

Pharmacological Study of Alkaloid Hirsutine-3-o-Glycopyranoside Isolated from Roots of *Cocullus hirsutus*

A.K Misra¹, P.Gouda²

¹Department of Chemistry, Rayagada(Autonomous) College, Rayagada, Odisha, India

²P.G Department of Chemistry, Khallikote(Autonomous) College, Berhampur(760001), Odisha, India

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ABSTRACT

Cocullus hirsutus belongs to the family Menispermaceae. It is a perennial herb (climber) growing widely in India and is used by the Indian tribes for a wide range of ailments. A good number of alkaloids and terpenoids have been isolated from the aerial parts of the plant. In our present work the root part of the plant have been used for isolation. Characterisation was done by taking their UV, IR, H-NMR, ¹³C-NMR, mass and elemental analysis. The antifungal and antimicrobial properties of the compounds were studied following standard procedures. The antimicrobial activity against clinically important bacteria such as Escherichia coli (MTCC 1195), Enterobacter aerogenes(MTCC 2823), Klebsiella pneumonia(MTCC 2405), Salmolnella typhi(MTCC 733), Proteus vulgaris(MTCC 1771) and Pseudomonas aeruginosa - gram negative(MTCC 2642), staphylococcus aureus(MTCC 1430), Bacillus cereus- gram positive(MTCC 1272) was done. The in vitro antimicrobial activity was performed by agar disc diffusion method. The result showed that the ethanol extract of the roots of *Cocullus hirsutus* highly affected the activity of Pseudomonas aeruginosa and Bacillus cereus. The inhibition against Staphylococcus aerues and Enterobacter aerogenes was moderate and remaining bacterial strains had no activity. The study suggests that the roots of *Cocullus hirsutus* obtained by infusion can be used in the treatment of various bacterial diseases.

Keywords: Menispermaceae, anti-bacterial, antifungal, pathogenic bacteria,

INTRODUCTION

Herbal medicines are gaining importance day by day due to their reduced side effects. *Cocullus hirsutus* belongs to the family Menispermaceae which has been used successfully in the indigenous system of medicines. It is a climbing shrub with bulky green leaves.

The aqueous extract of the aerial parts show significant diuretic activity and laxative effect in rats^{1,2}. The juice of the leaves used externally as cooling and soothing agent in purtigo, eczema and impetigo^{3,4}. The roots of *Cocullus hirsutus* possess anti-inflammatory and

analgesic property⁵. It has been reported that the leaf extract has a significant anti-hyperglycaemic property^{6,7}. Many chemically active compounds have been isolated from the leaf^{8,9} and root extracts¹⁰ of this plant and the biochemical properties possessed by them have been studied widely^{11,12}. In our present work a new alkaloid has been isolated and its antibacterial and antifungal properties have been studied. It is found that the isolated compound exhibits antifungal and antibacterial activities.

MATERIAL AND METHODS

About 3kg of the root parts of the plant *Cocullus hirsutus* has been collected from the hilly areas of Ganjam and Gajapati districts of Odisha, India and authenticated by comparing with the herbarium specimen (file no.108) of Khallikote College, Berhampur.

General instruments used: UV Shimadzee, UV-VIS 1700, H-NMR, ¹³C-NMR, GC-MS

The collected roots were pulverized and shade dried. These were powdered by grinding with the help of a grinder. The powder was kept submerged in the dry ethanol for fifteen days. It was then filtered and the filtrate was concentrated to dryness. The residue was treated with hot ethanol using soxhlet apparatus. Both the compounds were compared using TLC method and found to produce identical spots on the TLC. Both the contents



Fig 1. Photographic image of the plant *Cocullus hirsutus*

were mixed and evaporated to dryness. The dry extract was then treated with distilled water to remove water soluble constituents. The water insoluble part was dried perfectly and taken in benzene. It was subjected to column chromatographic method of separation using silica gel 60-120 mesh and benzene petroleum ether indifferent ratio as eluent. Compound C was recovered in considerable amount along with other two compounds (A& B) as minor constituents.

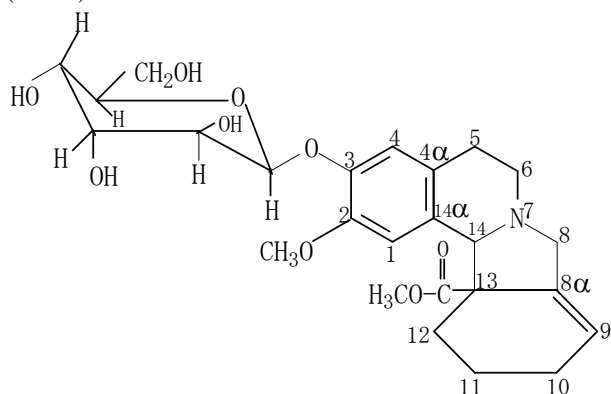


Fig 2. Structure of Hirsutine-3-O-glucopyranoside

The isolated compounds were tested for their anti-bacterial activities by using against *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Salmolnella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa* (gram negative), *Staphylococcus aureus* and *Bacillus cereus* (gram positive) pathogenic bacteria. The disc diffusion assay showed that the ethanol root extract of *Cocullus hirsutus* inhibit the activity of *Staphylococcus aureus* and *Enterobacter aerogenes* was moderate and the remaining strains *Escherichia coli*, *Klebsiella pneumonia*, *Salmolnella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa* (gram negative), *Staphylococcus aureus* and *Bacillus cereus* (gram positive) had no activity. Ethyl acetate and acetone extract showed very poor activity against the above mentioned bacteria (Table-1).

The isolated compounds A, B and C were also tested for their anti-fungal activity using *A.fumingatus*, *A.flavus* and *A.niger* under MDA, DDA and PSGI conditions. Compound A did not show any positive antifungal character whereas B & C responded to the test (Table-2).

Table 1: Antifungal study

Compound Name	A	B	C
	<i>A.fumingatus</i>	-	-
	<i>A.flavus</i>	-	-
MDA	<i>A.niger</i>	-	0.25
	<i>A.fumingatus</i>	-	0.125
DDA	<i>A.flavus</i>	-	0.1
	<i>A.niger</i>	-	0.2
PSGI	<i>A.fumingatus</i>	-	-
	<i>A.flavus</i>	-	0.25
	<i>A.niger</i>	-	0.15

Table 2: Antibacterial Study

Compound Name	A	B	C
<i>Escherichia coli</i>	0.125	0.25	0.12
<i>Enterobacter aerogenes</i>	-	-	-
<i>Klebsiella pneumonia</i>	-	-	-
<i>Salmolnella typhi</i>	0.6	0.5	0.6
<i>Proteus vulgaris</i>	-	-	-
<i>Pseudomonas aeruginosa</i>	0.115	0.125	0.11
<i>Staphylococcus aureus</i>	0.262	0.262	0.215
<i>Bacillus cereus</i>	-	-	-

RESULTS AND DISCUSSIONS

Analytical Methods: Column chromatography was carried out using silica gel 60-120 mesh (Ranbaxy). TLC was also carried out using silica gel G on a Bruker DRX 300 spectrophotometer, with TMS as an international standard.

From the root part of *Cocullus hirsutus* three compounds have been isolated of which hirsutine-3-O-glycopyranoside is the major product. The spectral data of the compound is specified below.

UV (CH_3OH), λ_{max} in nm: 220, 240 and 302 showed aromatic - * transition.

IR in KBr shows signals at 3400cm^{-1} for an -OH group at 1739.95cm^{-1} for > C=O group and aromatic absorption at 1464.10, 1373.44, 1174.75, 1033.94 and 723.37.

$^1\text{H-NMR}$ (300MHz) spectral data showed peaks at in ppm: 1.607(2H,m, $\text{C}_{11}\text{-H}$), 2.278(2H, $\text{J}=13\text{Hz}$, $\text{C}_{12}\text{-H}$), 2.762(2H, d, $\text{J}=10.1\text{Hz}$, $\text{C}_5\text{-H}$), 2.308(2H,d, $\text{J}=10\text{Hz}$, $\text{C}_6\text{-H}$), 4.130 (3H,s,2X-OCH₃), 4.138(1H, s, $\text{C}_{10}\text{-OCH}_3$), 5.341(1H,m, $\text{C}_9\text{-H}$), 7.20(1H,s, $\text{C}_1\text{-H}$) and 7.28(1H,s, $\text{C}_4\text{-H}$).

$^{13}\text{C-NMR}$ (CDCl_3 , 100MHz, ppm): 22.6(C_{11}), 27.14(C_{14}), 31.16(C_8), 31.51(C_6), 31.52(C_5), 39.72(C_{13}), 42.29(C_7), 50.03(-OCH₃), 51.35($\text{C}_{13}\text{-OCH}_3$), 56.68(C_6'), 56.68(C_1'), 60.08(C_3'), 76.72(C_2'), 77.04(C_4'), 77.36(C_5'), 122.55(C_1), 128.0(C_9), 129.95(C_3), 130.14(C_2), 173.79(>C=O group).

Mass (m/e): 491(M^+). 324, 311, 307(100%), 298, 293, 265, 256 and 245.

CHN: C: 63.3%; H: 5.9%; N: 2.5% against calculated

C: 63.0%; H: 6.0%; N: 2.3%

The above spectral and analytical data also corroborated with Laissegnes's Test. The structure of the isolated compound is depicted.

CONCLUSION

The compound Hirsutine-3-O-glucopyranoside is isolated from the plant species *Cocullus hirsutus* having antifungal and antimicrobial activities. It also has anti-diabetic and anti-hepato-protective activities. The root paste has been traditionally used by the tribal population as an antifungal medicine which has been supported by our experimental observations.

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