

Identification of Secondary Metabolites in *Senna Auriculata* Using Spectroscopic Methods

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ABSTRACT:

Senna auriculata [avarampoo in tamil] which is a backbone of all traditional plants are used to treat diabetes , nocturnal emissions, urinary discharge and other irritations. The crude was made with methanol extracts by soxhlet apparatus and thin layer chromatography is done . Since results were good, the sample was initiated to uv spectroscopy and fourier transform infrared spectroscopy in perkinelmer spectrum version 10.4.4 .

Keywords : *Senna auriculata*; Medicinal plant; Methanolic crude; TLC; UV; FTIR

INTRODUCTION

Traditional medicines play a major role in health care system. medicinal plants are like ‘back bone’ of those traditional plants. Plants, especially medicinal plants are using to treat various diseases in national level. At global level, different plants and its applications are helpful to society to control different diseases. These plants which have medicinal value and its secondary metabolites show enormous effect in traditional medical field. Due to side effects of modern medicines, people are slowly attractive towards the natural medicines. First India is origin of traditional medicine like siddha, Ayurveda, homeopathy which are natural based treatments without side effects. Again modern medicines are slowly replacing by natural medicines in many cases. According to report of health ministry of national and global levels, mostly in different countries people depends on the minor needs depends on the natural medicines. Total more than the thousand and five hundred drugs which are different varieties of natural herbs origin of different varieties.

SENNA AURICULATA is a herbal plant found in india from the family *FABACEA* .its synonym is *CASSIA AURIVULATA* LINN and commonly known by ‘avaramsenna’ , ‘avarampoo’ . ‘Tanner’s cassia . A shrub with large yellow bright flowers are distributed in different positions in different forest ranges which occupies a good place in traditional system. The plant has been reported to possess antipyretic , hepato protective , anti-diabetic , anti-peroxidative , anti-hyperglycemic and antimicrobial activity .

CLASSIFICATION:

- Kingdom : plantae
- Order : fabales

- Family :fabaceae
- Leo: caesalpiniodeae
- Folk :cassia
- Genous : cassia
- Species: cassia auriculata

These plants have different parts, among all parts the yellow colour flowers have more medicinal values, in India using in preparation of herbal formulations to treat various lifestyle disorders, mainly to control sugar levels in blood (Fig. 1).

The flowers are used to treat diabetes, nocturnal emissions, urinary discharges and irritations in throat. They are the one of the constituent of poly herbal formulation “DIASULIN” in concentration range of 40 mg/dl which is proven to have anti-diabetic activity.

The dried flower bud powder is used as substitute for tea called ‘ kalpa herbal tea ‘ which is consumed by patients suffering from diabetes mellitus , and mixture of ‘avaraipanchagachooram‘ used for ophthalmia, conjunctivitis and urinary infections . The flower extract of sennaauriculata has significant effect because it reduces the blood sugar and lipid level drastically in diabetic rats . The disease affected 4-5% of the population with diabetes type 1 or type 2 in recent years,

To analyse the plant metabolities,Gas chromatography technique use to analyse the various metabolites in plant extracts.



FIGURE 1: SHOWING SENNA AURICULATA

REVIEW OF LITERATURE:

L.vaidyanathan *et al.*, January 2014 identified Evaluation of in-vitro wound healing activity of sennaauriculata using chick embryo wound model. SirajNisa *et al.*, January 2014 identified Green Approach to Corrosion Inhibition of Aluminium By SennaAuriculata Leaves Extract In 1 N NaOH Solution. Sumithra . M and VasugiRaaja, February 2014 identified Antimicrobial Finishing of Denim Fabrics with SennaAuriculata . Guruprasadnille and K.R.C reddy , January 2016 identified various parts of sennaauriculata has antidiabetic , anthelmintic , hepatoprotective , anti fungal , antimicrobial , anti-inflammatory , antipyretic , antioxidant and antihyperlipidemic activities . Pandian Kumar *et al.*, January 2016 Enhanced Acaricidal activity of SennaAuriculata and Ocimumtenuiflorum Extracts with the Addition of Adjuvant against Red Spider mite, *Oligonychus coffeae* Nietner (Acarina : Tetranychidae) Infesting Tea. Gaurav Gupta *et al.*, September 2016 identified using animal model to detect this leaf extracts to detect the effect of prophylactic of leaves. Monisha. Met *et al.*, 2017 has done extraction of bio active compounds from sennaauriculata pods and leaves and its medicinal uses . Gayathri Nambirajan 2018 identified Antidiabetic activity of bud , leaves and flower in high fat diet [HFD] and streptozotocin [STZ] induced diabetic rats using LC-ESI/MS analysis . Ramasamy Rajesh Kumar *et al.*, July 2018 identified Characterization and Biological Application of silver nano particles produced from SennaAuriculata Leaf extract. Divya Kallingikalathil Gopi *et al.*, September 2018 has done Macro – Microscopic profiling of SennaAuriculata. Dr.M.ArulSheeba Rani March 2019 identified Influence of Pre-Sowing seed treatments on Germination pattern of SennaAuriculata ,(L.) Roxb.; (Family – Fabaceae).

COLLECTION OF SAMPLE:

SennaAuriculata plant was collected from aynavaram nursery of Chennai at 34 degree room temperature. The collected plant material sample were fresh and young flower. The flower sample were washed thoroughly with water and kept in sunlight for 72 hours to dry and grinded into fine powder.

MATERIALS AND METHODS:

APPARATUS REQUIRED:

Soxhlet apparatus , Filter paper, Condenser , Sterile test tubes, Autoclave , Sterile round flask , Weighing machine, Sterile Petriplates , Readymade TLC plates , Solvent system[mobile phase] , Sterile cuvette , Methanol, NMR tubes, DMSO , Chloroform , UV spectroscopy , FTIR Spectroscopy, NMR spectroscopy

THIN LAYER CHROMATOGRAPHY:

In Solvent system (mobile phase):Chloroform: Methanol (19:1)

PROCEDURE:

Readymade TLC plate was cut to the required size. A sample mixture was dissolved in methanol and spotted at one end of the TLC plate. The plate was kept in the beaker containing the mobile phase in such a way that the end near the sample application should touch the mobile phase. Allow the chromatogram to run about 1-2 hours. (3/4th level for TLC plate)The plate was dried at RT and viewed under UV light.Rf value of the sample can be determined by using the formula

Solute displacement(a-f)

Rf = -----

Solvent displacement (A)

ULTRAVIOLETSPECTROSCOPY:

PROCEDURE

First there is a need to stabilize the instrument, for this there is a need to maintain warm condition of minimum period to make stabilization process. Then clean the cuvette and fill the sample, which will act as blank. While keeping the blank in spectrophotometer should carefully observe to take blank readings. This is minimal absorbance, should be subtracted out from further samples. But this subtraction will happen in automatic mode in some instruments. Mostly while performing the energy related experiments this instrument is more suitable for this kind of experiments and its readings. In the kinetic related experiment first need to take initial sample reading and immediately add related chemicals to see the reactions, followed by stirring, small amount of the sample measurement by cuvette to take absorbance reading in uv-vis. To measure the absorbance of the sample at lambda maximum followed by the over time .

A calibration curve is used , to prepare the plot of sample concentration vs time ,to convert the value of absorbance into concentration. The graph are often fit with appropriate equation to work out the reaction rate constants (Figs. 4 to 7).

FOURIERTRANSFORMINFAREDSPECTROSCOPY[FTIR]:

Fourier transform infrared spectroscopy use for the spectral analysis of biological samples. Mostly it is useful of analytical studies of different biological samples in high end research areas. Especially to analyze the cytological and histological studies of samples which are related to spectrum studies. The major principle involved in the analysis of samples are relationship between molecular bonds with an electric dipole moment .In this principle natural vibrations are active by atoms movement.

Infrared spectroscopy is used to quantitatively detection of different atom vibrations of sample and its dynamic without perturbing the sample.

For biological samples , important spectral regions measured are typically the finger print region ($600-1,450\text{cm}^{-1}$) amide and amide II (amide I/II) region ($1,500-1,700\text{cm}^{-1}$) .

The higher -wave number region ($2,550-3,500\text{cm}^{-1}$) is associated with stretching vibrations such as S-H,C-H,N-Hand O-H, lower-wave number region typically correspond to the bending and carbon skeleton finger print vibrations 4 .Together, these regions comprise

biochemical finger print of structure and function of the interrogated cellular specimens. The typical biological IR spectrum with the molecular assignments are done (Figs. 8 to 11).

RESULTS :

The following are the results of each procedure

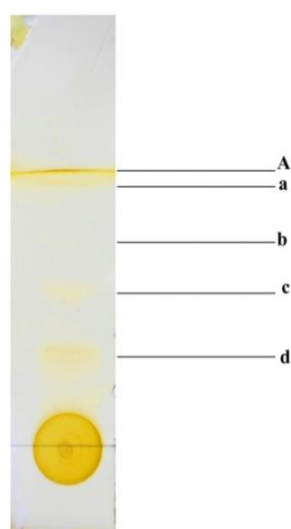


FIGURE 2 :BANDS SHOWING TLC PLATE

S.No.	Sample fractions	Solvent displacement(A) (cm)	Solute displacement(b) (cm)	R _f (b/a)
1	A	4.6	4.4	0.95
2	B	4.6	3.5	0.76

3	C	4.6	2.1	0.46
4	D	4.6	1.2	0.26

Table 1 : RF value of TLC plates

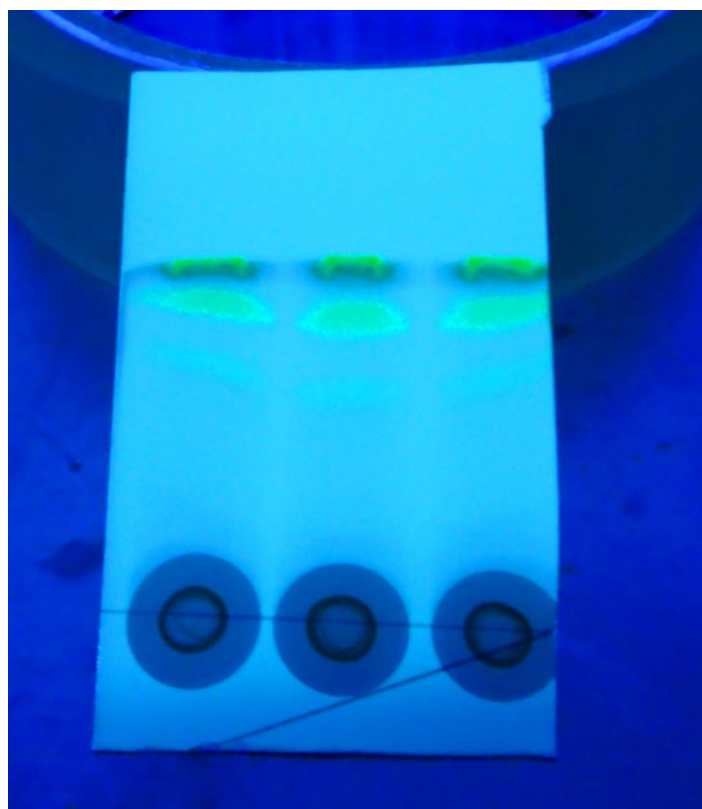


FIGURE 3 ; SHOWING TLC PLATE IN UV LIGHT

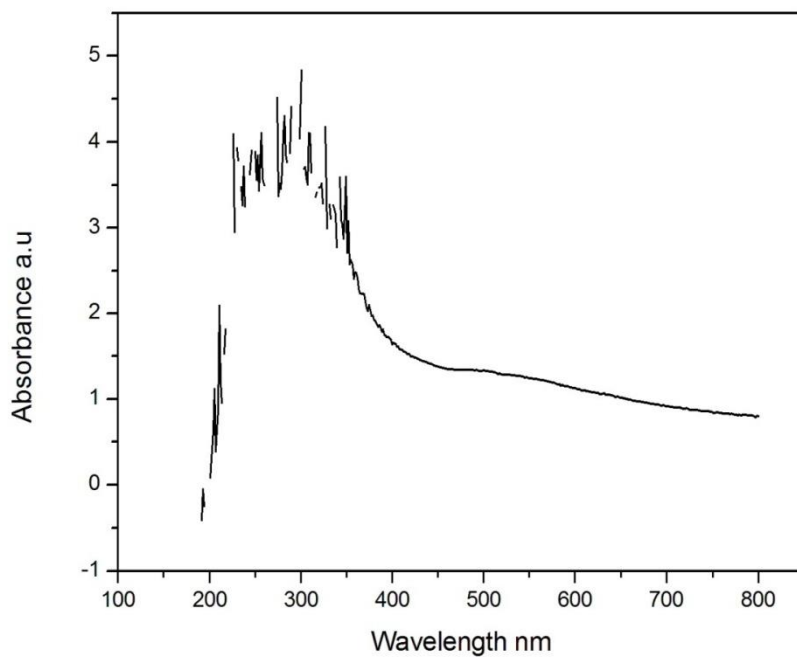


FIGURE 4: UV FOR CRUDE SAMPLE [CONTROL]

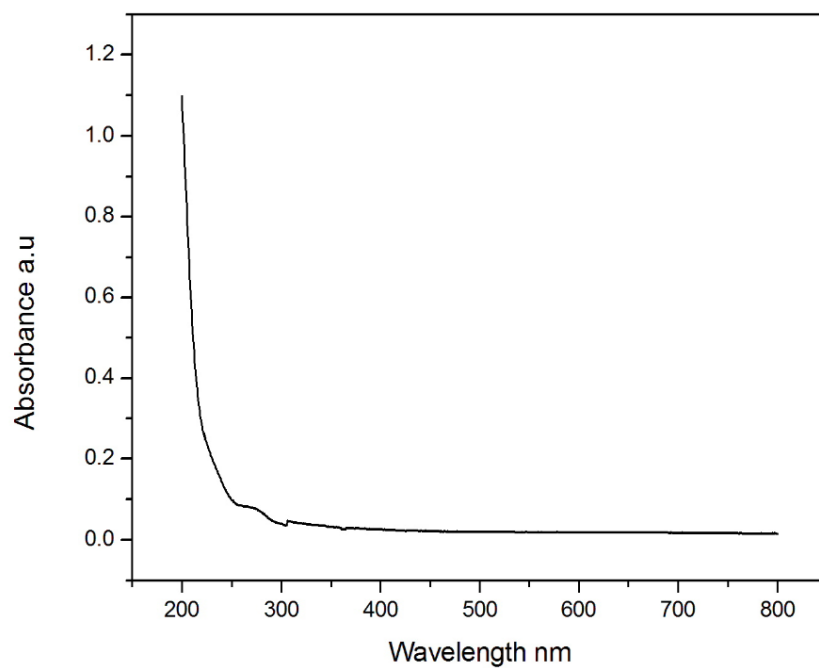


FIGURE 5 : UV FOR SAMPLE 1

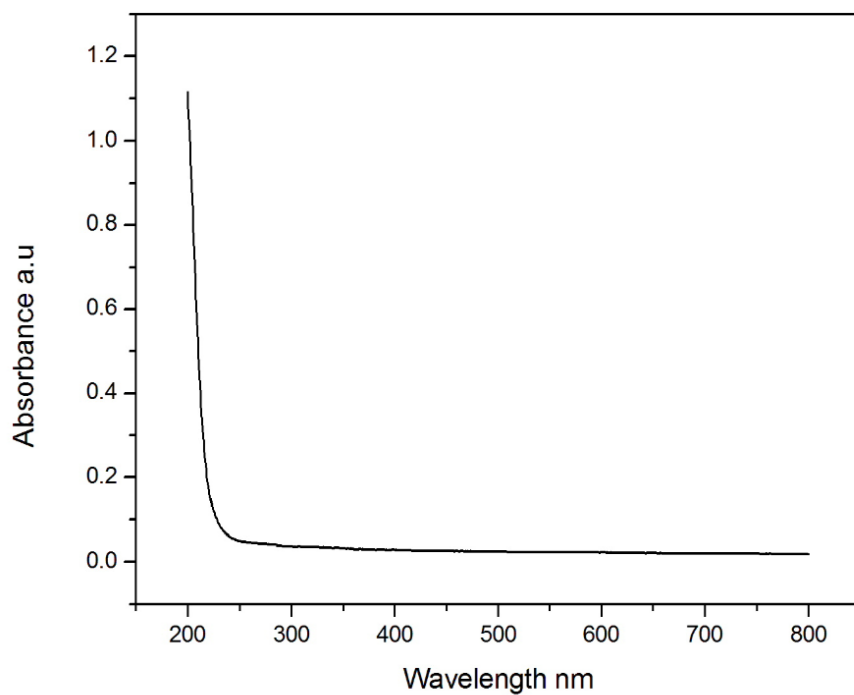


FIGURE 6 : UV FOR SAMPLE 2

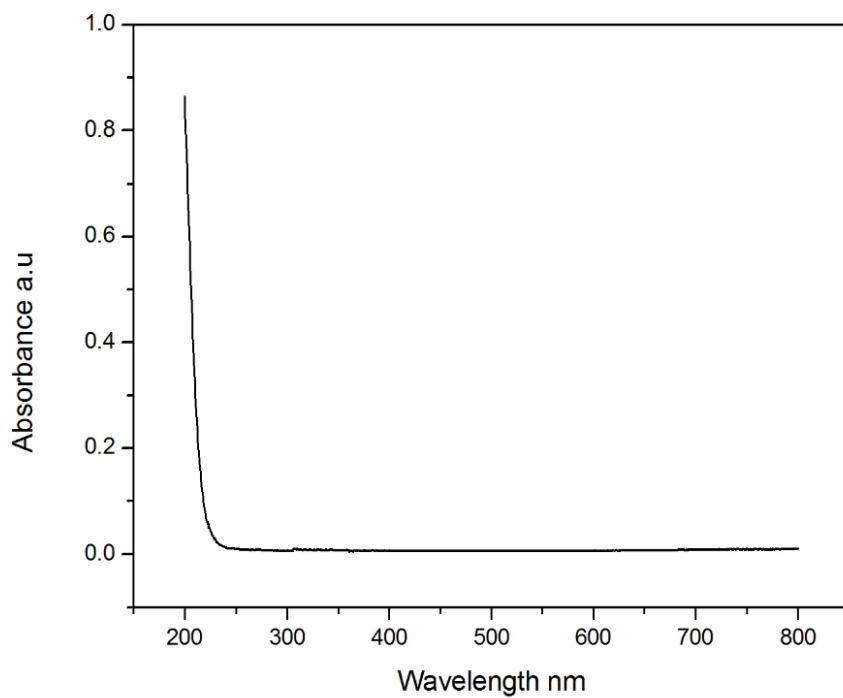


FIGURE 7:UV FOR SAMPLE 3

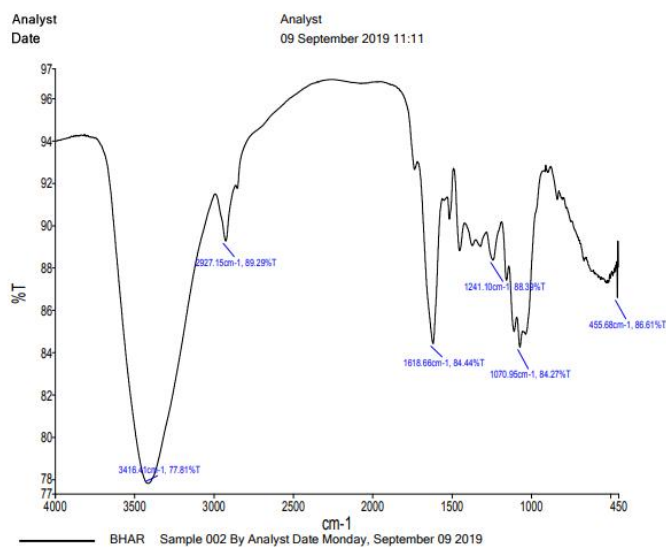


FIGURE 8 : FTIR FOR CRUDE SAMPLE [CONTROL]

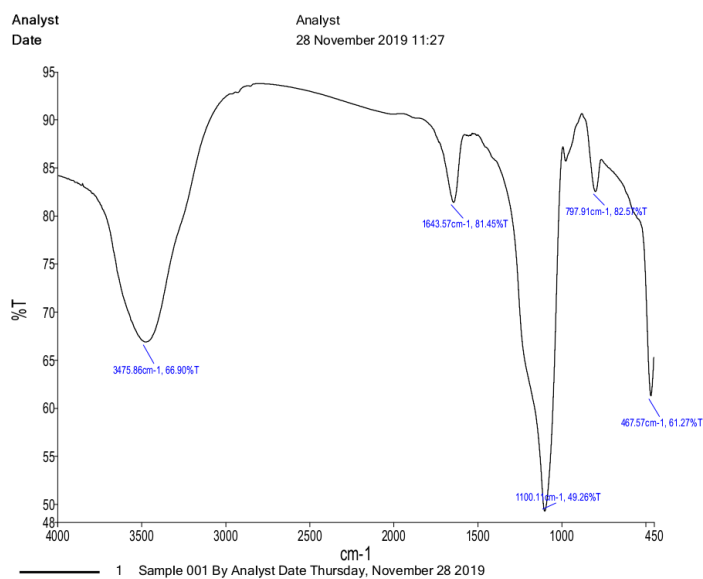


FIGURE 9 : FTIR FOR SAMPLE 1

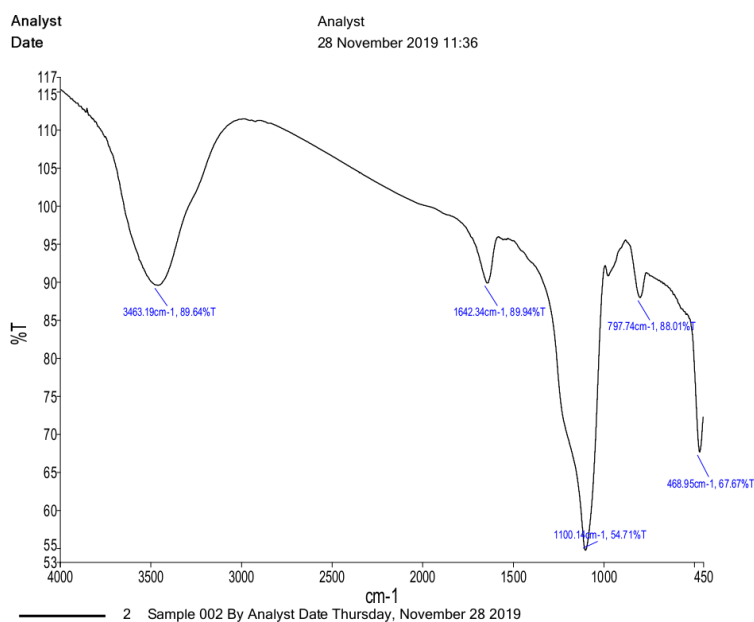


FIGURE 10 : FTIR FOR SAMPLE 2

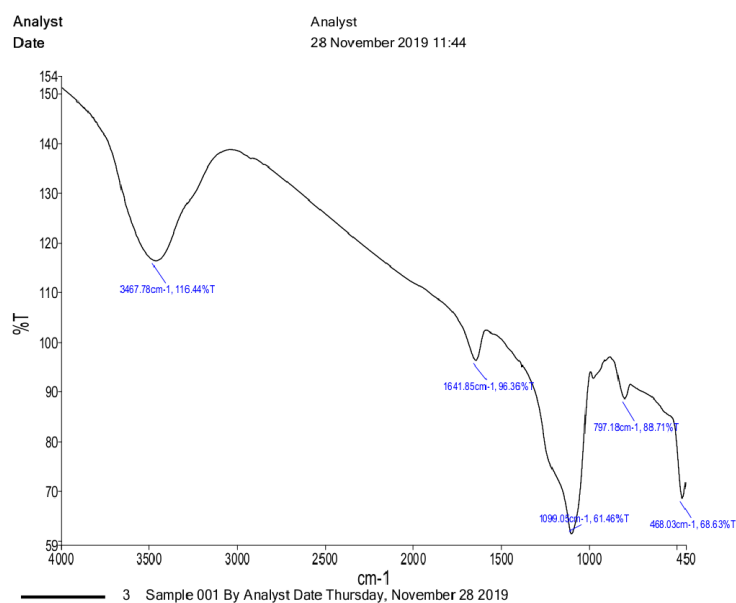


FIGURE 11 : FTIR FOR SAMPLE 3

CONCLUSION & DISCUSSION :

Senna auriculata is a secret gem which nature has given to us with so many uses and medicinal properties. Secondary metabolites were found through spectroscopic methods such as UV, FTIR. With the bands seen in TLC plates, this plant has many new compounds which

can have many useful properties. I have chosen three bands randomly and done the experiments.

FUTURE WORK :

Nuclear magnetic resonance [NMR] is also almost done to confirm the compounds and to find its structure which can also be a novel thing. In future I will publish those results with successful research work.

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