

Antibacterial activities of new 5,7-dihydroxy 13,14-dimethoxy flavanone from *Cyathula tomentosa*

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Abstract: From ethanolic extract of *Cyathula tomentosa* plant a new 5,7-dihydroxy, 13,14-dimethoxy flavanone has been isolated and characterized with help of FAB mass, ^1H , ^{13}C NMR, DEPT, HMQC and HMBC studies, compound gave positive tests against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *Mycobacterium smegmatis*.

Keywords: *Cyathula tomentosa*, 5,7-dihydroxy, 13,14-dimethoxy flavanone, antibacterial activities.

1. Introduction

Cyathula tomentosa (Kurru) belongs to family Amaranthaceae, is a perennial under shrub occurs throughout Garhwal Himalayas up to 600-2000 meter altitude, *Cyathula tomentosa* has been used in snake bite and has emetic properties [1]. From *Cyathula capitata* and *Cyathula officinal* ecdyson content as 0.046% and 0.057% isolated respectively [2] and *Cyathula prostrata* show antifungal free radical scavenging activities 2,2-diphenyl-1-picryl hydrazyl [DPPH] radical [3]. The chemical examnants at the basis of [4] has been reviewed. From ethanolic extract of *cyathula tomentosa* a new Disaccharide and flavone were isolated. The structure of compounds have been elucidated through. mass, ^1H , ^{13}C NMR, DEPT, HMQC and HMBC spectra with antibacterial activities.

2. Experimental

2.1. General:

^1H -NMR at (400 MHz), ^{13}C -NMR at (75 MHz) TMS as internal standard, using DMSO as solvent column chromatography was carried out on silica-gel 60-120 mesh (Merck). TLC was performed on percolated silica-gel. The eluting solvent was CHCl_3 -MeOH spots were visualized by 7% H_2SO_4 followed by heating.

2.2. Plant material:

The whole plant of *Cyathala tomentosa* was collected from Bacchehar District. Chamoli Uttrakhand in the month of October and identified by Department Botany, P.G. College Gopeshwar where voucher specimen was deposited.

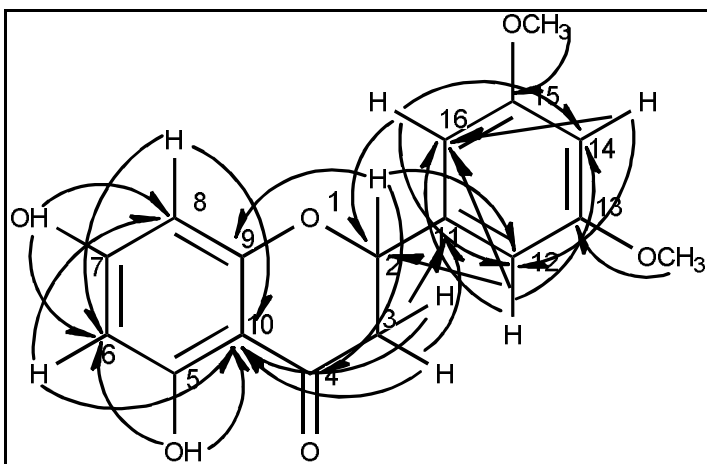
2.3. Extraction and isolation:

The air dried whole plant (3kg) was exhaustively extracted with 95% aqueous ethanol at 30-50°C (for 48 hours) on a heating mantle. The ethanol extract was concentrated to dryness under reduced pressure to yield a black brown residue(200g) and then adding to the top of the column prepared using 500g silica gel(60-120

mesh; Merch, Mumbai, india) in chloroform. The dry ethanolic extract was chromatographic over silica-gel using Methanol:Chloroform elution solvent at (30:70) afforded a new flavanone.

3. Result and Discussion

It was crystallized from MeOH as colorless crystallized solid M.P.278⁰C [approx]. On the basis of elemental analysis and its molecular formula deduced as C₃₀O₁₅H₃₈. It gave positive Molish test and Shinoda test indicated the presence of flavone. The molecular weight of compound was 300 amu derived from its FAB-MS showed molecular ion peak at m/z 301 fragment peaks 132, 154, 208 etc.



The ¹H NMR spectrum showed singlet at δ 6.77 and 6.41 indicated a tetra substituted aromatic function and singlet at δ 6.07 assigned for H-2 and doublet at δ 4.01 for axial and multiplies at 2.4 for equatorial for H-3 of flavanone group. Another two doublets at δ 7.99 (J=7.6Hz) and 7.06 (J=6.8Hz) were assigned for H-16 and H-15 whereas a singlet at δ 7.19 revealed H-12 of trisubstituted benzene ring. The position of one singlet at δ 13.11 indicated presence of one tri-substituted hydroxyl- group forming intermolecular hydrogen bond with carbonyl group assigned at position C-5 and one singlet at δ 9.11 for hydroxyl group assigned at position C-7. Further, the sharp singlet at δ 3.86 was assignable for two methoxy group.

¹H, ¹³C NMR, DEPT and HMBC data of compound in DMSO.

Position	δ _H (J,Hz)	δ _C	DEPT	HMBC
2	6.0s	77.7	CH	C-9, C-16, C-12
3	2.4m(eq) 4.0m(ax)	45.5	CH ₂	C-11 C-11, C-10
4	-	182.7	C	-
5	-	161.7	C	-
6	6.4s	97.1	CH	C-8, C-10
7	-	96.6	C	-
8	6.8s	92.7	CH	C-10, C-6
9	-	161.1	C	
10	-	104.4	C	
11	-	137.1	C	
12	7.2s	122.7	CH	C-14, C-16
13	-	156.6	C	
14	-	163.3	C	
15	7.0d(6.8Hz)	116.7	CH	C-11, C-13
16	7.9d(7.6Hz)	129.9	CH	C-2, C-12, C-14
OH-5	13.1s	-	-	C-10, C-6
OH-7	9.11s	-	-	C-9, C-5
OMe-13,14	3.8s	55.9	CH ₃	C-13, C-14,

These values were confirmed by ^{13}C NMR spectra which displayed 17 carbon signals. The highly downfield signals at δ 182.7 showed carbonyl signal. The downfield signals at δ 163.3, 156.6 and 161.7 were due to oxygenated substitution. These values further confirmed by DEPT, HMQC and HMBC spectra. The compound was identity with help of reported data of flavanone [4]. Hence it was identified as 5,7-dihydroxy, 13,14-dimethoxy flavanone .

The compound showed positive tests for some bacterial cultures by use agar well diffusion method [5].

(i) *Staphylococcus aureus*- solution of this compound showed 35 positive control (Rifampicin) and 11 mm zone of inhibition against *Staphylococcus aureus*. **(ii) *Staphylococcus epidermidis***- solution of this compound showed 17 positive control (Rifampicin) and 9 mm zone of inhibition against *Staphylococcus epidermidis*. **(iii) *Klebsiella pneumoniae***- solution of this compound showed 24 positive control (Rifampicin) and 11 mm zone of inhibition against *Klebsiella pneumoniae*. **(iv) *Mycobacterium smegmatis***- solution of this compound showed 20 positive control (Rifampicin) and 11 mm zone of inhibition against *Mycobacterium smegmatis*.

Acknowledgement:

Thanks are due to SAIF, CDRI-Lucknow for recording spectra, and SBSPG institute of Biomedical-Dehradun for antibacterial activities.

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