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Cytotoxicity and fragmentation pattern of *Datura metel* L. leaves using ultra-performance liquid chromatography-mass spectroscopy (UPLC-MS)

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ABSTRACT

Phytochemical screening, isolation, and cytotoxic testing with the brine shrimp lethality test have been carried out on *Datura metel* L. leaves. The extract obtained was screened for its phytochemical compounds. The results showed that the datura leaves contain flavonoids, alkaloids, steroids, and saponins. The cytotoxic activity test showed a high cytotoxic potential of datura leaf extract with an LC_{50} value of 46.1636 μg/ml. The compound isolation process was carried out using the preparative thin layer chromatography using chloroform: n-hexane (1:1 v/v) eluent. The stationary phase used was the octadecyl silica column. The motion phase used was acetonitrile: water (15:85) v/v isocratic elution system with positive ion mode. The chromatogram showed a retention time (tR) of 3.20 minutes and only showed one sharp peak. The result also showed that the isolate was a steroid belongs to the Withanolide group, namely Baimantuoluoline D. The isolate had a molecular weight of $[M^+]$ 504.0591 m/z with daughter fragments formed 477.2583 m/z $[M^+$ - CH3-OH] and 301.1780 $[M^+$-$C_9H_{11}O_4]$. There are more than 600 activities of the Withanolide group that has been documented. However, the activity showed that Withanolide isolated from it has potential as anti-microbial, anti-inflammatory, and cytotoxic.

Keywords: Datura, *Datura metel* L., Withanolide, UPLC-MS.

INTRODUCTION

Datura (*Datura metel* L.) is a typical Asian plant commonly found throughout South Asia's tropics to Southeast Asia (Indonesia). This plant has an annual cycle, characterized by thorny fruit, 0.40-1 m high. The leaves' size is ± 15 cm, and the colors of the flower are either purple or white (Gaire and Subedi, 2013). The use of datura as traditional medicine has been documented for centuries, and empirically, it is used as an asthma medication by utilizing its dried leaves. Datura

is also used as an anti-bacterial, antiseptic, narcotic, and sedative (Carpa *et al.*, 2017; Wu *et al.*, 2020).

Antony *et al.*, (2019) identify all parts of the datura plant: the roots, stalks, leaves, flowers, fruits, and seeds, containing terpene and alkaloid compounds. The most alkaloid content is found in its roots and seeds. For the terpene, the group consists of the Withanolide group, which is a steroid. The known alkaloid groups are atropine, hyoscyamine, scopolamine, and hyoscine. Besides, fatty substances and calcium oxalate were also found in the fruit and (Carpa *et al.*, 2017; Guo *et al.*, 2018; Antony *et al.*, 2019). Datura is classified as a toxic plant, with alkaloid compounds as the trigger factor. Several types of alkaloid compounds were found in hyoscyamine, atropine, and scopolamine (Iranbakhsh *et al.*, 2006; Carpa *et al.*, 2017).

Vadlapudi and Kaladhar (2012) researched datura leaf extract. Methanol and n-hexane extracts at a concentration of 2 mg/mL formed inhibition zones of 6 mm and 8 mm, respectively, against *Staphylococcus aureus*. Methanol and n-hexane extracts at a concentration of 2 mg/mL formed inhibition zones of 6 mm and 8 mm. The methanol and chloroform extracts at a 250 mg/mL concentration formed the same inhibition zone of 12 mm. Yang *et al.* (2014) successfully isolated nine new isolates of the Withanolid group. Isolate 5,7-dimethyl 6-hydroxyl 3-amine sitosterol isolated from datura leaves, showing inhibition of *P. aeruginosa*, *B. subtilis*, *S. typhi*, *K. pneumonia*, *S. aureus*, and *P. mirabis* (Okwu and Igara, 2009). One of them is the *Datura folisides*, a compound that shows a promising anti-inflammatory effect. Anti-inflammatory activity test used rat macrophages (murine) RAW 264.7 stimulated by Lipopolysaccharide (LPS). *Datura folisides A* isolate showed significant inhibition of nitrite production with an IC_{50} at 20.9 μM. The study on the cytotoxic potential of datura leaves has also been carried out by Bellila *et al.* (2011). The Datura leaf isolates of the Withanolide group, Withametelin, and 12α-hydroxydaturametelin B, showed

cytotoxic effects on human lung carcinoma cells (A549) and human colorectal adenocarcinoma cells (DLD-1). Both isolates showed cytotoxicity to A549 and DLD-1 cells with IC_{50} values of 7 and 2.0 µM, respectively. Therefore, further research is needed to identify compounds and their cytotoxic activities using the BSLT (brine shrimp lethality test). The compound's toxicity potential can be determined based on the number of deaths of the tested animals. The use of BSL T is also carried out to determine the safety limits of using a plant substance for medicinal therapy. The test results were declared toxic to *Artemia salina* Leach if the test sample had an LC of less than 50 1000 µg/ml.

Furthermore, the identification and isolation of secondary metabolites were carried out. The maceration stage was conducted by fractionation using the liquid-liquid partition. The isolation and purification used preparative thin-layer chromatography (TLC). The purification results obtained pure isolates, which were then identified using UPLC-MS (ultra performance liquid chromatography-mass spectroscopy). The UPLC-MS technique combines two methods in one analysis system. The isolate purity identification can use the UPLC chromatography system to detect compounds using Mass Spectroscopy by looking at the compound's fragmentation pattern and molecular weight. Another advantage of this method with LCMS is its high accuracy and sensitivity and the processing process and fast results (Jian, 2017; Yang *et al*., 2020).

MATERIALS AND METHODS

Extraction

In this study, extraction was performed by maceration using methanol 96% solvent. The extraction stage was conducted by soaking 150 grams of datura (*Datura metel* L.) leaf powder in 3000 mL n-hexane. Maceration was carried out for 3 days and stirred every 12 hours. The

maceration of the n-hexane extract was filtered. The filtrate obtained was concentrated using a rotary evaporator (IKA Germany) until it became a thick extract, and the remaining residue was evaporated in an oven at 45^0C for 48 hours. The residue that was evaporated was then macerated again using 3000 mL ethyl acetate. Maceration was carried out for 3 days and stirred every 12 hours. The result of the maceration of ethyl acetate extract was filtered. The obtained filtrate was concentrated using an evaporator until the extract became thick, and the remaining residue was evaporated in an oven at 45^0C for 48 hours. The evaporated residue is macerated. The maceration of the Methanol extract was filtered, and the filtrate obtained was concentrated using an evaporator. The concentrated thick extract was weighed afterward.

Phytochemical screening

Alkaloid identification

A total of 2 grams of the extract was put into a test tube, dripped with HCl 2N, then divided into several test tubes. Each tube was added with each reagent. If the extract forms a white or yellow precipitate after adding the mayerpositive reagent, the extract contains alkaloids. The extract contains alkaloids if an orange precipitate is formed after positive Dragendrof reagent (De Silva *et al.,* 2017).

Flavonoid identification

Several extracts were put into a test tube, then 10 mL of hot water were added and boiled for 5 minutes, then filtered. The filtrate obtained was taken 5 mL, added with magnesium powder and 1 mL of concentrated HCl and added with amyl alcohol, shook the mixture till separate. If an orange, red, or yellow is formed, it is positive for containing flavonoids (De Silva *et al.,* 2017).

Saponin identification

Several extracts were put into a test tube, then added 10 mL of hot water, cooled then shake vigorously for 10 seconds. Positive for saponins is indicated by forming foam as high as 1-10 cm for not less than 10 minutes, and with the addition of 1 drop of 1% HCl, the foam will be stable (Pandey and Tripathi, 2014).

Steroid and terpenoid identification

Several extracts were macerated with 10 mL ether for 2 hours, then filtered. The filtrate obtained was taken 5 mL and then evaporated in a cup. The residue obtained was added 2 drops of anhydrous acetic acid and added 1 drop of concentrated H_2SO_4. When produced, red is positive for steroids, while purple is positive for terpenoids (Pandey and Tripathi, 2014).

Cytotoxic test

To determine the activity of datura leaves, a cytotoxic test was carried out using the BSLT method. The first thing to do was to incubate the *Artemia salina* L. eggs in the *Artemia salina* L. egg hatching vessel for 24 hours of hatching and within 48 hours. Preparation of the standard solution in each extract by dissolving 100 mg of the extract in 100 mL of seawater to obtain 1000 ppm standard solution. The standard solution was diluted to a series concentration of 10, 20, 40, 80, and 160 ppm. Tests were carried out by inserting 10 larvae of *Artemia salina* L. in a test tube containing the extract. After 24 hours, the number of dead larvae was counted, and a probit analysis was performed to determine cytotoxic activity(Ramachandran *et al*., 2011).

Separation of active compound *Datura metel* L. leaves

The sample of datura (*Datura metel* L.) leaf extract was diluted with methanol for the dotting process. The mobile phase was prepared with a ratio of chloroform: n-hexane (1: 1), methanol: ethyl acetate (3: 2), N-hexane: ethyl acetate (2:3). The sample was dotted on the TLC plate and put into the chamber. The purpose of this process is to find the best eluent composition that will be used to separate the compounds contained in the datura leaf extract in the next thin layer chromatography process.

Separation by preparative TLC was performed using silica gel F254 with 20 cm × 20 cm. The plate was activated by heating in an oven at 100 °C for ± 30 minutes to remove any moisture on the plate. The concentrated extract was dissolved in the solvent, then 7 spots were dotted along the lower boundary line using a capillary tube. Then it was eluted using eluent, which produced the best separation on analytical TLC. Elution was stopped after the developer solution reaches the boundary line. The eluted plate was dried, and the stains were observed using a UV lamp with a wavelength of 254 nm and 366 nm.

Isolate identification

A total of 10 mg of isolates were weighed using analytical scales. Then dissolved by adding 10 ml of methanol to obtain a concentration of 1000 ppm, filtered, then used as a standard solution. A solution with a concentration of 10 ppm was made by pipetting 50 μL, each of which was put into a 5 mL volumetric flask and then added methanol to the marked line. The standard solution was filtered and put into the vial liquid chromatography. Then injected into the UPLC-MS QTOF (Waters) system with a mobile phase flow rate of 200 μL/minute, then viewed the fragmentation spectrum data on the mass spectroscopy data.

RESULTS AND DISCUSSION

Research has been carried out on datura leaves. This research was preceded by an extraction process using 96% methanol. The extract obtained was then used for phytochemical screening tests. Furthermore, the extract's toxicity test was performed using the BSLT method to perceive its potential toxicity. The isolation process used Preparative Thin Layer Chromatography (TLC) using the appropriate eluent. The isolates obtained were then identified by the UPLC-MS technique.

Phytochemical screening

About 200 g of datura leaf samples were extracted by the maceration method using 3000 ml of methanol as a solvent (Carpa *et al*., 2017). Maceration was carried out for 6 x 24 hours. The macerated filtrate is evaporated using a rotary evaporator until a thick extract is obtained. The extract was obtained with a weight of 28.19 g and a yield of 14.09% from this stage. The physical properties of the extracts obtained are blackish green, paste-shaped, and sticky.

The thick methanol extract produced from the extraction process was tested its phytochemical compounds, which aim to determine the secondary metabolite compounds present in the sample. The phytochemical test in this study included identifying alkaloids, flavonoids, steroids, saponins, and tannins. Phytochemical test results can be seen in Table 2.

Table 2 above shows that the datura leaves showed positively containing flavonoids, alkaloids, steroids, and saponins. Based on the analysis conducted, samples of datura leaves tested positive for containing flavonoids. It is indicated by the change in color to blackish-green when added with H_2SO_4 reagent. There is a change in color because flavonoids are phenolic compounds.

Therefore the color will change when an alkaline solution or ammonia is added (Hossain *et al*., 2013).

Datura leaf samples tested positive for alkaloid compounds. The deposits' formation indicates this in the three test tubes after the drops of Mayer, Wagner, and Dragendorf reagents. The reaction with Mayer's reagent formed white precipitate, Wagner reagent formed brown precipitate, and Dragendorf reagent formed red-orange precipitate (De Silva *et al*., 2017). Based on the analysis carried out, the datura leaves tested positive for containing steroid compounds. The color change can be seen after the Liberman Bauchard reagent addition, namely green (Tiwari *et al*., 2012; Pandey and Tripathi, 2014). Based on the analysis, the datura leaves tested positive for containing saponin compounds indicated by foam formation after the shaking process. The formation of foam indicates glycosides, which can form foam in the water (Tiwari *et al*., 2012; De Silva *et al*., 2017).

The same results were obtained by Dhawan and Gupta (2016), who carried out a phytochemical screening of datura leaves. The results showed that the methanol extract of datura leaves contains flavonoids, alkaloids, steroids, and saponins. The study also reported that the ethyl acetate and n-hexane extracts positively contained flavonoids, alkaloids, steroids, and saponins. Alabri *et al*. (2014) found that the methanol extract of datura leaves contains alkaloids, flavonoids, and saponins but negative in steroids. Many factors can influence these different results. Various factors cause secondary metabolite variability of the same species, including physiological variations, environmental conditions, geographical variations, genetic and evolutionary factors (Figueiredo *et al*., 2008). Given that the samples obtained also show different geographic locations and environments.

Secondary metabolites' content plays a role in providing antioxidant effects but through different biological mechanisms (Suresh and Nagarajan, 2009; Hossain and Nagooru, 2011). Most of the secondary metabolite components that have been isolated from datura plants that show biological activity are extracts dissolved with polar solvents (Gonzalez-Guevara *et al*., 2004; Alabri *et al*., 2014). Several research results on the flavonoid group show high potential biological activity as antioxidants, anti-inflammatory, anti-microbial, anti-cancer, and anti-allergic reactions (Chao *et al*., 2002; Thitilertdecha *et al*., 2008; Igbinosa, Igbinosa and Aiyegoro, 2009; Anyasor *et al*., 2010). Saponins are secondary metabolites that play a role in the plant defense system. Therefore, saponins show anti-microbial activity (Banso and Adeyemo, 2006; Ayoola *et al*., 2008). Phenolic compounds such as tannins and their derivatives are considered as compounds that act as antioxidants or free radical scavengers (Akharaiyi, 2001; Sekar *et al*., 2012; Vadlapudi and Kaladhar, 2012).

BSLT cytotoxicity test

The main solution from the thick methanol extract of datura leaves (*Datura stramonium*) was diluted to 5 concentrations, namely 10 ppm, 20 ppm, 40 ppm, 80 ppm, and 160 ppm, to be used in concentration orientation tests. Negative controls were seawater and shrimp larvae without the extracts. It was occurred to determine seawater and other larval mortality factors (Ramachandran *et al*., 2011). Thus, it can be as certained that the addition of the extract only causes the death of larvae.

After the concentration orientation test was carried out to obtain the percentage of larval mortality in the range of 10% -90%, the test concentrations were 160 ppm, 80 ppm, 40 ppm, 20 ppm, and 10 ppm. This experiment was performed in 3 repetitions (triple) to obtain more accurate

data. Larvae that were 48 hours old were put into vials containing 10 different concentrations of each. After 24 hours of extract addition, the larvae mortality was observed. Table 3 shows the results of the cytotoxic test of datura leaf extract.

The mortality results of *Artemia salina* Leach larvae in each vial were compared, including negative control vials. The total larvae used in each concentration with 3 repetitions of the experiment was 30 individuals. Therefore, the total larvae used in the entire experiment was 180. The percentage of mortality is obtained by multiplying the average mortality rate for larvae by 100. The use of *Artemia salina* larvae in this BSLT test is because shrimp larvae have an affinity with mammals. Such as having the same DNA-dependent RNA polymerase as that of mammals (Bagheri *et al*., 2010).

The eggs of *Artemia salina* Leach used were shrimp larvae that were 48 hours old. At the age of 24 hours, the new larvae will enter the first instar-phase, where the larvae cannot eat because their mouth and digestive tract are not yet fully formed. Meanwhile, at the age of 36-48 hours after hatching, the larvae will metamorphose into a second instar where the larvae already have a perfect mouth and digestive system. Thus, the larval environment extract enters the larvae body and causes death in the larvae (Chanda and Baravalia, 2011). Based on standard criteria, larvae are said to be dead if they do not move for 10 seconds of observation. Observation of larval mortality is carried out after 24 hours after giving the extract (Bagheri *et al*., 2010). The value above is entered into the straight-line equation y = bx + a, where the y value is the 50% probit value of the death percentage, then X is the concentration log, and the antilog X is LC_{50} (Figure 1).

The results of the toxicity test of the datura leaf extract are shown in the graph above. It can be concluded that the higher the concentration of an extract, the higher the mortality rate of the larvae. Calculation of LC_{50} using the straight-line equation Y = 2.6537X + 0.5833, then input the

number 5 in the Y value to obtain LC_{50} 46.1636 μg/ml. An extract indicates a strong cytotoxic presence and can further analyze if the LC50 value is <100 ppm. Calculation using this method produces LC50, which is included in the toxic category. It indicates that datura leaf extract has a very cytotoxic ability and can potentially further analyze (Chanda and Baravalia, 2011; Ramachandran *et al*., 2011).

The biological response of plants does not come from one component but a mixture of various bioactive components from plants (Baravalia *et al*., 2012). The BSLT test results showed a correlation with the results of the phytochemical screening test. Phytochemical screening showed the presence of flavonoids, alkaloids, steroids, and saponins. Flavonoids and alkaloids are thought to form a complementary effect to kill shrimp larvae (Subhadra *et al*., 2011; Alabri *et al*., 2014). They work by acting as stomach poisoning. Therefore, when these compounds enter the larvae body, their digestive organs will be disturbed. This compound will inhibit the taste receptors in the larvae mouth. This results in the larvae failing to get a taste stimulus, thus they are unable to recognize their food, and as a result, the larvae die of starvation (Padua *et al*., 1999; Djali *et al*., 2018; Khairunnisa *et al*., Diana, 2018). Meanwhile, other bioactive compounds that cause shrimp larvae death are steroids. Steroids and saponins in plants are toxic to insects, bacteria, and fungi and can be used as drugs to prohibit tumor cell growth in plants and animals (Rohmawati and Sutoyo, 2018; Nugrahaningsih *et al*., 2019).

BSLT is very sensitive because the Artemia larvae have fragile skin, making it easy for the solution to diffuse. Furthermore, the rapid growth of Artemia larvae resembles cancer cells' growth, making it easier for researchers to detect changes in biological responses (Mirzaei and Mirzaei, 2013; Thangapandi and Pushpanathan, 2014). Several previous studies have concluded a direct relationship between toxic activity in BSLT and antiproliferative effects (Suzery and

Cahyono, 2014; Handayani *et al*., 2018; Sandrawati *et al*., 2019). BSLT has a synergistic correlation with cytotoxic activity in some solid human tumors and pesticide activity. It has led to the discovery a new class of natural pesticides and active antitumor agents (Chanda and Baravalia, 2011). Therefore, it might be suggested that the BSLT is an inexpensive, easy to master, and suitable preliminary test for predicting cytotoxic activity (McLaughlin *et al*., 1998).

Separation and identification of compounds

The fractionation of the compound components in the chloroform extract of datura leaves began with determining the eluent (mobile phase) through TLC. The stationary phase used was the G60 F254 silica plate. Observations of the stains on the TLC plate were carried out using 254 nm and 366 nm UV lamps. The use of UV 254 lamps will cause fluorescent silica gel. It distinguishes it from Silica Gel, which binds to the compound (stain) and will appear blurry and differently clear (Fried, 2017). The search for the mobile phase (eluent) begins with a single eluent and then a combination of eluents to get the best separation shown through the separation stage between spots. TLC's mobile phase optimization is carried out until a suitable solvent is obtained based on a trial and error system (Coskun, 2016). The result is a combination of 2 solvents, namely chloroform: n-hexane with a ratio (1: 1 v/v). The combination of these solvents produces spots that are relatively far apart. Thus, it is used as an eluent in preparative thin-layer chromatography (TLC). From the calculation results, the Rf stain value is 0,61. The Rf value is still vulnerable to the recommended Rf value of 0.2-0.8 (Gandjar and Rohman, 2012).

The isolation process using TLC with a stationary phase of silica gel G60 F254 using a mobile phase of chlorophyll-n.hexane (1:1 v/v). The elution result consists of 1 dominant band and is separated from the other bands that are far apart to facilitate the scraping process. The results

were observed under 254 nm and 366 nm UV lamps. The visible band at UV 254 nm is due to the interaction between UV rays and the plate's indicator, namely silica gel F254 (Spangenberg *et al*., 2011). The plate will glow in UV light 254, while the stained area will cover the plate's light. Thus the stain can be seen (Fried, 2017). Whereas at a wavelength of UV 366, it will show the band's fluorosis and indicate the presence of a conjugated double bond (Wang *et al*., 2012; Ferey *et al*., 2017).

The results of the KLTP scraped off were then refined using the same method. The isolates produced yellowish stains after being sprayed with cerium sulfate (Figure 1). Cerium sulfate functions as a chelating agent. Thus, stains can appear in visible light (Harbone, 2001; Wall and Smith, 2005; Pandey and Tripathi, 2014). The main compound bands which have been predicted to be located are scraped off. The scraped powder was extracted with methanol and filtered, then evaporated to obtain the isolate powder's crystals. Figure 2 shows the results of the isolate purification process by performing the TLC.

To confirm the purity of the isolates, an evaluation was carried out using TLC (Coskun, 2016). The isolates obtained were eluted with 3 different types of eluents, polar, semi-polar and non-polar. Figure 3 shows one of the semi-polar elution of the mobile phase of methanol: n.hexane (1: 1 v/v) as a result of the isolate purity evaluation process.

The TLC technique was used to confirm the purity of the isolates. Using eluent with 3 different variations, namely polar, semi-polar and non-polar with variations in the polar mobile phase of methanol: n.hexane (4: 1 v/v), semi-polar methanol: n.hexane (1: 1 v/v)) non polar methanol: n.hexane (1: 4 v/v). The elution evaluation results of the isolates' purity showed that only one stain appeared in the 3 eluent concentrations. It indicates that the isolates obtained contain only one compound based on the TLC method (Coskun, 2016). Isolates are said to be pure should

they are eluted using three variations of polar, semi-polar, and non-polar mobile phases, showing the consistency of only having one spot (Bajpai *et al.,* 2016). The stains formed also showed that the polar eluent Rf was below 0.28, semi-polar Rf was in the middle 0.61, and non-polar Rf was above 0.85. It occurs due to the eluent's influence, where the polar eluent will cause the isolate not to be eluted upwards because hydrogen bonds are formed between the isolate and silanol on the TLC plate. When the eluent's polarity is reduced, the dimer interaction between the isolate and the TLC plate is reduced, causing the isolate to be more susceptible to Rf (Rossing and Chiaverina, 2000; Waksmundzka-Hajnos *et al.,* 2008; Marston, 2011).

Wasnik *et al.*, (2009) identified Withanolide using the TLC, documented the Rf. The approximate of Withanolide was 0.61 with methanol: n: hexane (50:50%) as eluent. The spray reagent used to identify phytosterol groups in plants uses Cerium Sulfate. The alcohol group steroid group's presence is characterized by yellow to brown stains in visible light and light blue fluorescence of 366 nm UV rays (Harbone, 2001; Sarker and Nahar, 2012). Based on the isolate chromatogram results by spray, the cerium sulfate reagent shows a yellow-brown color. It indicates the presence of phytosterol compounds. The color that occurs is due to a substituted hydroxyl group on the steroid ring with a positively charged Ce (Rossing and Chiaverina, 2000; Harbone, 2001).

Isolate identification using LCMS

The initial step before carrying out the LCMS analysis is to optimize the instrument and its mobile phase. The purpose of optimizing the LCMS tool is to see the most suitable conditions for analyzing paracetamol in herbal samples. Based on the LCMS conditions' optimization results (Table 1), the optimal conditions for analyzing paracetamol are best obtained by using an isocratic

elution system. According to Yu *et al*. (2016), in the isocratic elution system, the mobile phase used is regulated in a constant concentration that is pumped into the column. This study's type of column is the type of octadecyl silica column (ODS or C_{18}) with the silica gel component in it. This C_{18} column has a reversed-phase column and can produce the best separation with high levels of purity and accuracy (Jian, 2017).

In this analysis, an isolate chromatogram was obtained, which showed a sharp peak at the retention time (tR) of 3.20 minutes. According to Termopoli *et al*. (2019) the use of LCMS in the qualitative analysis was carried out by looking at the chromatogram peaks. The sharper the chromatogram peak indicates that the compound only consists of one compound. The chromatograms of the isolates are shown in Figure 4.

Retention time (tR) is the time required for the analyte, which starts during the injection process until the column's separation process. The separation response will be sent in the form of a signal read by the detector. Small peaks formed around it indicate that impurities are present (Niessen and Correa, 2017). These impurities usually come from solvents or during the sample preparation process (Termopoli *et al*., 2019). However, the presence of impurities did not have a significant effect on the peaks of the isolates. It can be seen from the chromatogram peaks that are relatively far apart.

Furthermore, specific isolates were identified using a mass spectrometer to analyze these isolates' molecular ions and fragmentation patterns. The mass spectrometer detector will identify the compound eluted from the LCMS column by ionizing it first, then measuring the mass ratio (m/z) and molecular fragments into small pieces. The results of the detection of isolates using a mass spectrometer are as shown in Figure 5.

Table 4 shows the detected molecular weight and fragmentation patterns. The isolate is

assumed to be a Baimantuoluoline D (m/z 504) compound, a steroid derivative from the Withanolide group (Xia *et al*., 2019). The Withanolide family has been detected in more than 600 different plants (Misico RI *et al*., 2011). Withanolide is formed from the Ergostane skeleton with side-chain modifications in the δ-lactone ring-substituted on the C_{22} and C_{26} carbon chains (Guo *et al*., 2018). Withanolide derivatives have been known to have anti-inflammatory, antitumor, cytotoxic, and immunomodulatory activities (Xu *et al*., 2018; Wu *et al*., 2020). The isolate is known to have a molecular weight of [M^+] 504,0591 m/z with a base peak of 477,2583 m/z. The daughter fragments formed 477.2583 m/z [M^+ ¬-CH_3-OH] and 301.1780 [M^+ ¬-$C_9H_{11}O_4$] (Figure 4). At the 477.2583 m/z fragments, there was a breakdown of hydroxy at Ring A of C_1 carbon and methyl at C_{19}. The termination of the hydroxy bonds is due to non-bonding bonds on the C_1 carbon, thus easier to be released. Meanwhile, the effect of Lewis acid on the proton of the neighboring hydroxy group (C_1) is to form hydrogen bonds, which result in the instability of C_{18} carbon. The formation of the 301.1780 m/z fragment is more due to the instability of the δ-lactone ring in the Ergostane framework due to epoxy in carbon substitution C22 C_{26} (Niessen and Correa, 2017; Yang *et al*., 2020).

The mass spectrum is an accurate, valid, and decisive identification because it can directly identify the structure of an unknown compound in a complex mixture even with a minimal concentration (Evard *et al*., 2016). Based on the mass spectra data obtained, the isolates analyzed showed 504, 427, 301 m/z. This pattern is in line with that obtained by Yang *et al*. (2020), who confirmed that one of the secondary metabolites in datura leaves is *BaimantuoluolineD* with a molecular weight of 504.5 m/z with ion fragments formed 504,477.301 m/z (Niessen and Correa, 2017; Yang *et al*., 2020). This fragmentation pattern confirms that the isolate is a *Baimantuoluoline D*.

The fragmentation pattern that is formed is also not abundant because the ionizer system used is ESI (electron spray ionization). The electron ionization (EI) system uses the Bombardement technique with a potential energy of 70 eV, which causes many fragments to be formed (Wei *et al*., 2019). In the ESI system using Electrospraying technology, the isolate's molecular ion is obtained by evaporation, where the charged liquid particles are reduced in size, and the electric-charge becomes closer together. The reduction in grain size due to evaporation continues to occur, to the point where the Coulombic repulsive force overcomes and opposes the granules' cohesive force, resulting in desolvation or the breakdown of the solvent (Banerjee and Mazumdar, 2012; Schröder, 2012). The sample in the granules will be released/desorbed out in the form of $[M + H]^+$ or $[M-H]^-$. The formed fragments are not as many as in the EI system because the potential difference given is only 3-5 eV (Chen *et al*., 2011).

Research conducted by Yang *et al*. (2020) managed to find 85 with an olide isolates. One of them is the *Daturamentaline* compound found in all parts of plants. *Daturafoliside* compounds are found in all parts of the plant except in the seeds. Based on literature studies, it shows the leaves' antifungal potential, especially related to the withanolide content in datura leaves (Dabur *et al*., 2004; Dabur *et al*., 2004; Chukunda *et al*., 2019). Antifungal activity in datura is found in leaves and fruit, while other parts are very low. Roots are generally less active than leaves, fruit, and stems (Javaid and Saddique, 2012). Geographical differences significantly affect the existence of secondary metabolites in plant organs (Al-Snafi, 2017).

Apart from datura, Withanolide is also found in the roots of *Withania somnifera*, which is well-known ginseng from India (Misra *et al*., 2008; Trivedi *et al*., 2017). Several studies also show the Withanolide groups such as β-hydroxy-2,3-dihydro-withanolide F, withanolide A, withaferine A, and within one withanolide D, ixocarpalactone A, withanolide S, and thiowithanolide

(Chatterjee *et al*., 2010; Trivedi *et al*., 2017). Besides, it shows that isolates from *Withania somnifera* have various pharmacological activities including antioxidants, anticancer, immunomodulating, hepatoprotective, neuroprotective, anti-inflammatory, anti-inflammatory, anti-microbial, hypoglycemic (Budhiraja *et al*., 2000; Singh *et al*., 2010; Chen *et al*., 2011; Gorelick *et al*., 2015)

CONCLUSION

The phytochemical screening results showed that the datura leaves contained alkaloids, flavonoids, steroids, and saponins. The cytotoxic activity of datura leaf extract obtained LC_{50} 46.1636 μg/ml. The isolate obtained from datura leaves is *Baimantuoluoline D*, a steroid base framework and is classified as a steroid derivative compound from the Withanolide group. The LCMS results support that the isolate has a molecular weight of [M $^{+}$] 504.0591 m/z with the daughter fragments of 477.2583 and 301,1780 m/z. This research helps to find a way to isolate the withanolide group compounds, one of which is *Baimantuoluoline D*. Furthermore, activity tests are needed on *Baimantuoluoline D* isolates to develop medicinal compounds and to perceive their usefulness.

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