



## Structure elucidation of Maksiterone-A from methanolic extract of *Tinospora cordifolia* and used as antidepressant agent

Arun Kumar

Department of Chemistry, Hindu Post Graduate College Zamania, Ghazipur, India

### ABSTRACT

*Tinospora cordifolia* is an annual or perennial Ayurvedic plant which is used in several traditional medicines to cure various diseases. Common names are Amrita, Guduchi, Shindilakodi (Tamil) and Gulancha. Makisterone-A is extracted from the stem of *Tinospora cordifolia* by Soxhlet by repeated washing with predistilled methanol (organic solvent) under reflux. TLC and Column Chromatography techniques are used to isolate and purify Makisterone-A and its structure elucidated by Infra-red and NMR spectroscopy techniques. Makisterone-A was found to be very effective in reducing the depressant potential.

**Keywords:** *Tinospora cordifolia*, Makisterone-A, Antidepressant agent.

### INTRODUCTION

Trees and plants are of paramount importance for human life, not only in the present time but also in the remote past as well. The early man depended on them for his physical needs such as sources for food, shelter, clothing, medicines, ornaments, tools and for spiritual needs like magic or ritualistic practices. Among plants of economic importance, medicinal and aromatic plants have played a vital role in alleviating human suffering. Uses of medicinal plants in the industrialized societies have been traced from the extraction and development of several drugs and chemotherapeutic drugs from these plants as well as from traditionally used rural herbal remedies (1).

Plant species have served as sources of medicine for millennia. Many medical practitioners with training in pharmacology and or pharmacognosy are well aware of a number of modern therapeutic agents that have been derived from plant species. Natural products are chemical compounds derived from plants and these are helpful as dietary supplements and for cure of a variety of ailments. Modern Chemist employs isolation techniques guided by bioassay to isolate the active compounds in pure form (2). The plant mainly contains alkaloids, glycosides, steroids, sesquiterpenoid, aliphatic compound, essential oils, mixture of fatty acids and polysaccharides (3). In the present study a new compound Makisterone-A was isolated from the methanolic extract of *Tinospora cordifolia* and identified by I.R and NMR spectral studies.

*Tinospora cordifolia* is an Ayurvedic plant which belongs to family Menispermaceae has important medicinal values. It is widely known as Guduchi, Giloy or Amrita. In India it is known by its various vernacular names, the most commonly used ones are Amrita, gulbet, gurchara (Hindi), Gulvel (Marathi), Amudom, chindil (Tamil). *Tinospora cordifolia* is a glabrous, succulent, climbing shrub distributed throughout tropical Indian subcontinent. The stem rather succulent with long filiform fleshy aerial root from the branches. Its bark is grey-brown or creamy white in colour, its leaves are membranous and flower is small. It appears yellow or greenish yellow when plant is leafless. Male flower is clustered and female usually solitary.

This plant has been widely used in the Indian System of Medicine (Ayurveda) as Rasayana for the treatment of diabetes, jaundice, general weakness, gout (4). A variety of constituents have been isolated from this plant and their

structure were elucidated. They belong to different classes such as alkaloids, diterpenoids lactones, glycosides, steroids, phenolics, sesquiterpenoids, aliphatic compounds and polysaccharides. Some of the important constituents reported so far are Tinosporide (5), Furanolactone diterpene (6), Cordifolloside (7), Choline (8), Lignans (9), Giloinsterol (10), Tinosporic acid (11), Tinosporidene (12), and arabinogalactan (13).

## EXPERIMENTAL SECTION

### Plant material

Fresh stem of *Tinospora cordifolia* was collected from Medicinal Plants Garden, Patanjali, YogPeeth, Hardwar, Dehradun, India. The plant was identified by Acharya Balkrishna, Ayurved Scholar, Patanjali Yogpeeth, Hardwar, Dehradun, India.

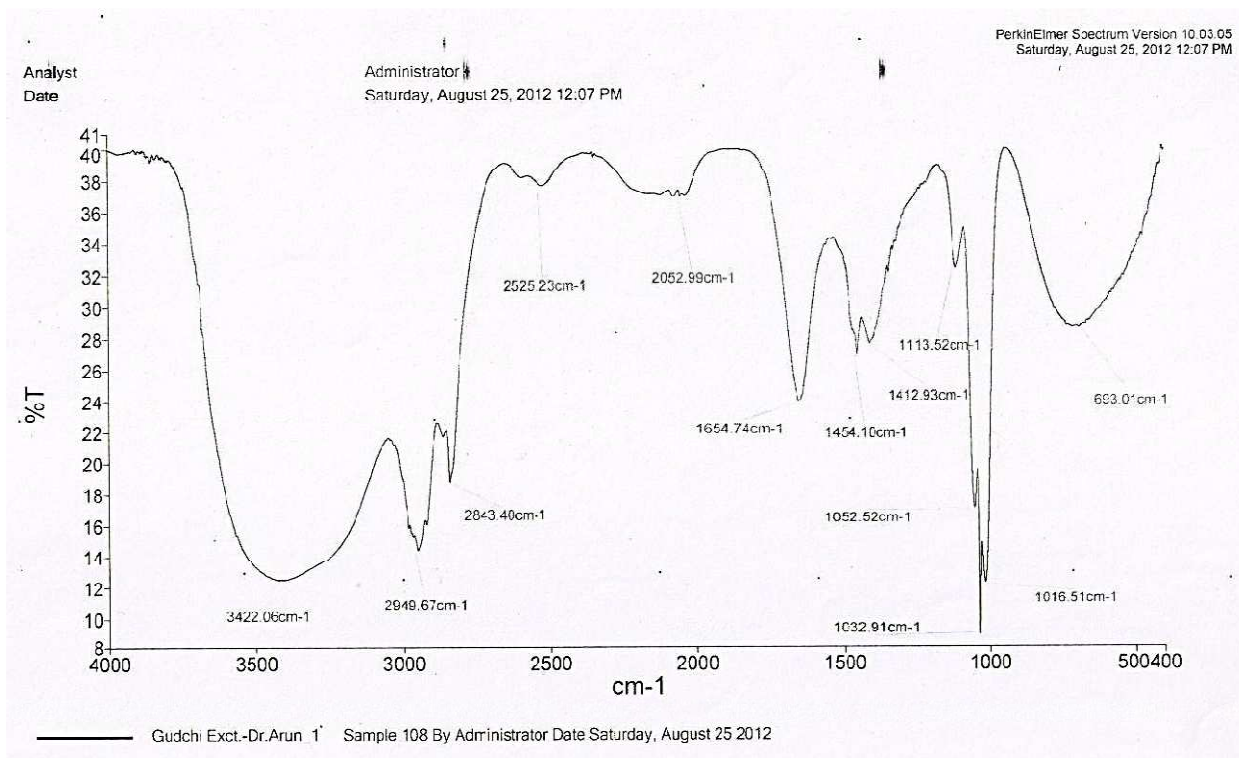
### Preparation of methanolic extract of *Tinospora cordifolia*

The fresh stem collected, washed with distilled water were shade dried for five days and crushed to coarse powder. The powder is employed in Soxhlet extractor for the extraction of natural product by repeated washing with predistilled organic solvent (methanol) under reflux. 100g dried herb powder was taken in a cellulose thimble in the extraction chamber suspended on a 5L RB flask containing 2L methanol (solvent). It is designed such that when the solvent surrounding the sample exceeds a certain level it over flows and trickles back down into the boiling flask. A refluxing condenser with separating funnel help in recondensations of the excess solvent. The flask is heated and the solvent starts evaporating which then moves up into the condenser where it is condensed and trickles into the extraction chamber. At the end of extraction, the flask containing the solvent and the extract is removed wherefrom the solvent is distilled off. This residual extract is weighed and percentage of the organic extract is calculated. This is the crude separated fraction which may contain several compounds. The pure organic constituents were obtained after TLC and Column Chromatography. The structures were elucidated by infrared and NMR spectral studies.

### Infrared spectra

These were recorded in KBr (AR) using Perkin Elmer Spectrum, FT-IR Spectrophotometer in the range 400 – 4000  $\text{cm}^{-1}$ . A pinch of sample and 50mg KBr thoroughly grounded in an agate mortar and the mixture was put on the pallet holder and pressure was applied through the hydraulic machine to make a thin film. Identity of the compound was confirmed by matching the spectra with those reported in Aldrich library (14).

Figure 1: I.R. Spectra of isolated compound from *Tinospora cordifolia* (guduchi)



## NMR Spectra

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on 200 and 30 MHz JEOL AL 300 FTNMR spectrometer using  $\text{CDCl}_3$  and  $\text{D}_2\text{O}$  solvents and TMS as an internal standard. Spectra were matched with those reported in Aldrich Library (15).

Figure.2:  $^1\text{H}$  NMR spectra of isolated compound from *Tinosporacordifolia* (guduchi)

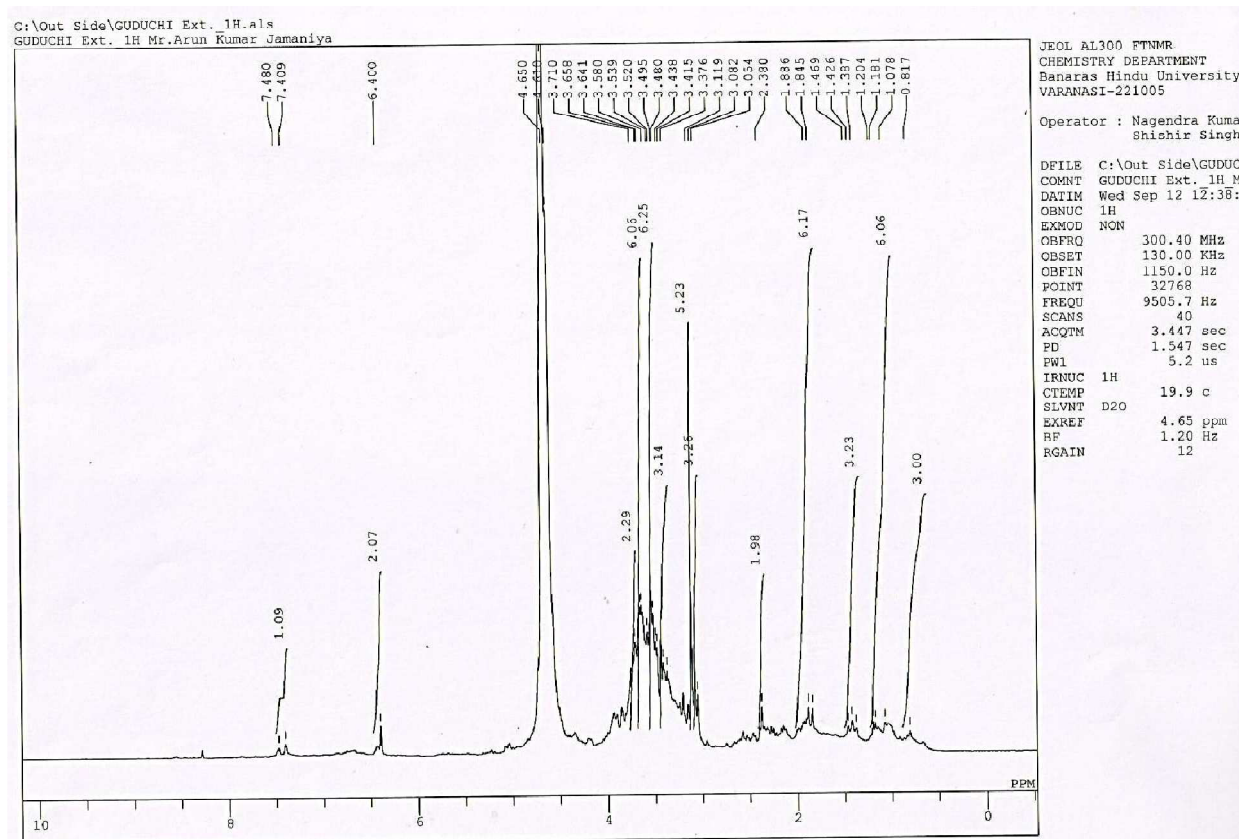
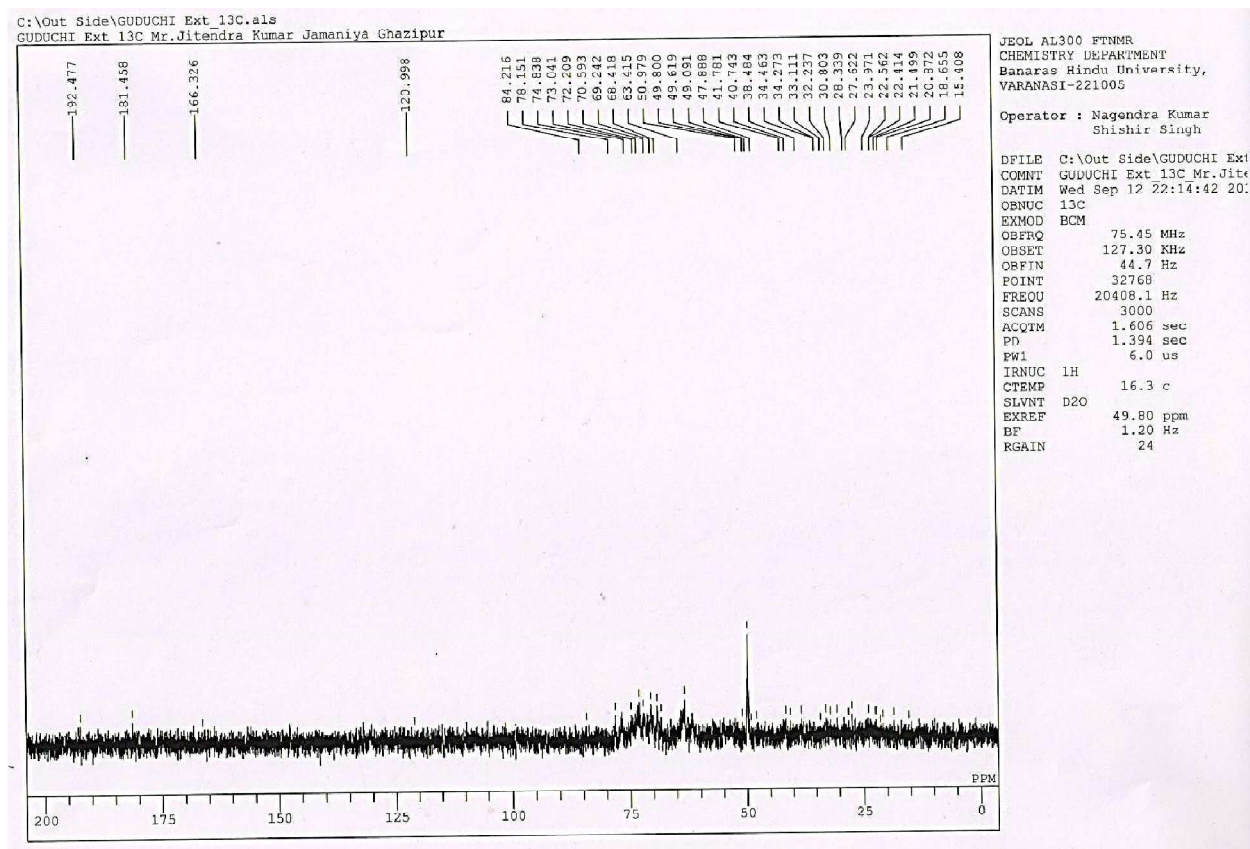


Figure- 3:  $^{13}\text{C}$  NMR spectra of isolated compound from *Tinospora cordifolia*(guduchi)Table- 1:  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR data for makisterone A

C	$\delta$	H	$\delta$
1	37.8	2	4.2 (br, t)
2	68.0	3	4.24 (br, s)
3	68.1	5	3.04 (dd)
4	32.3	7	6.30
5	51.3	9	3.62 (m)
6	203.6	17	2.99 (t)
7	121.6	18-Me	1.27
8	166.1	19-Me	1.10
9	34.4	21-Me	1.62
10	38.6	22	4.02
11	21.1	24	-
12	31.7	26-Me	1.34
13	48.1	27-Me	1.32
14	84.2	28-Me	1.09 (d)
15	32.0		
16	21.3		
17	49.9		
18	17.9		
19	24.4		
20	77.0		
21	21.5		
22	74.7		
23	34.4		
24	41.4		
25	72.2		
26	26.3		

27	28.2		
28	15.8		

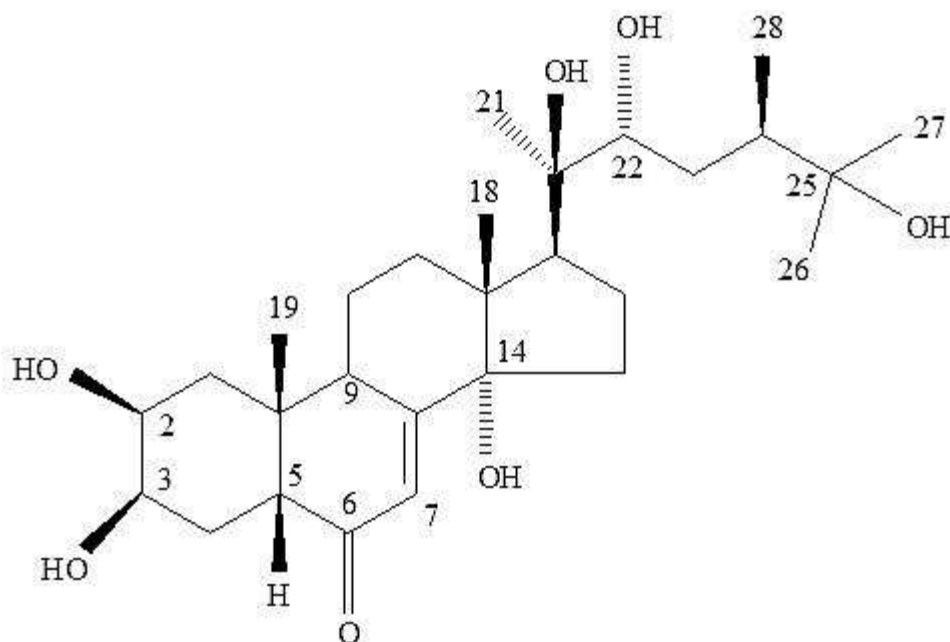


Figure 4 : Makisterone-A

#### Identification of functional group by IR spectral studies

IR spectrum (Figure 1) of the isolated compound registered a broad band at  $3422\text{ cm}^{-1}$  and stretching band at  $1654\text{ cm}^{-1}$ . This indicates the presence of OH group and carbonyl (C = O) group. The spectrum also shows peak at  $693\text{ cm}^{-1}$ ,  $1016\text{ cm}^{-1}$  and  $1032\text{ cm}^{-1}$  which indicates the presence of cyclic ring stretching.

#### Structure elucidation of active constituent by $^1\text{H}$ NMR and $^{13}\text{C}$ MNR spectral studies

$^1\text{H}$  NMR spectrum (Figure 2) of the isolated compound show 29 peaks. The spectrum show 6 peaks for 6 methyl proton from  $\delta 1.09$  to  $\delta 1.62$ . The spectrum show ten  $\text{H}_a$  proton at  $\delta 3.99$ , six  $\text{H}_b$  proton at  $\delta 3.56$ , three  $\text{H}_c$  protons at  $\delta 4.61$ .  $^1\text{H}$  NMR spectrum shows the presence of 46 protons.

$^{13}\text{C}$  NMR spectrum (Figure 3) of the isolated compound shows a peak at  $\delta 166$  for the presence of carbonyl (C=O) carbon. There are 35 peaks from  $\delta 15.40$  to  $\delta 192.47$  including 7 peaks for reference compound.  $^{13}\text{C}$  NMR spectrum shows the presence of 28 carbon.

#### Identification of active constituent

All spectral data in (Table-1) confirms that the isolated compound is 2 $\beta$ ,3 $\beta$ ,14 $\beta$ ,20 $\beta$ ,22R,25 – hexahydroxy – 5 $\beta$  – 24Rergost – 7 – en – one, which is commonly called Makisterone – A (Figure 1).

The molecular weight of the isolated compound is 478.66 and molecular formula  $\text{C}_{28}\text{H}_{46}\text{O}_6$ , needle shaped crystal m.p.  $263^{\circ}$ - $256^{\circ}\text{C}$ .

## DISCUSSION

Phytochemicals are defined as bioactive non-nutrient plant compounds found in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major chronic diseases(16). The medicinal values of plants i.e their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds produce a definite physiological action on the human body(17). Medicinal plants contain many antioxidants such as vitamin, carotenoids, flavonoids, polyphenols. Several reports say that these compound possess remarkable antitumor, antidiabetic, antioxidant activity and antidepressant activity (18,19,20).

Mental depression represents a major public health problem worldwide. The high prevalence of suicide in depressed patients (up to 15%) coupled with complications arising from stress and its effects on the cardiovascular system have suggested that it will be the second leading cause of death by the year 2020. The use of alternative medicines is increasing worldwide day by day. Thus, there is a constant need to identify newer natural antidepressants with greater efficacy, and to explore their potential over synthetic antidepressant. *Tinospora cordifolia* (Family: Menispermaceae), a well known plant of Indian Medicinal System, was selected for evaluating antidepressant like activity in laboratory animals, since this plant has been reported to possess antistress activity(21). According to the Ayurvedic System of medicine, *Tinospora cordifolia* is an antispasmodic, analgesic, antipyretic, antiperiodic, bitter tonic, blood purifier, antidiabetic and digestant. The chemical constituent Makisterone-A is ansteroid widely used as antidepressant agent. Dhingra and Goyal study the action of antidepressant potential of *Tinospora cordifolia* in mice(22).

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