamini / Jalyamini is a climbing shrub found in the tropical & subtropical parts of India, South China, Africa, Arabia and Ceylon. Ayurveda describes the uses of its roots to destroy "Kapha & Vata", lessen bile and in burning sensation it enriches the blood and is useful in urethral discharges. It is also used as refrigerant, laxative, in chronic rheumatism, venereal diseases, fevers and in syphilitic cachexia<sup>2,3</sup>. Alcoholic extract of the roots have shown significant analgesic, anti-inflammatory<sup>4</sup>, hypoglycemic and cardio tonic effects<sup>5</sup>. A number of phytoconstituents, including alkaloids & sterols have been reported<sup>6-12</sup>. i.e. 2-sitosterol, ginnol, a monomethylether of inositol, hirusidol, hirusitine, isotrilobine, magnoflorine, colclaurin, cohirsutinine and shaheenine.

The plant is well reputed in traditional system of medicine; it is being used by local

tribal people to treat various diseases. Present investigations were carried out to justify its uses in the indigenous system of medicine.

**MATERIALS AND METHODS****Plant Material**

The root of *Cocculus hirsutus* were collected from Tilli village, Sagar, (M.P.) in the month of November. Its identity was confirmed from the department of Botany, Dr. Harisingh Gour Vishwavidhyalaya, Sagar. The collected roots were washed, dried in shade and crushed to coarse powder.

**Extraction**<sup>13-14</sup>

Powdered plant material (2 kg) was defatted with petroleum ether (40-60<sup>0</sup>) in a soxhlet apparatus. Petroleum ether extract was concentrated using vacuum to remove the solvent. Petroleum ether extract (PTE) so obtained (yield 2.6%) was kept in a cool

place. Defatted powdered material was air dried to complete evaporation of petroleum ether and was macerated with ethanol (95%) for 24 hours and filtered. Ethanolic extract (ETE) was dried under reduced pressure to remove solvent completely (yield 10.6%).

Ethanolic extract (100 grams) was treated with 5% H<sub>2</sub>SO<sub>4</sub> and filtered. The aqueous acidic extract was extracted with chloroform and chloroform layer was discarded. It was then basified with ammonia to P<sup>H</sup> 10 and extracted with chloroform. Chloroform layer was separated. Solvent evaporated and total crude alkaloids (CAE) were collected (yield 7.8%).

The presence of alkaloids in ethanolic extract and crude alkaloidal fraction was confirmed using qualitative chemical tests and thin layer chromatographic methods.

### ANTIMICROBIAL ACTIVITY<sup>15-19</sup>

The antimicrobial activity of the extracts PTE, ETE, and CAE was tested *in vitro* using *Staphylococcus aureus*, *Escherchia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*, collected from department of Microbiology, Dr. Harisingh Gour vishwavidhyalaya Sagar. The growth Media were nutrient agar and nutrient broth. Different concentrations of extracts were prepared using dimethyl sulphoxide (DMSO) as a solublizing agent. Concentrations prepared for PTE and ETE were 50,100, 200 and 300 mg and for CAE, 10, 25, 50 and 100 mg respectively. The molten nutrient agar was incubated with a 0.2 ml suspension of the test organism using sterile Pasteur pipette. Agar suspension was rotated for even distribution of the organisms and the mixture quickly poured into sterile petri dises. The seeded plates were allowed to solidify after which fine wells were bored in each plate with a cork

borer (6 mm), with the aid of sterile Pasteur pipette each well was filled with different concentrations of the extracts and the vehicle (DMSO). The Petri disches were incubated at 37<sup>0</sup>c for 24 hours. The experiments were carried out in triplicate and the average diameter of zone of inhibitions were recorded.

Results were expressed as mean  $\pm$  standard deviation. The significance of difference was determined by student 't' test (P<0.05).

### RESULTS AND DISCUSSION

The antimicrobial activity of the roots of *Cocculus hirsutus* indicate that the ethanolic extract and crude alkaloidal fraction have significant antimicrobial activity against the test microorganisms. The zones of inhibition in diameter (mm) recorded for petroleum ether extract (PTE) ethanolic extract (ETE) and crude alkaloidal fraction (CAE) are presented in table-1. PTE is not found to significantly effective against the test microorganisms in selected concentrations while ETE shows better.

Activity against *Salmonella typhi* and *Staphylococcus aureus* than *Pseudomonas aeruginosa* and *Escherchia coli* in the concentrations of 50, 100, 200 and 300 mg respectively. CAE has more potent significant activity in the concentrations of 10, 25, 50 and 100 mg and gives better results against *Salmonella typhi* and *Staphylococcus aureus* that two other test organisms. The activity is found to be concentration dependent in ETE and CAE. The results also indicate that the CAE is significantly effective in less concentrations than the ETE. Since ETE and CAE both have shown the significant activity, it can be interpreted that the antimicrobial activity against test microorganisms is due to any one or more alkaloids of the plant.

Present findings support the applicability of *Cocculus hirsutus* in traditional systems for its claimed uses like fever, inflammation, urinary and vaginal infectious diseases. Now-a-day microorganisms acquiring

resistance to many commonly used antibiotics. Hence there is a need to search for new antibiotics. Plants continue to be a rich source of therapeutic agents and present study reveals the potential value of *Cocculus hirsutus*.

**Table – 1: Antimicrobial activity of the roots of *Cocculus hirsutus***

S. No.	Microorganisms	Zone of inhibition in diameter ± SD (mm)											
		PTE				ETE				CAE			
		50 mg	100 mg	200 mg	300 mg	50 mg	100 mg	200 mg	300 mg	10 mg	25 mg	50 mg	100 mg
1	<i>Staphylococcus aureus</i>	2020±	2.46±	2.46±	2.25±	4.66±	5.06±	5.83±	6.40±	5.63±	6.58±	8.16±	8.40±
		0.152	0.057	0.152	0.153	0.152	0.230	0.057	0.173	0.208	0.321	0.208	0.173
2	<i>Escherchia coli</i>	2.46±	2.33±	1.90±	2.13±	3.76±	4.70±	6.13±	7.73±	4.16±	5.66±	6.60±	8.10±
		0.057	0.208	0.100	0.115	0.152	0.173	0.057	0.208	0.115	0.152	0.100	0.115
3	<i>Pseudomonas aeruginosa</i>	2.10±	2.00±	2.23±	2.00±	3.50±	4.10±	4.26±	4.73±	4.06±	4.86±	4.96±	5.56±
		0.264	0.100	0.251	0.200	0.300	0.100	0.208	0.288	0.057	0.057	0.115	0.233
4	<i>Salmonella typhi</i>	2.40±	2.26±	2.26±	2.13±	5.43±	6.86±	7.73±	8.90±	6.00±	7.00±	8.86±	9.33±
		0.280	0.115	0.152	0.208	0.251	0.057	0.115	0.100	0.152	0.100	0.057	0.115

PTE : Petroleum ether extract, ETE : Ethnolic extract, CAE: Crude alkaloidal extract, \*P<0.05

## REFERENCES

1. Chopra, R.N., Nayar, S.L., Chopra, I.C., "Glossary of Indian Medicinal Plants", National Institute of Science Communication, New Delhi, IV Edition (reprint), 72, (1996).
2. Kritkar, K.R. and Basu, B.D., "Indian Medicinal Plants", B.S. Mahendra Pal Singh, Delhi, Vol-1 II Edition (reprint), 86 (1995).
3. Nadkarni, K.M., Nadkarni, A.K., Chopra, R.N., "Indian Materia Medica", Popular Prakashan Bombay, Vol – 1, 362 (1995).
4. Nayak, S.K., Singhai, A.K., *Ind. J. Nat. Prod.* 9(1), 12 (1993)
5. Satyanarayana, K., Mangathayaru, V., Sreekanth, J., Venkateswarlu, V and Kokate, C.K., *Ind. J. Pharm. Sci.*, 63(1), 30(2001).
6. Merchant, J.R., Naik, K and Hirwe, S.N., *J. Ind. Chem. Soc.*, 39, 411 (1962).
7. Ahmad, V.U., Mohammad, F. V., Rasheed, T., *Phytochemistry*, 26, 793, (1987).
8. Tripathi, V.J., Ray, A.B. and Dasgupta, B., *Ind. J. Chem.* 14B, 62 (1976).
9. Ahmad, V.U., Rasheed, T. and Iqbal, S. *Phytochemistry*, 30, 1350 (1991).
10. Rasheed, T., Khan, M.N.I and Zhadi, S.S.A. *Fitoterapia*, 62, 157 (1991).
11. Ahmad, T., Khan, M.N.I., Zhadi, S.S.A and Durrani, S., *J.Nat. Prod.*, 54, 582, (1991).
12. Ahmad, V.U. and Iqbal, S. *J. Nat. Prod*, 55, 237, (1992).
13. Evans, W.C., "Trease and Evan's Pharmacognosy", W. B. Saunder Company Noida, Delhi, XIII Edition, 343, (1989).
14. Brain, K.R. and Turner, J.D., "The Practical Evaluation of Phyto Pharmaceuticals", Wright scientechnica, Brister, 99 (1975).
15. Lovain, V., "Antibiotics in Laboratory Medicin", Willians, Baltimore London, 7, (1980).
16. Carter, S.J. (Editor) "Cooper and Gunn's Tutorial Pharmacy", CBS Publishers & Distributors, Delhi, VI Edition, 317, (1986).
17. Pandya, K.K., Patel, R.B. and Charkravarty, B.C., *Indian Drugs*, 27 (8), 415, (1990).
18. Grisanapan, W., Wuthi – Udomlert, M., Caichompoo, W., *Phytomedicine*, 7 (suppl-II), 85 (2000).
19. Portillo, A., Vila, R., Freixa, B., Adzet, T. and Canihual, S., *J. Ethnopharmacology*, 76 (1) 93 (2001).