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ANALYSIS OF SILDENAFIL AND ITS DERIVATIVES IN JAMU (HERBAL MEDICINES) USING LC/MS/MS SPECTROSCOPY

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Abstracts. Jamu (herbal medicine) is a traditional Indonesian medicine used to nourish, treat, and restore of health, as well as for beauty and fitness. A healthy Jamu is safe when consumed and free from chemicals (in Indonesia it's called BKO). BKO is a medicinal chemical compound which is intentionally added in jamu (herbal medicine), which aims to increase the accelerate effects of Jamu or herbal medicine from the usual. The aims of this study are to extract, isolate, identify and elucidate the structure of the BKO of sildenafil and it's derivatives in two herbal preparations circulating in the city of Banda Aceh. Extraction, isolation and identification of sildenafil BKO and its derivatives in jamu were done by using column chromatography (CC) and thin layer chromatography (TLC) meanwhile, the structure elucidation of sildenafil and it's derivatives were done by Fourier-Transform Infra-Red (FT-IR) and liquid chromatography/mass spectroscopy (LC/MS/MS). The results of extraction, isolation and identification with a solvent ethyl acetate: methanol: ammonium hydroxide (25%) (100:15:1.5 $^{v}/_{v}$ showed that two jamu circulating in Banda Aceh positively contained BKO sildenafil derivatives, namely thiosildenafil. The FT-IR analysis showed a strong absorption of thiocarbonyl (C=S) group at 1288-1242 cm^{-1}, which is a typical uptake of the thiosildenafil compound. The Spectroscopy LC/MS/MS analysis using electron spray ionization (ESI-MS/MS) showed that the isolate have a similar spectra with thiosildenafil compound $[TSLD+H]^+$ at 491 m/z and $[TSLD+Na]^+$ at 513 m/z and other derivate of thiosildenafil, namely propoxyphenylhydroxylethylthiosildenafil $[PHTSLD+H]^+$ at 533 m/z.

Keywords: Jamu, sildenafil, thiosildenafil, propoxyphenylhydroxyethylthiosildenafil

I INTRODUCTION

Drug Chemicals (in Indonesia it's called BKO) are synthetic drug compounds or chemical products derived from natural materials commonly used in modern medicine. The addition of BKO in traditional medicine preparations is still common, and it is very dangerous because it does not go through safety tests, efficacy and quality, improper quantities, no clear warnings and inappropriate use rules [1]. Sildenafil or known Viagra® (Pfizer) is a BKO that is often found in herbal medicine and includes a class of strong- drugs, used as an inhibitor *phosphodiesterase-5* (PDE-5) for the treatment of erectile dysfunction in men [2]. This drug works by increasing the levels of cyclic guanosine monoposphate (cGMP) in the *corpus cavernosum* indirectly by inhibition of the enzyme PDE-5 by increasing nitrogen oxide. This effect is utilized by patients with erectile dysfunction [3]. Traditional medicines or jamu are ingredients or ingredients in the form of plant materials, animal materials, mineral materials, sari preparations or mixtures of these substances which have been hereditary for treatment, and applied in accordance with the norms prevailing in the community. Jamu its have small side effects at a low cost, but have a disadvantage that the resulting therapeutic effects take a long time [4]. This is what makes jamu manufacturers to add BKO into their products, with the aim of speeding up the desired therapeutic reactions [5]. In several studies that have been done, it was found that the presence of synthetic compounds that have similarities in chemical structure with sildenafil and often identified in small quantities contained in jamu (herbal medicine) special for men who circulate in the market or purchase online [6, 7]. Generally the modification of this sildenafil structure lies in the piperazine ring and the presence of position exchange in the lactam portion between sulfur and oxygen. The derived compounds of sildenafil can be developed into new drug discovery, but not selected as clinical treatment. This is because the threat of health hazards that consumers are not aware of the content of BKO, which has not been tested safely to be used in medicine [8, 9]. Sildenafil analysis qualitatively and quantitatively has been done by using several methods with Thin Layer Chromatography (TLC), Column Chromatogphy (CC), UV-Vis Spectrophotometer, FT-IR, NMR, HPLC, LC/MS and LC/MS/MS. The results of the analysis are not only sildenafil found, but also

sildenafil derivatives. In this study, sildenafil and it's derivatives were analyzed in jamu (herbal medicine), where extraction and isolation using the CC method, followed by identification using the TLC method, while the elucidation of the structure of compounds obtained from jamu using the method FT-IR and LC/MS/MS spectroscopy methods.

II METHODOLOGY

In this study using 2 herb preparations circulating around the city banda Aceh and standard sildenafil (Viagra®, Pfizer) (Figure 1). The method of selecting samples by purposive sampling with criteria of jamu that are not registered in BPOM, or registered with product name and registration number is fictitious.

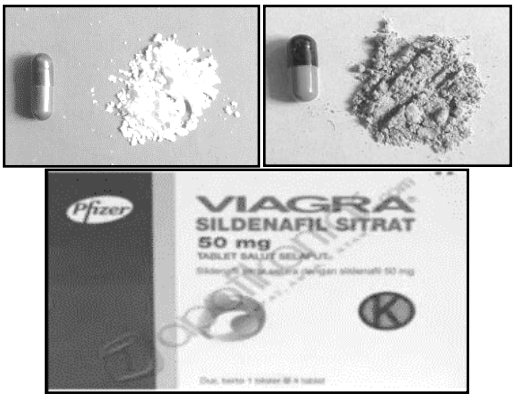


Figure 1 Picture of (a) Jamu 1 (b) Jamu 2 and (c) Standard Sildenafil

Extraction and Isolation by Column Chromatography (CC)
Accurately weighed ±2 g powder of jamu, then mixed with a little silica gel powder 60 (0.2-0.5 mm), stir until homogeneous. The columns are cleaned, then inserted a 60 (0.2-0.5 mm) silica gel powder that has been dampened with a solvent, ethyl acetate: methanol: ammonium hydroxide (25%) (100:15:1.5 $^{v}/_{v}$. Furthermore, that powdered of jamu have been mixed with silica gel powder 60 (0.2-0.5 mm), and stream the solvent into the column. The fraction obtained is accommodated in vials and evaporated. Fractions were collected based on the same Rf value as the standard sildenafil [10].

Identification by Thin Layer Chromatography (TLC)
The fraction results obtained from extraction and isolation by family planning were then identified by TLC by means of being sprayed in the stationary phase using silica gel G-60 GF_{254} plate and mobile phase using ethyl acetate: methanol: ammonium hydroxide (25%) (100:15:1.5 v/v). Observed stain patterns formed between sample jamu and standard sildenafil [10].

Elucidation of Structure

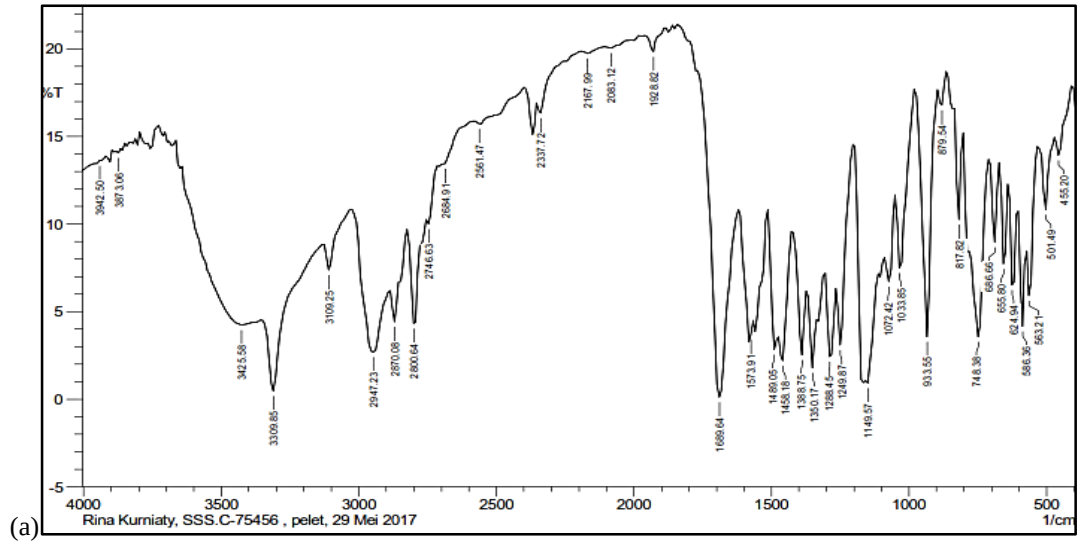
FT-IR Analysis
The analysis was performed by KBr pellet method, using Shimadzu IR Prestige-21 *fourier-transform infra-red* (FT-IR) with spectral range 4000-400 cm^{-1}.

LC/MS/MS Analysis
The analysis is done by using LC/MS/MS equipped with The Waters Vevo TQD (Tandem Quadrupole Detector). The mobile phase it used 0.1% formiat acid and methanol (35:66), flow rate 0.3 mL/min, and volume of injection 2 µL. Reserved phase used C18 Acquity, flow rate 0.3 mL/min, volume of injection 2 µL for 7 min., low-noise, off-axis, long-life photomultiplier detector. This technique is equipped with ionization ES^{+} mode, capillary voltage 3 kV, cone voltage 25 V, desolvition temperature 500ºC and source temperature 1000 L/hr.

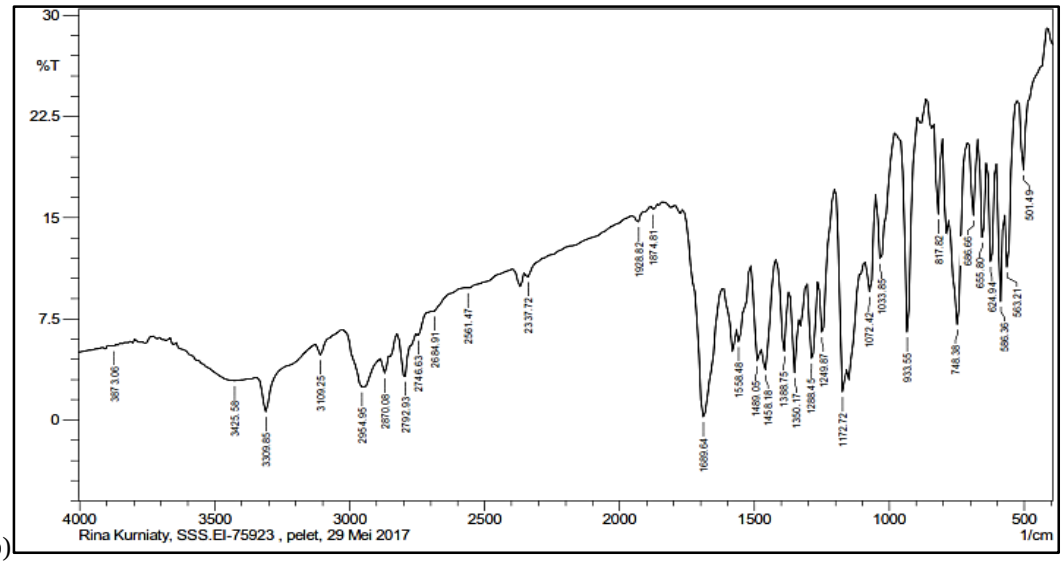
III RESULTS AND DISCUSSION

Organoleptically sample of jamu 1, that is on the color and odor have differences with the sample of jamu 2. Jamu or tradisional medicines, usually has its own distinctive color and odor based on the result of mixture of natural ingredients or simplicia that form part of plant, like rimpang, leaf, bark and fruit. In the sample of jamu 1 has no color and unique smell of natural ingredients, but has a white powder color, so it is suspected in this jamu containing synthesis drug packed with herbal medicine label. The results of isolation and extraction by column chromatography on the sample of jamu 1 and sample jamu 2, were each obtained 3 fractions. Furthermore, the three fractions of each sample jamu are reanalyzed using the TLC method. The fraction of the sample jamu has a stain pattern and the same Rf value as the standard sildenafil is reunited. Further purification of the fraction is carried out, so that the pure fraction isolates obtained from the sample of jamu 1 as much as 130 mg and sample of jamu 2 as much as 87 mg (Table 1).

FT-IR Analysis
In (Figure 2 and Table 2), the FT-IR analysis of samples jamu 1 shows the presence of a functional group of SO_2 stretch with spectral range in the region of 1172 cm^{-1}, aromatic C=C bond at 1558 cm^{-1}, C=O stretch at region 1697 cm^{-1}, saturated C-H stretch at region 2870 cm^{-1}, unsaturated C-H stretch at region at 3109 cm^{-1}, secondary N-H stretch at region 3309 cm^{-1} and OH stretch at region 3425 cm^{-1}. Sample jamu 2, also shows the same FT-IR spectra with functional group of SO_2 stretch at region 1149 cm^{-1}, aromatic C=C bond at region 1573 cm^{-1}, C=O stretch at region



(a)



(b)

Figure 2 Results of FT-IR analysis sample of (a) jamu 1 and (b) jamu 2

1689 cm^{-1}, saturated C-H stretch at region 2947-2870 cm^{-1}, unsaturated C-H at region 3109 cm^{-1}, secondary, N-H *stretch* at region 3309 cm^{-1} and O-H *stretch* at region 3425 cm^{-1}. Result of FT-IR analysis on sample of jamu 1 and jamu 2, still on the characteristic of the spectral range of FT-IR sidenafil (Table 2), except in the 1700-1500 cm^{-1} spectral range aromatic the C=C bond, but this spectral range still shows the characteristics of the sildenafil spectra range [11]. The C=S (thioceton) bond hose of the sample of jamu 1 and sample of jamu 2 it's in an area of 1288-1242 cm^{-1}, which shows the spectral characteristic of the compound of the sildenafil derivative, namely thiosildenafil [12].

Analisis LC/MS/MS

Figure 3, shows the results of ESI(+)-MS/MS spectra of sildenafil ion (SLD) molecules obtained from Viagra® tablets extracted using a methanol solvent. Identification of detected ion SLD form from ESI(+)-MS/MS at spectra m/z 475. Results of collision induced dissociation (CID) from collision process at MS/MS stage will form the structure $[SLD+H]^+$ at m/z 475 as the main fragment [13]. The molecular weight of a generated compound can provide valuable information, in which the analytical ions will be fragmented by the presence of colliding molecules. The voltage applied to the analytical ions to increase energy, in order to be able to collide so as to create more fragmentation [14].

Table 1 Rf value of standard sildenafil and sample of jamu 1 and jamu 2

Sample	Rf Value	Fraction	Weight (mg)
Standar sildenafil	0.61	-	-
Jamu 1	0.61	1	130
Jamu 2	0.61	1	87

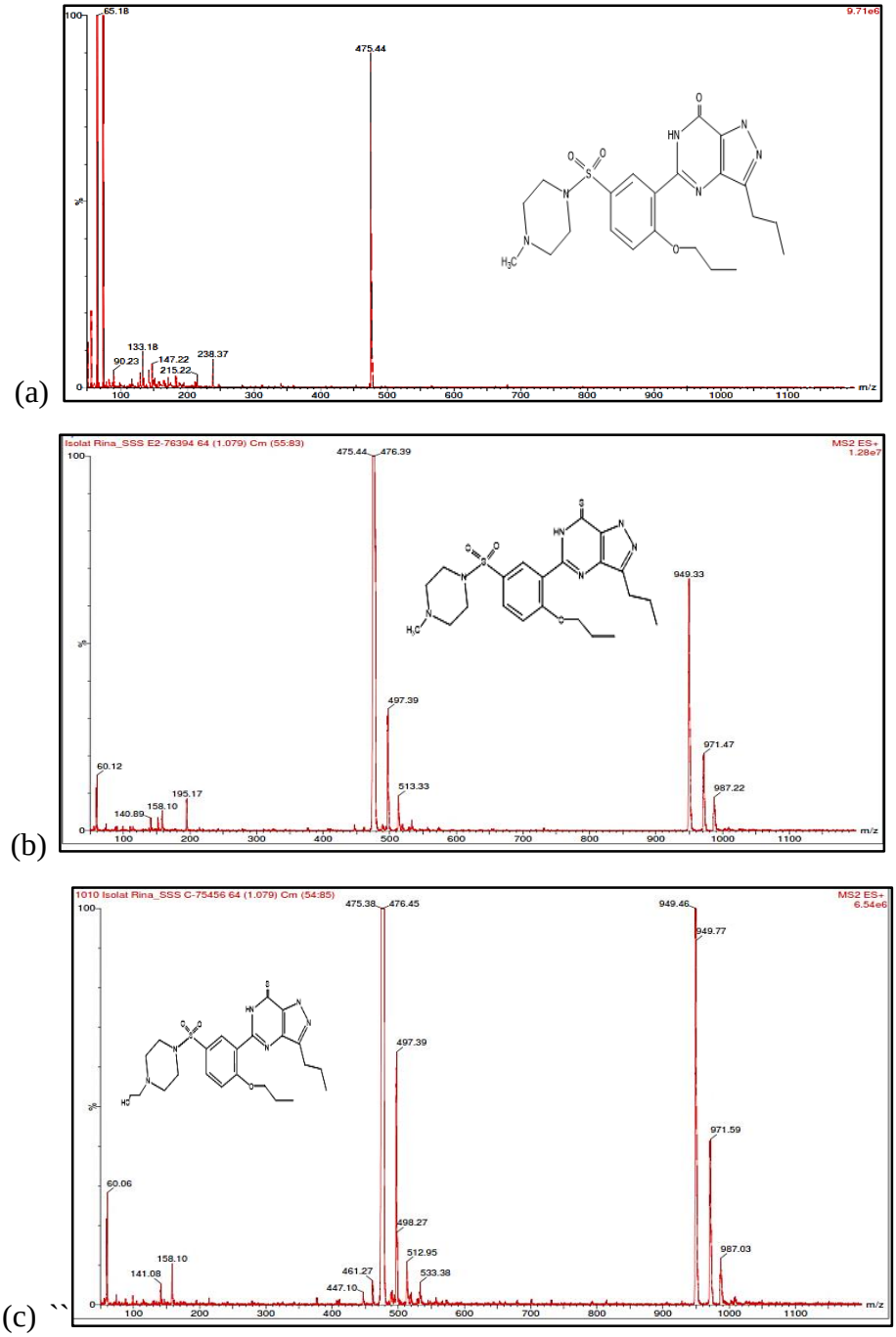


Figure 3. Spectra ESI(+)-MS/MS of sildenafil its derivatives in sample of (a) sidenafil (b)jamu 1 (c) jamu 2

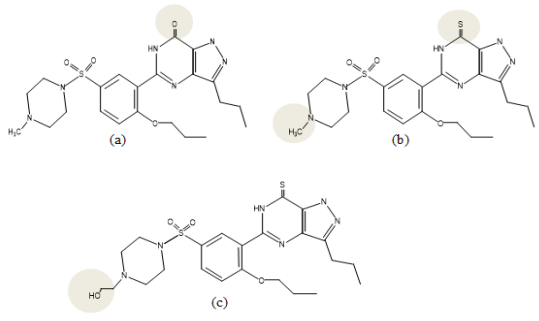
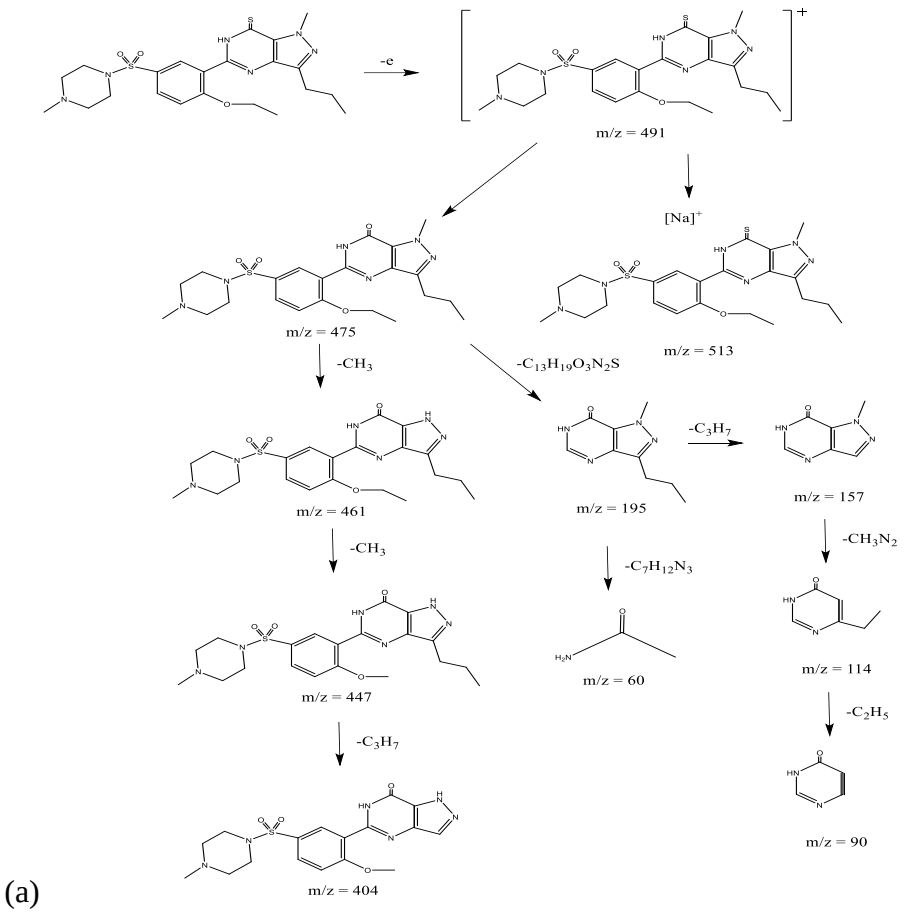
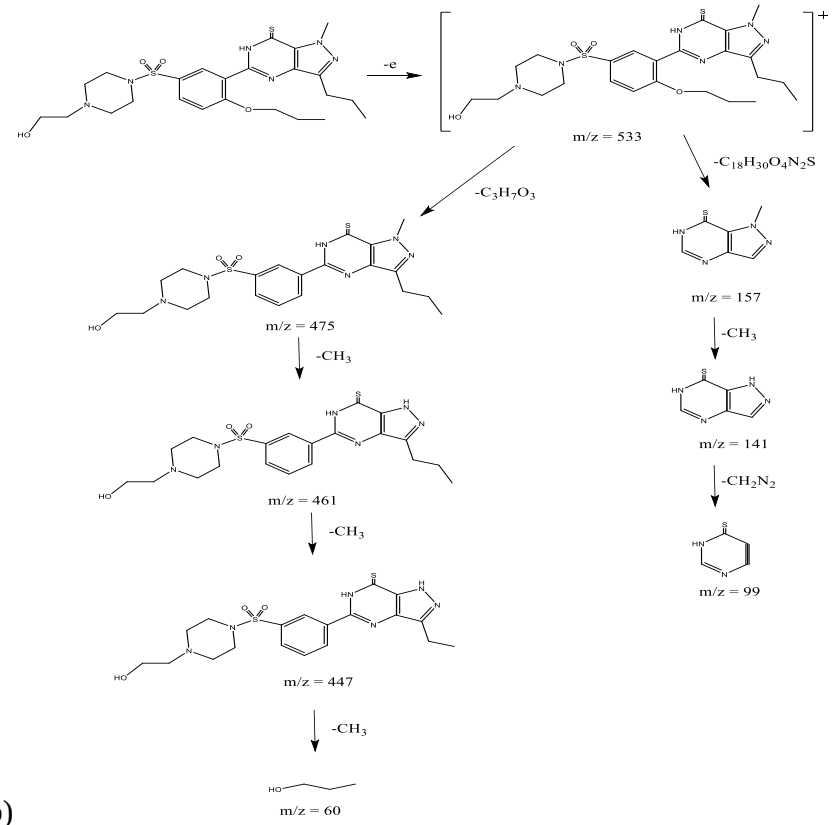


Figure 4. Structure of (a) sildenafil, (b) thiosildenafil, dan (c) *propoxyphenylhidroxyethylthiosildenafil* [6, 12]



(a)



(b)

Figure 5. Fragmentation (a) Thiosildenafil ($C_{22}H_{30}N_6O_3S_2$) at m/z 491 and (b) Propoxyphenylhidroxyethylthiosildenafil ($C_{24}H_{34}N_6O_4S2$) at m/z 533

Table 2 FT-IR spectrum analysis results of sildenafil based on literature [11, 12]

No	Fungsional Group	Characteristic Absorption Range (cm^{-1})
1.	SO_2 stretch	1172.74
2.	C=C aromatis	1582.62
3.	C=O stretch	1702.21
4.	-C-H stretch	2962.71
5.	=C-H stretch	3029.26
6.	N-H stretch	3299.3
7.	O-H stretch	3617
8.	C=S	1288-1242

The results of ESI (+)-MS/MS spectral data (Figure 3) in the sample jamu 1, show that the presence of compounds having ESI (+)-MS/MS ionic characterizations similar to those of sildenafil derivatives, namely thiosildenafil $[TSLD+H]^+$ at m/z 491 and $[TSLD+Na]^+$ at m/z 513. The presence of sildenafil ion characteristics $[SLD+H]^+$ at m/z 475 and $[SLD+Na]^+$ at m/z 497 [6]. Fragmentation of other ions formed are m/z 195, m/z 158, m/z 140 and m/z 60 (Figure 5). The molecular structure form of thiosildenafil ($C_{22}H_{30}N_6O_3S_2$) has a molecular mass of a compound it larger than the structure molecule of the sildenafil, which mass 16 it's an oxygen atom [6]. The results of the ESI (+)-MS/MS (Figure 3) spectral data from the sample of jamu 2, also show the presence of similar ionic compounds of thiosildenafil derivatives, namely propoxyphenyl-hydroxyethylthiosildenafil $[PHTSLD+H]^+$ at m/z 533 and the presence of ionic characteristics $[SLD+H]^+$ at m/z 475, $[SLD+Na]^+$ at m/z 497, and $[TSLD+Na]^+$ at m/z 513 [6]. Fragmentation of other ions are m/z 461, m/z 447, m/z 158, m/z 141 and m/z 60 (Figure 5). The compound of this thiosildenafil derivative has a molecular structure it's $C_{24}H_{34}N_6O_3S_2$. The structural differences of these two compounds are the ethoxyl and methyl functional groups present in thiosildenafil replaced by the propoxy and hydroxyethyl groups in piperazine ring, there by forming a compound derived from thiosildenafil [12].

CONCLUSION

Sample of jamu 1 and jamu 2 circulating in Banda Aceh city positive containing sidenafil derivatives, identified by using CC, TLC, FT-IR and LC/MS/MS spectroscopy methods**.** The results of FT-IR analysis shows the functional group of C=S (thioceton) at 1288-1242 cm^{-1}, which is characteristic of functional group of sildenafil derivative, namely thiosildenafil. The results of LC/MS/MS analysis on sample of jamu 1, showed that the presence of compounds having similar structural properties of the sildenafil derivative, namely thiosildenafil ($C_{22}H_{30}N_6O_3S_2$) with fragmen tation $[TSLD+H]^+$ at m/z 491. While, on the sample of jamu 2 shows the existence of compounds of thiosildenafil derivatives, namely propoxyphenylhydroxyethylthiosilde nafil ($C_{24}H_{34}N_6O_4S_2$) with fragmentation $[PHTSLD+H]^+$ at m/z 533.

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