

## PHYTOCHEMICAL, ANTIBACTERIAL, ANTIOXIDANT AND ANTICANCER ACTIVITY STUDY OF *M. candidum* LEAF ACETONE EXTRACT

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### ABSTRACT

*M. Candidum* has been frequently used as a traditional medicine to treat various diseases such as diarrhea, dysentery, haemorrhoids, cuts and wounds, toothache, and stomach ache. This research was aimed to identify the activity of *M. candidum* acetone extract as an antibacterial, antioxidant, anticancer and phytochemical. Antibacterial activity test was performed in vitro against each of the two Gram-positive and Gram-negative bacteria by paper disc diffusion method followed by determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The antioxidant activity of the extract was tested against 2,2-diphenyl-1-picrylhydrazyl (DPPH), while the cytotoxic activity of the extract was evaluated against MCF-7 cells. Furthermore, the identification of secondary metabolite content was determined by <sup>1</sup>H-NMR spectroscopy. Activity test results revealed that acetone extract of *M.Candidum* leaf was active against four pathogenic bacteria, such as *P. acne* ATCC (27853), *S. saprophyticus* ATCC (49907), *S. Mutans* ATCV (35668), *C. freundii* ATCC 8090) with inhibition diameter of 5.70 ± 0.17 - 11.23 ± 0.23 with MIC values of 1250 - 2500 µg /mL and MBC between 1250 - > 5000 µg /mL. *M.candidum* acetone extract has antioxidant and cytotoxic activity with IC<sub>50</sub> value = 22.4761 µg / mL and IC<sub>50</sub> = 601.09 ug / mL respectively. Also, the results of phytochemical tests indicated that *M. candidum* acetone extract contained terpenoids and aromatic compounds.

**Keywords:** *M. candidum*, Antibacterial, Antioxidant, Anticancer, Phytochemical.

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### INTRODUCTION

Natural compounds play an important role in the development of medicinal substances. Many compounds that came from natural ingredients have transformed into drug candidates, and even most of the drugs used today are derived from natural compounds, such as Quinine, theophylline, penicillin G, morphine, paclitaxel, digoxin, vincristine, doxorubicin, cyclosporin, and vitamin A.<sup>1</sup> One of the approaches used to obtain natural compounds that have potential as medicinal compounds are performed through the assessment of potentially therapeutic plants through the ethnopharmacology approach.<sup>2</sup>

Plants of the *Melastomataceae* family are widely used for traditional medicine. Currently, the number of *Melastoma* species has not been exactly reported, however, an estimation has been reported to be 80-90 species.<sup>3</sup> In the Southeast Asia region, the genus *Melastoma* consists of 22 species,<sup>4</sup> one of which is *Melastoma candidum* that is commonly used as a medicinal plant in North Sumatra, Indonesia. *M. candidum* has the local name of "Senduduk" and the synonym name of *Melastoma malabathrum subsp. normale* (D. Don) K.Mey, *Melastoma polyanthum* Blume. *M. candidum* is an easy plant to grow and is commonly found in the province of North Sumatra, Indonesia. In North Sumatra, especially for Karo ethnicity, the *M.candidum* plant has been used as a traditional medicine to treat abscesses, thrush,

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diarrhea, bone fractures and oukup.<sup>5,6</sup>In addition to Bangladesh, India and Malaysia, *M.candidum* has been used to treat diarrhea, dysentery, haemorrhoids, cuts and wounds, toothache, and stomach ache.<sup>4</sup>

*M.candidum*, as traditional medicine is widely known, that several studies have been carried out to investigate it. In detail, *M.candidum* plant extract has various pharmacological effects, such as antibacterial, antiviral, anti-parasitic, antioxidant, cytotoxicity, anticoagulant, platelet-activating factor inhibitory, wound healing, anti-ulcer, anti-diarrheal, anti-venom, anti-inflammatory, anti-nociceptive, and anti-pyretic.<sup>4,7</sup> Therefore, this research was aimed to observe and report the potential of *M.candidum* plant, which is commonly found in North Sumatra, as an antibacterial agent, antioxidant, anticancer based on its phytochemical test.

## EXPERIMENTAL

### Plants extract Preparation

Samples of *M. candidum* were obtained from herbal drug stores, CV. Sempurna Sambu, Medan, Indonesia. A total of 100 g of the dried sample was mashed, and then extracted by maceration process using a 500 mL 100% (v/v) acetone solvent for 3 x 24 hours at room temperature. Then, it was filtered with Whatman filter paper no.2 (Whatman International Ltd, Middlesex, England). The filtrate was evaporated at low pressure using a rotary evaporator (Heidolph VV 2011, Schwabach, Germany) at a temperature of 50°C, until the crude extract was obtained.

### Antibacterial Agents

As much as 100 g of *M.candidum* extract was dissolved in 1 mL DMSO. Then, it was diluted 10 times to obtain a 1% solution in 10% DMSO (Sigma Aldrich), which was equivalent to 10,000 µg/mL. Also, chloramphenicol (500 µg/mL) was used as an antibiotic standard.

### Antibacterial Strains and Inoculums Preparation

The four American Type Culture Collection bacteria used for the test bacteria consisted of two Gram-positive bacteria: *Propionibacterium acne* ATCC (27853), *Staphylococcus saprophyticus* ATCC (49907), and two Gram-negative bacteria: *Streptococcus mutans* ATCV (35668) and *Citrobacter freundii* ATCC 8090. The inoculum was prepared based on the growth method by taking 3-5 isolated bacterial colonies with the same morphological type from the culture plate and a sterilized cotton bud was used. Then, it was suspended to 4-5 mL of 0.9% NaCl. Furthermore, the turbidity of the suspension was adjusted to the turbidity of 0.5 McFarland.

### Antibacterial Activity

M02-A11 paper disc diffusion method with Clinical and Laboratory Standards Institute Reference method (CLSI) was used for a preliminary test. A total of 100 µL inoculums of bacterial species were added to the Mueller Hinton Agar (MHA) plate evenly, using a sterile spreader. Then, the sterile paper disk blank (6 mm diameter disc, oxoid) was placed on the surface of the inoculum layer on the MHA plate in an even manner. After that, a total of 15-20 µL of the test solution was dripped on paper discs, then aerobically incubated at 37°C for 24 hours.<sup>8</sup>

### Determination of Minimum Inhibitory Concentration (MIC) Value

The microdilution method (M07-A9) was used for the determination of MIC, using a microplate (96-well).<sup>9</sup> The first well was filled with Muller Hinton Broth (MHB, oxoid) media which served as a negative control. The second well was filled with media and inoculum as a positive control. Wells 3 to 12 were filled with a series of sample concentrations. In each microplate well, as much as 100 µL of MHB liquid media containing inoculum was inserted. Into well 12, a total of 100 µL MHB liquid was inserted and moved to well 11. A similar step was performed towards well 11 to well 3, resulting in an equal amount of MHB liquid. Lastly, microplate was incubated at 37°C for 24 hours.<sup>10</sup>

### Determination of Minimum Bactericidal Concentration (MBC)

MBC determination was conducted according to<sup>11,12</sup>. Each of 10 µL mixture in each well on the microplate of MIC test results was inoculated into the MHA plate. The MHA plate was incubated at 37°C for 24 hours or until the growth was seen in positive control.

### Antioxidant Activity Test

Antioxidant activity tests were carried out following.<sup>13,14</sup> The sample was reacted with DPPH, and then left for 30 minutes. Then, it was analyzed by UV-Vis spectrophotometer at a wavelength of 517 nm. Methanol was used as a blank and the absorbance value of each concentration variation was recorded along with the IC<sub>50</sub> value.

### Cytotoxic Testing of MCF7 Cells

The cytotoxic activity test was carried out by the method as described by<sup>14</sup> with a slight modification, namely the positive control used in this test was Cisplatin and the use of presto blue reagent.

### Cell Inhibition Percentage

The percentage of cell inhibition from each sample concentration obtained was calculated by using a formula as follows:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance Control} - \text{Absorbance Sample})}{(\text{Absorbance Control})} \quad (1)$$

### Phytochemical Test

Phytochemical analysis was performed by NMR spectroscopy. The <sup>1</sup>H-NMR spectrum was determined with the Agilent DD2 spectrometer which operates at 500 MHz (<sup>1</sup>H).

## RESULTS AND DISCUSSION

### Antibacterial Activity

Antibacterial activity test was carried out in vitro and the preliminary test was performed using the paper disc diffusion method. MHA media (Mueller Hinton Agar) as recommended by CLSI, FDA and WHO was used for antibacterial testing. This agar media has also been shown to provide good and reproducible results for Davis and Stout.<sup>15</sup> The potential antibacterial properties of the extract were determined from the clear zone around the filter paper as displayed in Fig.-1.

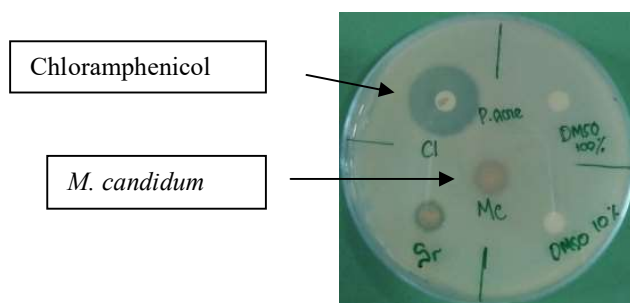


Fig.-1: Clear Zone Diameter of *M. Candidum* extract towards *P. Acne*

Preliminary test of the antibacterial activity against the extract of *M. candidum* acetone was conducted by using the disk diffusion method. The test results showed the existence of antimicrobial activity against four bacteria tested with inhibition zone diameters between  $5.7 \pm 0.17$  mm -  $11.23 \pm 0.23$  mm. Inhibition test data are summarized in Table-1.

Table-1: The Antibacterial Activity of Acetone Extracts of *M. Candidum* by Disc Diffusion Method

Gram-positive Bacteria	Diameter $\pm$ SD (mm)	Gram-negative Bacteria	Diameter $\pm$ SD (mm)
<i>P. acne</i> ATCC (27853)	$11.23 \pm 0.2082$	<i>S. mutans</i> ATCV (35668)	$10.26 \pm 0.1157$
<i>S. saprophyticus</i> ATCC (49907)	$7.53 \pm 0.256$	<i>C. Freendii</i> ATCC (8090)	$5.7 \pm 0.1723$

Based on Table-1, *M. candidum* extract showed activity against all test bacteria. The tendency of antibacterial activity test of *M. candidum* extract was in line with the results of a study conducted by<sup>16</sup>.

There are two types of antibacterial activity, namely bacteriostatic and bactericidal. Bacteriostatic is inhibiting bacterial growth while bactericidal is killing bacteria. The determination of the MIC value was intended to determine the lowest inhibitory concentration of a bacterium. On the other hand, the determination of MBC was intended to determine the lowest concentration of killing a bacterium. Micro dilution method was used for MIC and MBC determination. Acetone extract of *M.candidum* can inhibit bacterial growth with an MIC range between 625 - 2500 ( $\mu\text{g/mL}$ ). While the concentration of extract to kill bacteria was in the range between 1250-> 5000 ( $\mu\text{g/mL}$ ). Data on MIC and MBC values are summarized in Table-2.

Table-2: MIC and MBC Values of *M. Candidum* Acetone Extract

Bacteria	<i>M. candidum</i>		Chloramphenicol	
	MIC ( $\mu\text{g/mL}$ )	MBC ( $\mu\text{g/mL}$ )	MIC ( $\mu\text{g/mL}$ )	MBC ( $\mu\text{g/mL}$ )
<i>S. saprophyticus</i> ATCC (49907)	2500	2500	0.48	56.30
<i>P.acne</i> ATCC(27853)	1250	>5000	0.48	31.50
<i>S. Mutans</i> ATCV(35668)	1250	5000	0.97	125
<i>C.freundi</i> ATCC (8090)	2500	>5000	1.90	62.50

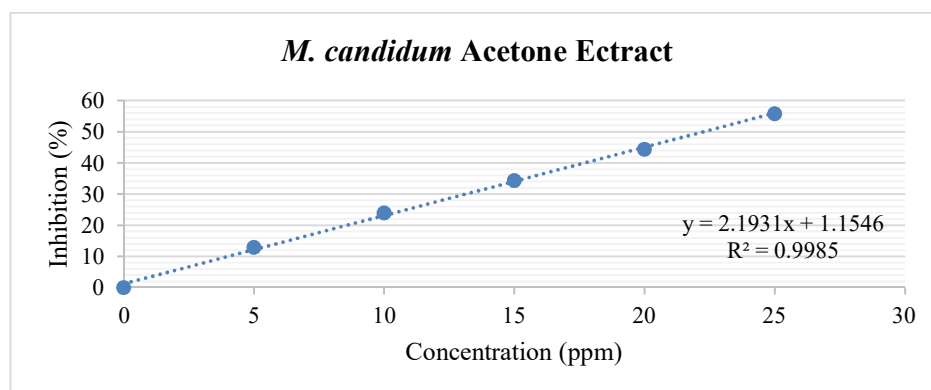
An extract can be categorized as active, moderate and weak if the MIC value was less than 100  $\mu\text{g/mL}$ , range of 100 < MIC < 625  $\mu\text{g/mL}$ , and more than 625  $\mu\text{g/mL}$  respectively.<sup>17</sup> Thus, *S. Mutans*ATCV 35668, *S. saprophyticus*ATCC 49907, *P.acne*ATCC 27853 and *C.freundi* ATCC 8090 were included in a weak category. These results are in line with studies of the MIC values of water extract, ethyl acetate and benzene against *E. coli* (MDR), *S. aureus* (MDR), *K. pneumonia*, *B. cereus*, *V. cholera* in the range of 650-800  $\mu\text{g/mL}$  with the weak category and MBC value of the three extracts against the bacteria were in the range of 700-950  $\mu\text{g/mL}$ .<sup>16</sup>

### Antioxidant

The determination of antioxidant activity was performed based on  $\text{IC}_{50}$  values (inhibition concentration 50).  $\text{IC}_{50}$  is a concentration that is needed to inhibit free radicals by 50%. The absorbance measurement results are summarized in Table-3 and displayed in Fig.-2.

Table-3: Anti-oxidant Activity Test using DPPH Method towards *M. candidum* Acetone Extract

Concentration (ppm)	Absorbance		% Inhibition		$\text{IC}_{50}$ (ppm)
	Trial-1	Trial-2	Trial-1	Trial-2	
0	0.9333	0.8765	0.0000	0.0000	
5	0.8128	0.7976	12.9112	9.0017	
10	0.7096	0.7000	23.9687	20.1369	22.4761
15	0.6127	0.5911	34.3512	32.5613	
20	0.5191	0.5011	44.3802	42.8294	
25	0.4125	0.3793	55.8020	56.7256	

Fig.-3: The Graph of Anti-oxidant Activity of *M. candidum* Acetone Extract

An antioxidant is categorized as very strong, strong and moderate if the  $IC_{50}$  value is  $<50 \mu\text{g/mL}$ ,  $50-100 \mu\text{g/mL}$ , and  $101-250 \mu\text{g/mL}$  respectively.<sup>18</sup> Based on these criteria, the antioxidant activity of *M.candidum* acetone extract is categorized as very strong with  $IC_{50}$  value of  $22.4761 \mu\text{g/mL}$ .<sup>19,20</sup> There is a slight difference in  $IC_{50}$  value from previous researches, because the solvents used to extract are different, so the extracted metabolites were not the same.

### Anti-cancer

The results of absorbance measurements of *M. candidum* acetone extract on MCF7 breast cancer cells are described in Table-4.

Table-4: The Absorbance of *M. candidum* Acetone Extract Test towards MCF7 Cell

Control Cell	Sample Concentration ( $\mu\text{g/mL}$ )	Sample Absorbance	Inhibition (%)
0.5299	7.81	0.6003	-13.29
	15.63	0.5649	-6.61
	31.25	0.5614	-5.95
	62.50	0.5183	2.20
	125.00	0.4885	7.81
	250.00	0.4211	20.53
	500.00	0.2660	49.81
	1000.00	0.0977	81.55

Based on data described in Table-4, a linier regression graph can be produced and resulting in an equation of  $y = 0,0937x - 6,3224$  (Fig.-3). Thus, based on the equation,  $IC_{50}$  value was obtained to be  $601.09 \mu\text{g/mL}$ .

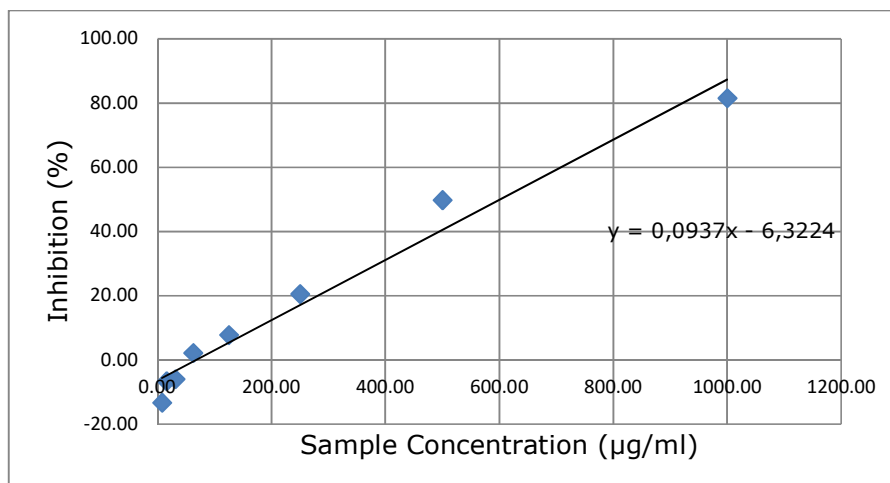


Fig.-3: Absorbance Curve of *M. candidum* Acetone Extract towards MCF5 Cell

Thus, the acetone extract of *M. candidum* leaves with a concentration of  $601.09 \mu\text{g/mL}$  was able to kill 50% of MCF7 cells. Based on The National Cancer Institute (NCI), an extract has strong anticancer potential if it has an  $IC_{50}$  value  $<30 \mu\text{g/mL}$ , moderate with  $30 < IC_{50} < 100 \mu\text{g/mL}$  and inactive with  $IC_{50}$  value  $> 100 \mu\text{g/mL}$ .<sup>21</sup> Therefore, based on these criteria, the acetone extract of *M. candidum* leaves is inactive as an anticancer.

These results are in line with research conducted by<sup>22</sup> which tested the methanol extract of *M.malabatum* leaves on MCF-7 cells and obtained the  $IC_{50}$  values  $> 400 \mu\text{g/mL}$ . The results of this study are also following the results of<sup>23</sup> who tested the ethyl acetate and methanol extract of *M.malabatum* and obtained concentrations of  $500 \mu\text{g/mL}$ , causing morphological changes in MCF7 cells. Whereas the



methanol extracts of *M.malabatrums* did not show anticancer activity. Although the acetone extract of *M.candidum* leaves is less active against MCF-7 cells, it is still potential as an anticancer based on observations using a microscope (Fig.-4), which shows that at a concentration of 250  $\mu\text{g/mL}$ , a huge amount of cells have died, while at concentrations of 500  $\mu\text{g/mL}$ , almost all cells were dead. These observation results are indicated from appearing dark, not glowing and the cell membrane looks broken or somewhat faint, while the morphology of living cells appears to shine brilliantly and the membrane boundary with the media is visible.

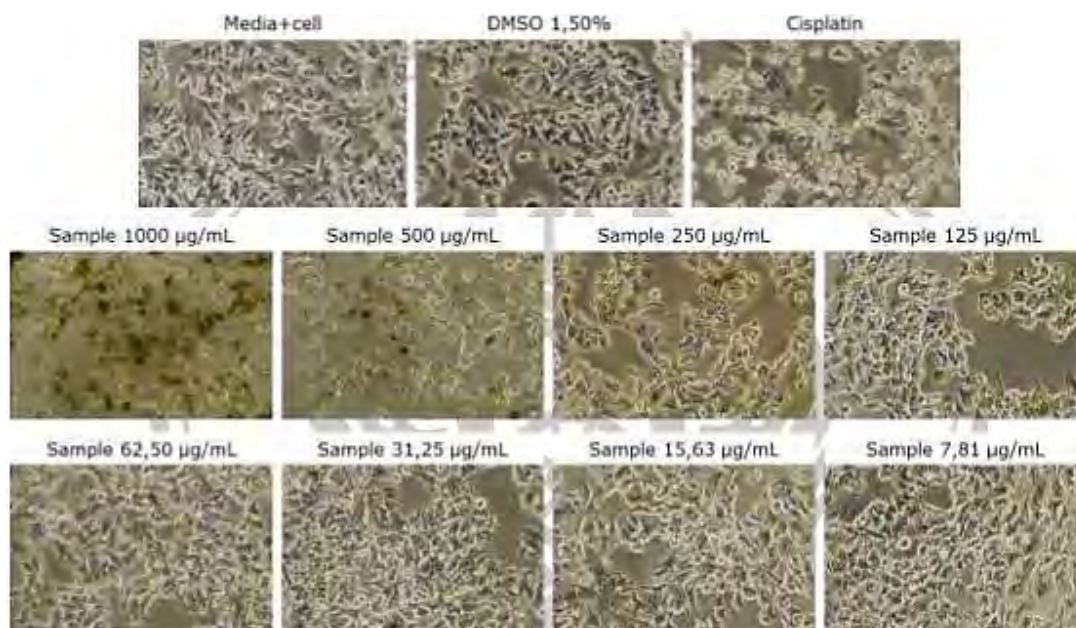


Fig.-4: MCF7 Cell Morphology Images for Various Extract Sample Test Results

### Phytochemical Study

Based on  $^1\text{H-NMR}$  data of *M.candidum* extract with  $\text{CD}_3\text{OD}$  solvent, a chemical shift  $\delta\text{H}$  7.5 - 7.7 ppm (figure 5) which is the signals of aromatic proton benzene derivatives is observed. Signals of  $\delta\text{H}$  3.1 - 4.2 ppm are signals from  $-\text{CH-O}-$  and  $\text{C-OH}$  from a glycoside. Thus, the acetone extract of *M.candidum* leaves contains aromatic compounds and aromatic compounds that bind a glycoside. From the previous research results, aromatic compounds which were found in *M.candidum* are including flavanoid and tannins.<sup>24</sup> Flavanoid compounds that have been successfully isolated from *M.candidum* extracts were kaempferol, quercetin, narigenin and flavanoid that bind to a glycoside namely Quercitrin, Cyanidin-3-glucoside, Cyanidin-3,5-diglucoside, Kaempferol-3-*O*- $\alpha$ -Lrhamnopyranoside, Kaempferol-3-*O*- $\beta$ -Dglucopyranoside, Kaempferol-3-*O*(2'',6''-di-*O*-p-trnas-coumaryl)- $\beta$ -glucoside. Also, tannin was hydrolysed in the form of monomers, dimers, and oligomers, namely 1,4,6-tri-*O*-galloyl- $\beta$ -D-glucoside, 1,2,4,6-tetra-*O*-galloyl- $\beta$ -D-glucoside, strictinin, casuarictin, pedunculagin, nobotanin D, pterocarinin C, malabathrins (A, B, C,D, E and F), nobotanins B, G, and H and nobotanin J, casuarinin, (-)-epicatechingallate, (-)-epicatechin, stachyurin, procyanidin B-5 and B-2, stenophyllanins A and B, alienanin B, and brevifolincarboxylic acid.<sup>4</sup>

Based on Fig.-5, extracts of *M.candidum* leaf acetone also contain terpenoid compounds or steroids which are characterized by the presence of a signal at  $\delta\text{H}$  0.6 - 2.3 ppm. This signal corresponds to  $\text{CH}_3$  and  $\text{CH}_2$  groups, which is a characteristic signal of terpenoid or steroid compounds. The terpenoid compounds that have been successfully isolated were including Ursolic acid, 2-Hydroxyursolic acid, Asiatic acid,  $\alpha$ -Amirin, Uvaol and Bertulinic acid which are triterpenoids.<sup>4</sup>

The medicinal properties of a plant are caused by its secondary metabolite content. Based on the result, *M.candidum* acetone extract contains aromatic compounds and terpenoids. The compounds act as an antibacterial, antioxidant and anticancer. The mechanism of action of flavonoids in inhibiting bacterial

growth causes damage to bacterial cell wall permeability.<sup>25</sup> This damages cell membrane, causing the release of various important components from within bacterial cells such as proteins, nucleic acids and nucleotides. While the mechanism of action of terpenoid compounds in inhibiting growth bacteria disrupt the membrane function.<sup>26-28</sup> Flavanoid is a compound that can donate hydrogen atoms, so that it can reduce the DPPH molecule, thus having the ability as an antioxidant.

Likewise, terpenoids which have hydroxyl groups can donate atoms hydrogen to free radicals. Triterpenoids on the red meranti (*Shorea singkawang* .Miq) bark tested with DPPH radicals have strong antioxidant activity with IC<sub>50</sub> values of 82 ppm.<sup>29</sup> The failure of *M. candidum* actone extract to exhibit its cytotoxicity effect suggested that the antagonist effect of the compounds presented in the extract play an important role in not affecting the cell proliferation.

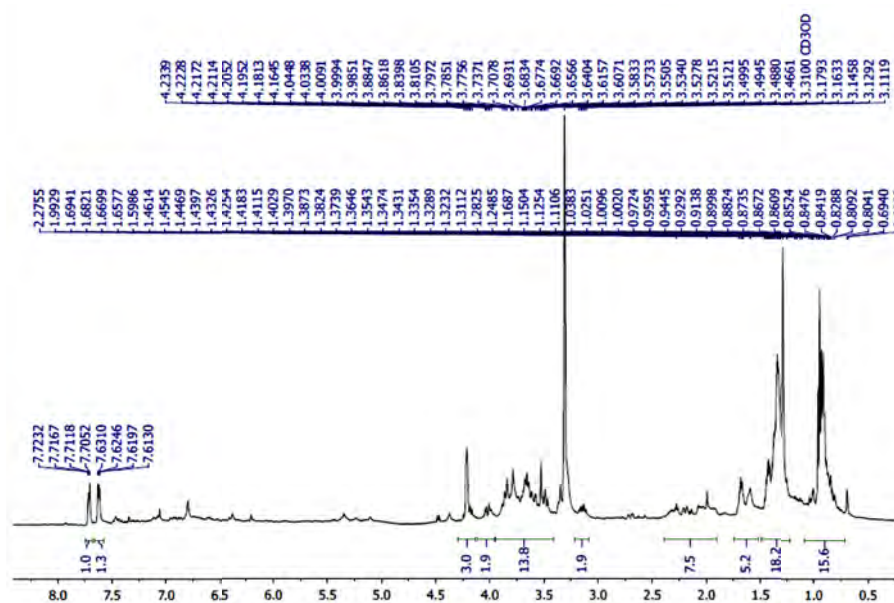


Fig.-5: <sup>1</sup>H- NMR Spectrum Result of *M. candidum* Extract

## CONCLUSION

The acetone extract of *M.candidum* exhibits a broad spectrum of antibacterial activity (both against Gram-positive and Gram-negative bacteria). It also shows a very strong antioxidant activity and potential as an anticancer. Particularly, acetone extract of *M.candidum* leaves contains aromatic compounds and terpenoids, which can turn *M.candidum* to be a potential medicinal plant.

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