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Antibacterial Activity Determination, Phytochemical Screening and Structure Elucidation of Flavonoid Compound Isolated from Indigenous Medicinal Plant (*Kyoe-sa-gauk*)

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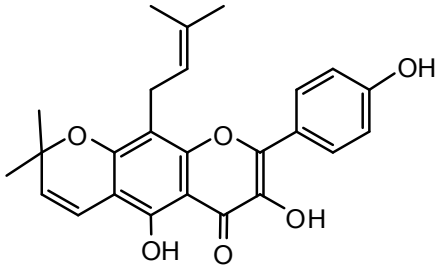
ABSTRACT

Antibacterial activity of an indigenous medicinal plant *Chrozophora plicata (*Vahl) A. Juss. Ex Spreng, under family Euphorbiaceae, Myanmar name (Kyoe-sa-gauk), was determined in different solvent extracts with the selected micro-organisms namely *Bacilus substilis, Pseudomonous aureus,* and *Pseudomonous aurigenosa*. Ethylacetate and ethanol extracts showed the higher activity than the remaining extracts. Phytochemical constituents of the plants were also examined by phytochemical methods that confirm the presence of alkaloid, glycoside, steroid, and polyphenolic compounds. An unknown compound could be isolated from the plant by column and thin layer chromatographic separation techniques. Its structure could be elucidated by spectral analysis consisting of ^{1}H NMR, ^{13}C NMR, DQF COSY, DEPT, HSQC, PFG HMBC, and EIMS respectively.

Molecular Formula	$C_{25}H_{24}O_6$
Molecular Mass	420
Class of compound	Flavonol



INTRODUCTION

Up to now, man has been relying plants and forests. The plants and forests are essential for human beings. From the plants, foods are obtained. Plants also provide timber, cane and bamboo for housing, flowers for gardening. Besides, plants play very important role in keeping the human environment clean. They restore the atmospheric oxygen by the process called photosynthesis. Plants support medicines for sick people, and so forth. Among the various aspects of the plant, we have been specializing in "Natural Product Chemistry" or "Phytochemistry" since the earliest day of the twenty first century. In this respect, the indigenous medicinal plant known as Kyoe-sa-gauk was chosen for this research work.

The plant is well known in the treatment of diseases caused by bacteria, cancer and tumor. Present work includes determination of the anti-bacterial activity of crude plant extract, isolation of an unknown compound from the plant, re-determination of the anti-bacterial activity of pure compound and structure elucidation of this isolated pure compound by spectral interpretation.

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EXPERIMENTAL

Aerial parts of the plant were washed with water. Then they were chopped into small pieces and dried in the well ventilated shade.

(1) Determination of the anti-bacterial activity of crude plant extract

Anti-bacterial activity of the crude plant extract in five solvent systems was determined by agar well diffusion method with three selected organisms. These tests were done in DCPT, Insein, Yangon.

Table 1. Results of anti-bacterial activity determination

Solvent system	*Bacelus substilis*	*Pseudomonous aureus*	*Pseudomonous aurigenosa*
Pet-ether	nil	low	low
Benzene	nil	low	nil
Chloroform	medium	low	medium
Ethylacetate	high	medium	high
Ethanol	medium	high	medium

(2) Determination of the phytochemical constituents

The classes of compound present in the plant were suggested according to the phytochemical methods described in Bulletin of the American Society of Botany. Phytochemical determination is the initial and primary task in field of Natural Product Chemistry. From the results obtained one can nicely estimate that what kind of the class will account for observed activity of the plant.

Table 2. Results of phytochemical determination

No.	Class	Reagents	Remark
1	Alkaloid	1) Mayer. 2) Dragendoff	Present
2	Glycoside	Lead acetate in slight alkaline medium	Present
3	Steroid	conc. Sulphuric acid	Present
4	Phenolic	10% Ferric chloride	Present
5	Polyphenolic	10% Ferric chloride and potassium ferricyanide	Present
6	Reducing sugar	Benedict's solution	Absent
7	Coumarin	TLC separation and observed under UV radiation	Absent
8	Terpene	Aceticanhydride, chloroform, conc. sulphuric acid	Absent

(3) Isolation of an unknown compound

Aerial parts of the air dried sample 250 g was percolated with 500 mL of ethylacetate for a month. During the percolating, the glass container was frequently shaken to have the maximum extraction in short period. Then the solution was filtered through Whatmann filter paper. The filtrate was evaporate to get 4.6 g of ethylacetate crude extract.

Resulting crude extract was fractionated by column chromatography over silica gel 70 – 230 mash. Mixture of n-hexane and ethylacetate in ratios of n-hexane only, 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, and ethylacetate only were employed as eluents. The contents of the each of the fractions obtained were checked by thin layer chromato-graphy. Fractions with the nearly same contents were combined to yield seven combined fractions.

Among them, major combined fraction-5 (1.02 g) was further fractionated by column chromatography using n-hexane and ethylacetate 9 : 1, 8 : 2, 7 : 3, and 4 : 6 respectively. An unknown compound 230 mg was obtained. It was recrystallized out from n-hexane and ethylacetate 4 : 6. The unknown compound 80 mg was obtained in pure state. Yield of this compound is found to be 0.032% on the original sample weight.

(4) Re-determination of the anti-bacterial activity of pure unknown compound

50 mg of the pure unknown compound were used for the anti-bacterial activity determination. This compound responds high activity on all the tested organisms.

Table 3. Results of anti-bacterial activity of pure unknown compound

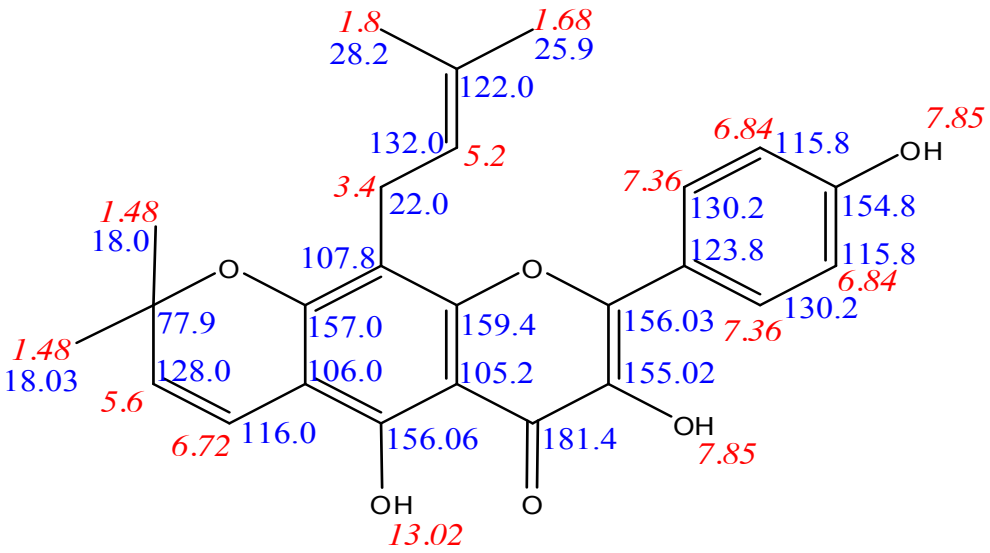
Bacelus substilis	*Pseudomonous aureus*	*Pseudomonous aurigenosa*
high	high	high

(5) Structure elucidation of pure unknown compound

30 mg of pure unknown compound were consumed in the measurements of spectral properties for structure elucidation. From the interpretation of the resulting sets of spectra- ^{1}H NMR, ^{13}C NMR, DQF COSY, DEPT, HSQC, PFG HMBC and EIMS,- the structure of unknown compound having the high anti-bacterial activity could be precisely elucidated.

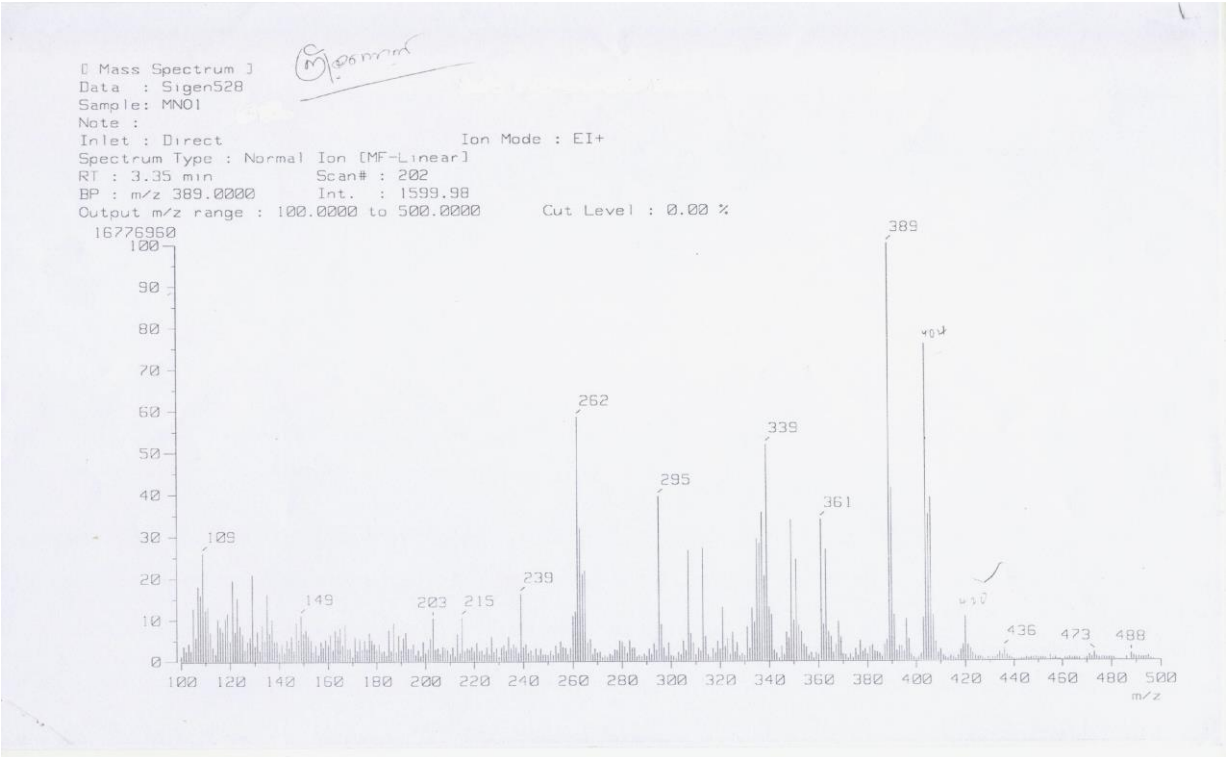
Number of protons (24), number of carbons (25) and number of oxygen atoms (6) were deduced by chemical shift values of protons and carbons from ^{1}H NMR and ^{13}C NMR spectra. Molecular formula $C_{25}H_{24}O_6$ was assigned with additional spectra HSQC, and EIMS. Molecular mass of this compound (420) is observed in EI mass spectrum. Then the molecular structure was elucidated by those kinds of spectra in combination with DQF COSY

and PFG HMBC spectra which represent proton to proton and proton to carbon correlations respectively. The elucidated molecular structure of the unknown compound was described below.

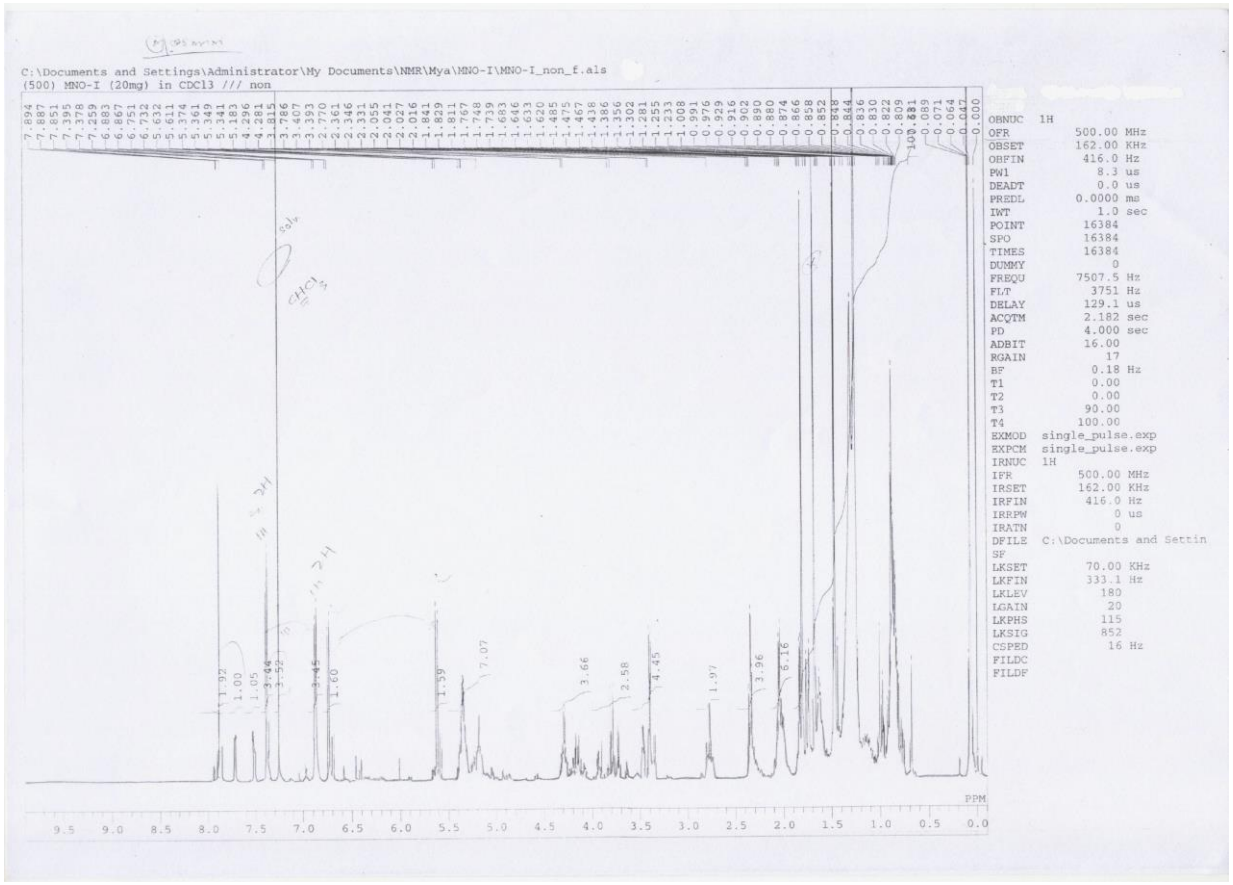


Basic skeleton of the compound is found to be flavonol.

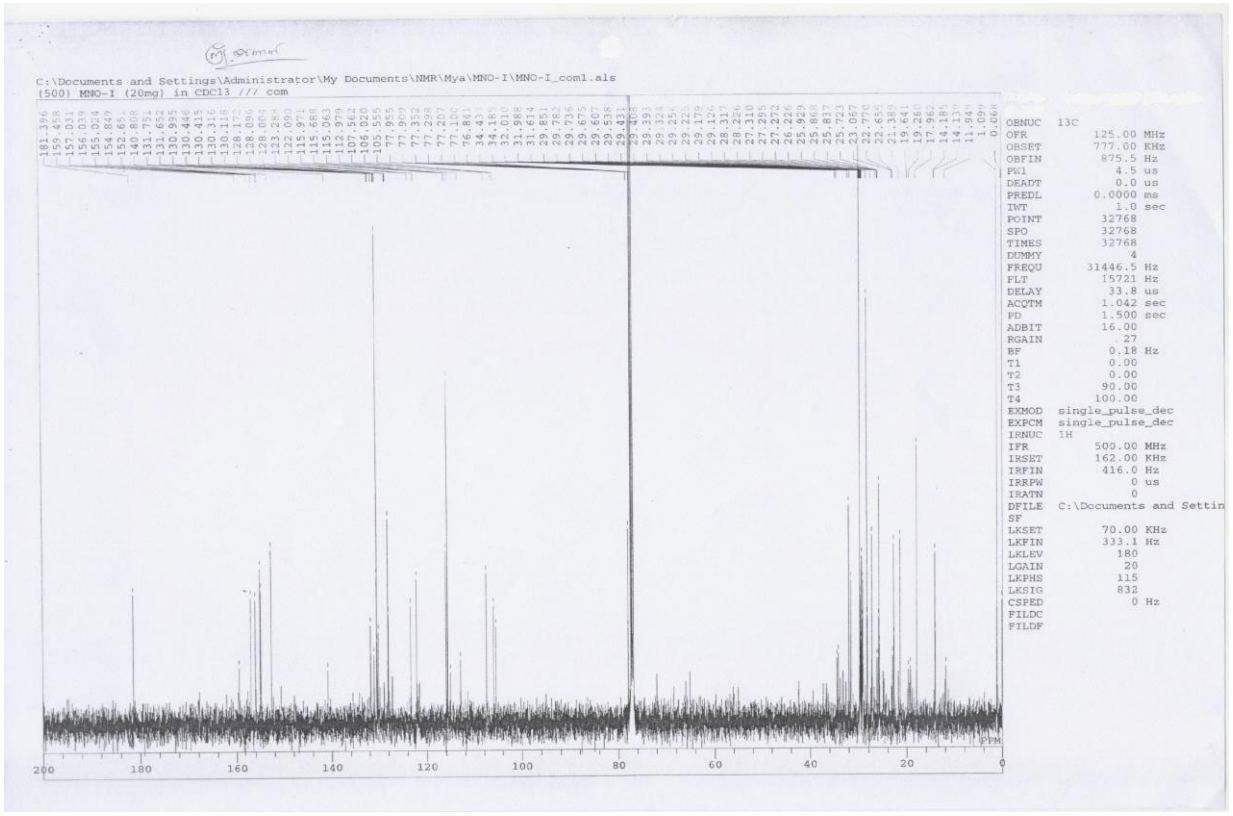
In the structure, the italic numbers represent the assigned chemical shift values of protons. Where, plane text numbers represent the chemical shift values of carbon atoms.



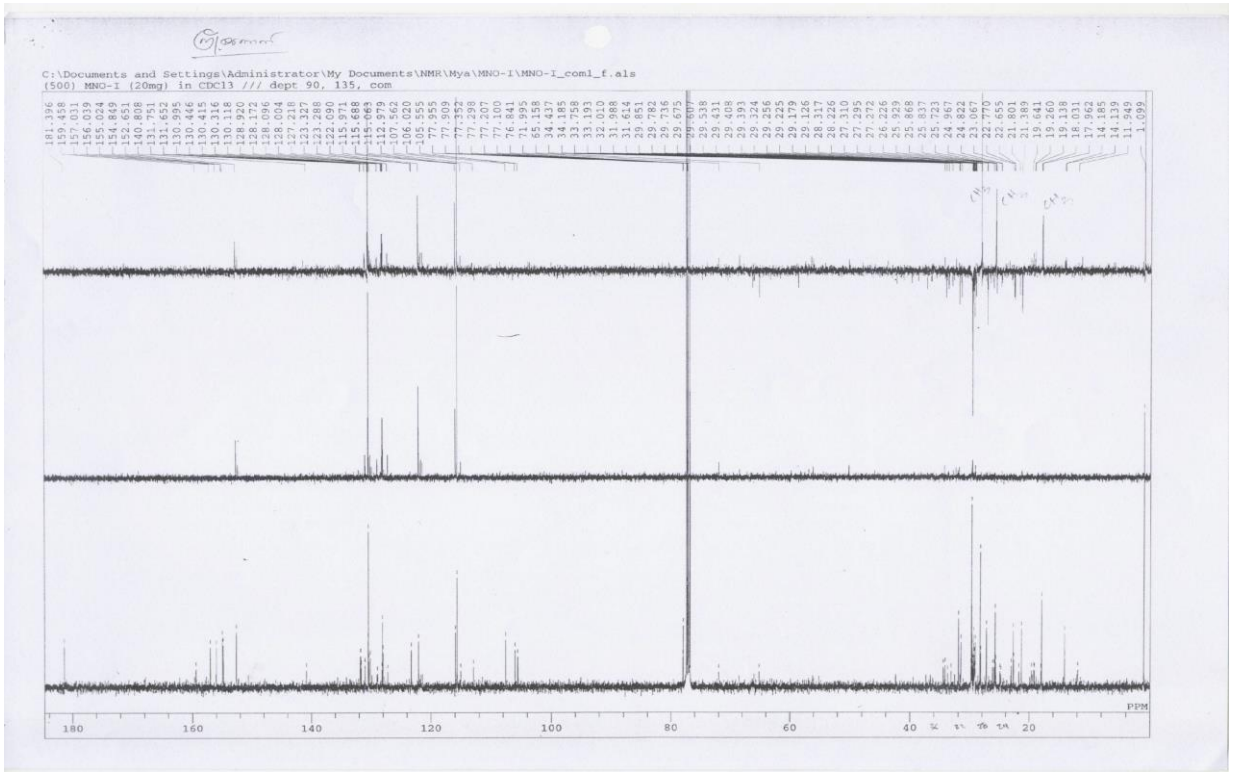
EI Mass Spectrum



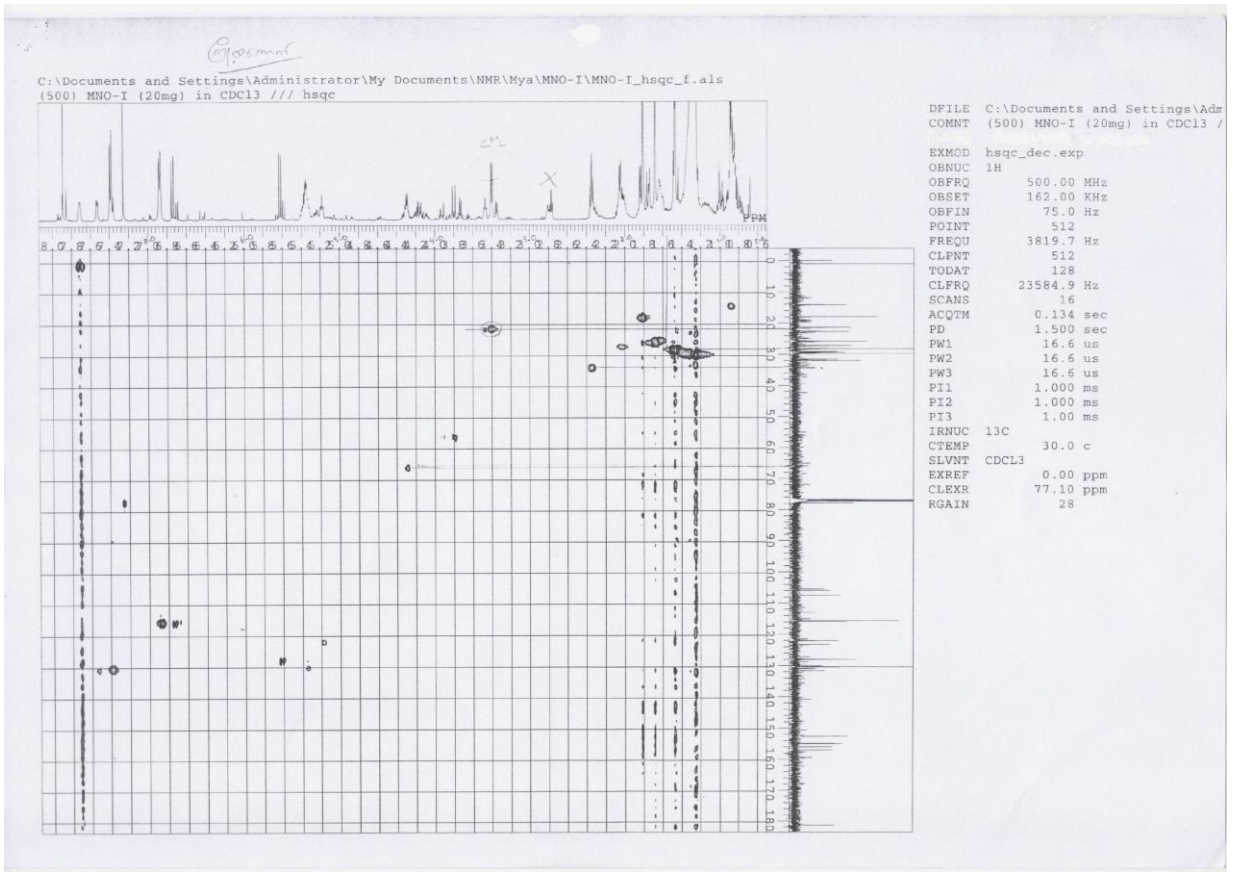
[1]H NMR Spectrum



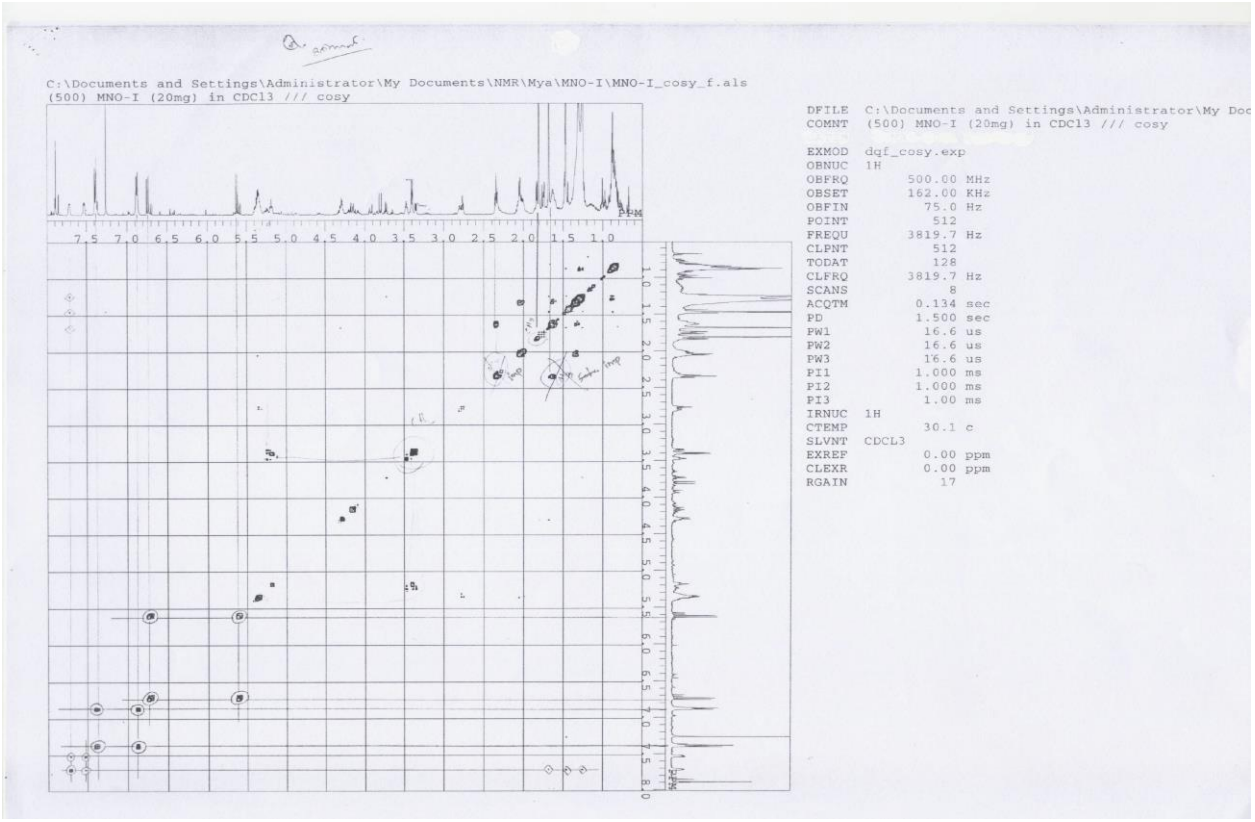
[13]C NMR Spectrum



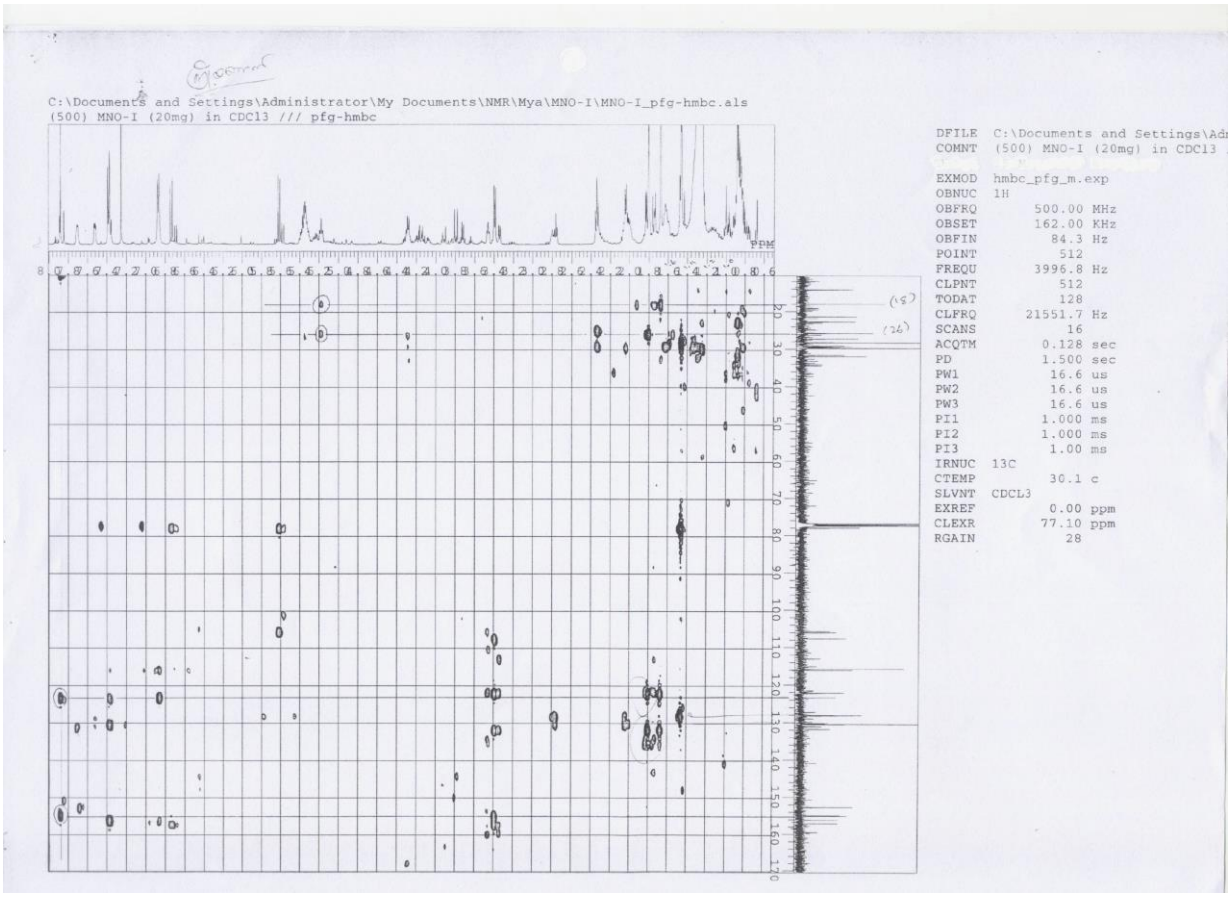
DEPT Spectrum



HSQC Spectrum



DQF COSY Spectrum



PFG HMBC Spectrum

RESULT AND OUTCOME OF RESEARCH

From the Myanmar indigenous medicinal plant, Kyoe-sa-gauk, 8-Prenylated-6-pyrano flavonol having high anti-bacterial activity on *B. substilis*, *Pseu. aureus* and *Pseu. aurigenosa* could be introduced.

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