Bioactive compounds in Pinang Yaki (*Areca vestiaria*) fruit as potential source of antifertility agent

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Abstract: Fruits of Pinang Yaki (*Areca vestiaria*) are used by the people around Bogani Nani Wartabone as contraseption for men. Extracts from the fruit contain tannin, triterpenoid, flavonoid and saponin which are potential as bioactive compounds. This research aimed at exploring the fractions or bioactive compounds contained in the fruit. The extract was prepared by fractionation using hexane. The fractions were separated and analysed by gas chromatography mass spectrometry (GC-MS) technique. The fractions revealed the presence of five compounds. These compounds were identified by interpretation of mass spectra and comparing their retention time and covate indexes with those from literature. The five compounds are pentadecane, methyl-dodecanate, methyl-tetradecanoate, hexadecanoic acid and methyl-octadecanate.

Keywords: *Areca vestiaria,* bioactive compounds, fractionation, gas chromatography, hexane, Pinang Yaki.

INTRODUCTION

Most of the country has a problem with the increasing of birth rate. Uncontrolled population will cause negative impacts to demography and make it difficult to be a wealthy country. Indonesia is the fourth-largest country with regard to population sizes after China, India and the United States. Indonesian population in 2014 was 252.20 million with growth rate 1.4% per year (Badan Pusat Statistik 2016), so the projection of the population will be 400 million in 2050. To focus on potential and quality of human resource development, the nation has to be able to control the population growth. For Indonesian government, the demography is important because it is highly related to life quality and welfare.

In an effort to suppress the population growth rate, the Indonesian government took a policy through the Family Planning Program. According to the survey of Health and the Indonesian Democratic issued by the national Family Planning Agency in 2003, from 27 million acceptors in Indonesia, 90% were female, male participation was very low, only about 1.3%. While in Malaysia, the participation of men who undergo family planning program has reached 15%. Furthermore, also reported that the active involvement of men in family planning programs was still very low and limited only by the use of contraceptives condoms (1.11%) and vasectomy (1.35%).

Low participation in male birth control program is caused by the limited option of male contraception. To increase participation of men in birth control, there must be various contraception options available, thereby men have alternatives in choosing the most suitable one. One dificulty in controlling fertility in men is that a man produces million of sperm cells a day, but a woman produces one egg in a month. Injection or pill should control the production of sperm without decreasing libido and causing dangerous side effects. One alternative of male contraception is by using natural compound from plants. Indonesia has a wet tropical climate and rich in plant species. In finding ideal contraception for men, those medicinal plants must prevent fertilization and meet other requirements such as save, reversible, fast, easy to use and without side effects, especially for sex and libido.

Pinang Yaki (*Areca vestiaria*) has long been used by people around the Park Nani Wartabone as male contraception. They take the meat pieces from the fruits and boiled with 1 cup of water. Water decoction is then drunk as a contraceptive drug. Pinang Yaki is one member in *Areca* genus. It has general characteristics such as grow individually or in clusters, thin stem and has rings. This plant has long leaf-sheaths with oval lamina, monoecious and monocotile, ovale shape fruit with orange to red color (Simbala and Tallei, 2010). Phytochemistry analysis showed that Pinang Yaki fruit extract contained tannins, flavonoids, hydroquinones, triterpenoids and saponines. Based on extract characters, Pinang Yaki fruit contained 6.10% water, 0.70% ash, 5.78% water rendements and 16.46% organic solvent rendements (Simbala and Tallei, 2010). This research aimed at screening bioactive compounds of Pinang Yaki fruit for anti-fertility. The results are expected to support World Health Organization (WHO, 1988) programs in finding new methods (contraception) for birth control in

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men that save, effective, reversible and without side effects.

MATERIAL AND METHODS

Collection of plant material and authentication

Pinang Yaki was obtained from Bogani-Nani Wartabone National Park, North Sulawesi. Species identification was conducted at Herbarium Bogoriensse, Balitbang Botani – Puslitbang Biologi - LIPI -Bogor.

Preparation of extract

The fruit was assured to be free from disease and thoroughly washed with running tap water. The fruit was then rinsed with distilled water and air dried. The dried fruits were homogenized using a mixer grinder to obtain coarse powder. The coarse powder was macerated with methanol. The methanol macerated substances were subsequently extracted with n-hexane solvent using Soxhlet extractor to separate the non-polar compounds from the methanol extract. The resulting hexane extract was concentrated using a rotary evaporator to obtain a thick extract with less water content and dried at 50^{o}C. The hexane fractions were subjected to examination using gas chromatography mass spectrometry (GC-MS).

RESULTS

The hexane fractions of Pinang Yaki fruit revealed the presence of 5 compounds in the form of chromatograms, as followings:

Pentadecane

Gas Chromatography Mass Spectrometry (GC-MS) analysis showed that the compound with retention time 17,326 has MS spectrum described in fig. 1. The spectrum showed molecule ion (M^+) m/z 212 and basic peak m/z 57 and also peak pattern has m/z 14 amu differences.

Based on characteristics of MS in fig. 1, the compound with retention time 17,326 was suspected as pentadecane. Molecular weight of pentadecane was 212g/Mol, confirmed with MS spectrum which, showed molecular ion peak (M^+) on m/z =212. In the fig. 1, there were several hydrocarbon compound characteristics such as small molecular ion peak, peak clusters with 14 amu differences and basic peak on m/z 57. Fragmentation of pentadecane compound is showed in fig. 2. Basic peak m/z 57 was representative of butane ion $(CH_2CH_2CH_2CH_3)^+$.

Basic peak was on m/z 57 which is a representation of butane ion with secondary carbocation (fig. 3). GC-MS analysis showed compound with 17,326 retention time which is pentadecane (an alkane hydrocarbon). The structure showed in fig. 4.

Methyl dodecanoate

GC-MS Analysis showed that compound with retention time 17,689 has a MS spectrum as shown in fig. 5. The spectrum described that molecular ion (M^+) m/z 214 and basic peak was m/z 74 and peak pattern has m/z 14 amu differences. According to MS spectrum in fig. 5, suspected compound with retention time 17,689 is methyl dodecanoate, an aliphatic ester. Molecular weight of methyl dodecanoate is 214 g/mol, confirmed by MS spectrum which shows peak of molecular ion (M^+) on m/z =214. There were several characteristics of methyl ester compound such as a small peak of molecular ions, occurrence of peak clusters with 14 amu differences, occurrence of basic peak on m/z 74.

Fig. 6 shows fragmentation of methyl dodecanoate. Basic peak on m/e 74 was a representation of methoxyethanol compound as a result of McLafferty rearrangement with β-cleavage reaction (fig. 7). Based on GC-MS analysis, compound that has retention time 17,689 is methyldecanoic. The structure is described in fig. 8.

Methyl tetradecanoate

The result of DC-MS analysis showed that compound with retention time 20.125 has MS spectrum as described in fig. 9. The spectrum showed molecular ion (M^+) m/z 242 and basic peak was m/z 74 with m/z 14 amu differences.

Based on characteristics of MS spectrum in fig. 9, suspected compound with 17,689 was methyl tetradecanoate, an aliphatic ester. Molecular weight of methyl-tetradecanoic is 242g/mol, confirmed with MS spectrum that showed peak of molecular ion (M^+) on m/z = 242. In the picture there are several characteristics of methyl ester compound such as small molecular ion peak, occurrence of clusters with 14 amu differences and occurrence of basic peak on m/z 74. Molecule fragmentation of methyl tetradecanoate is shown in fig. 9. Basic peak on m/e 74 was a representation of methoxy-ethanol ion compound that are a McLafferty rearrangement with β-Cleavage reaction (fig. 10). Based on GC-MS analysis, the compound with retentintion time 17.689 was methyl tetradecanoate. The structure is described in fig. 11.

Hexadecanoic acid

GC-MS analysis showed compound with retention time 22,748 has MS spectrum as described in fig. 12. The spectrum showed molecular ion (M^+) m/z 256, basic peak m/z 73, specific peak m/z 60, and peak pattern with 14 amu differences.

According to MS spectrum in fig. 12, suspected compound with retention time 22.748 is hexadecanoic acid. Molecular weight of hexadecanoic acid is 256 g/mol, confirmed by MS spectrum which showed peak of molecular ion (M^+) on m/z = 256. In the picture there were several characteristics of carboxylic acid such as

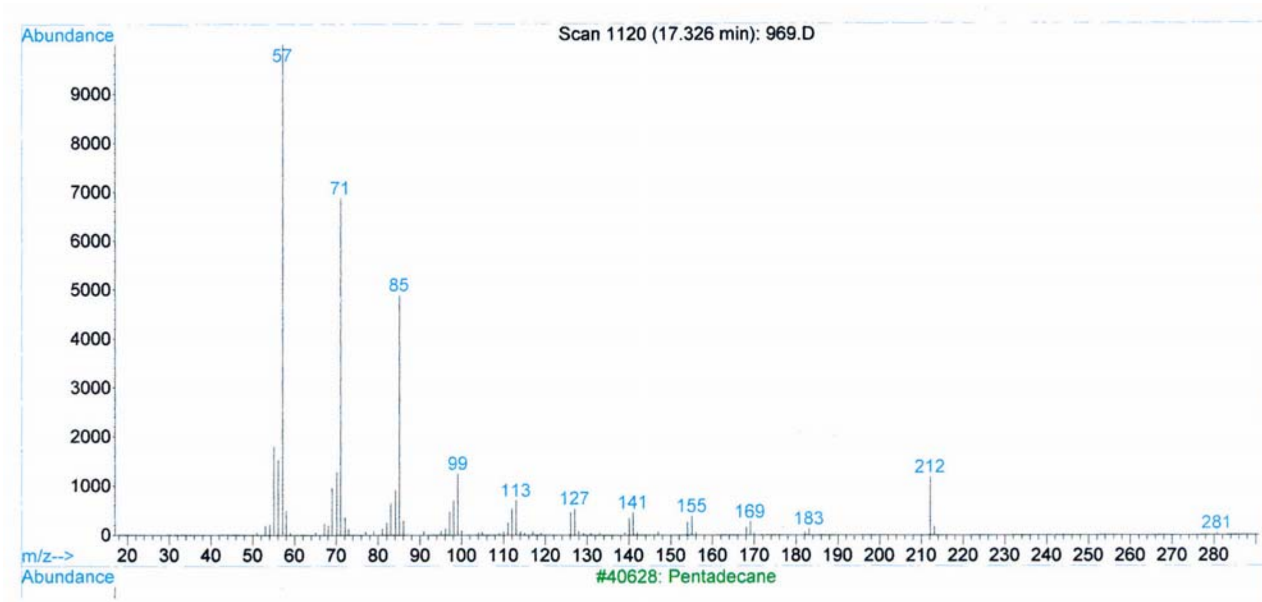

Fig. 1: Mass spectrum of pentadecane

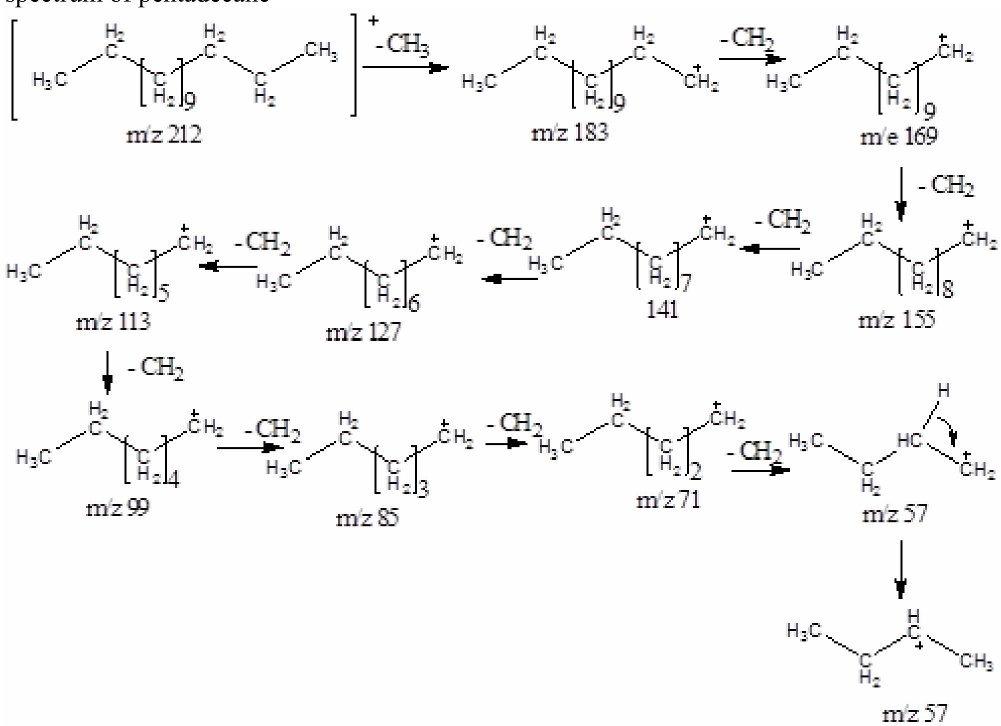

Fig. 2: Fragmentation of pentadecane compound

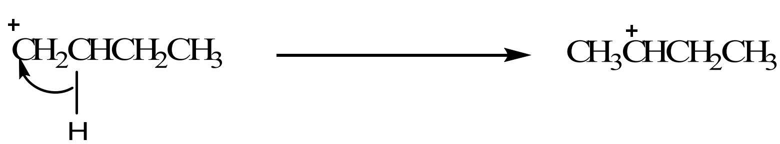

Fig. 3: Butane ion

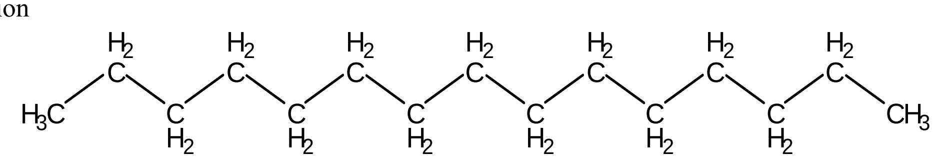

Fig. 4: Structure of pentadecane

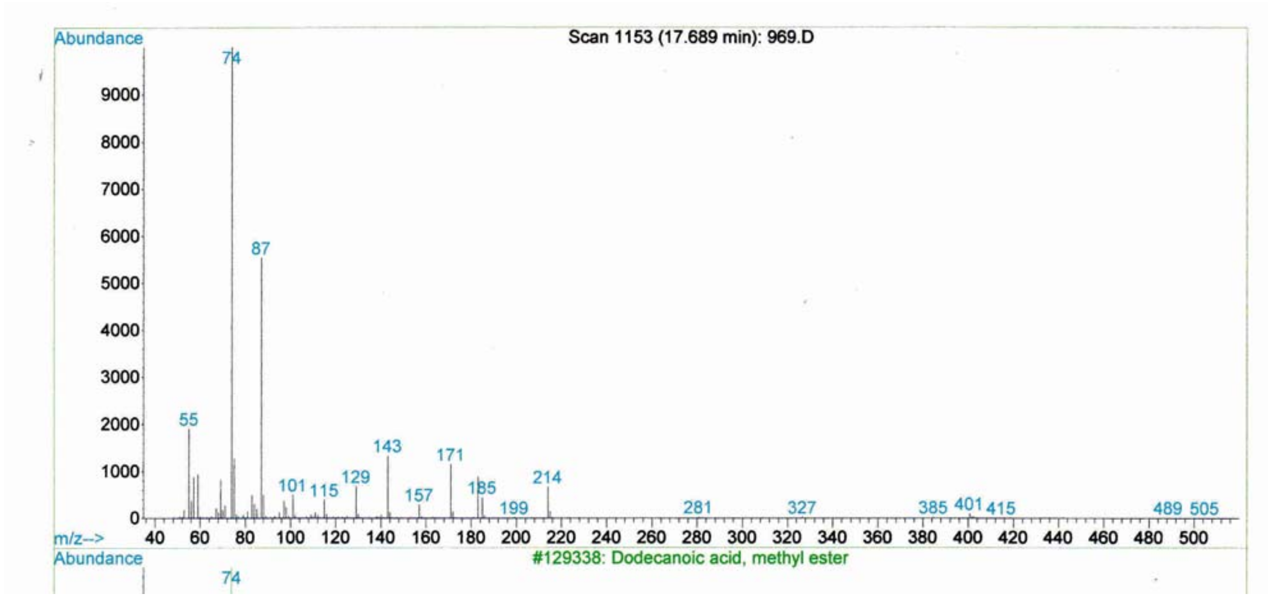

Fig. 5: Mass Spectrum of methyldodecanoic

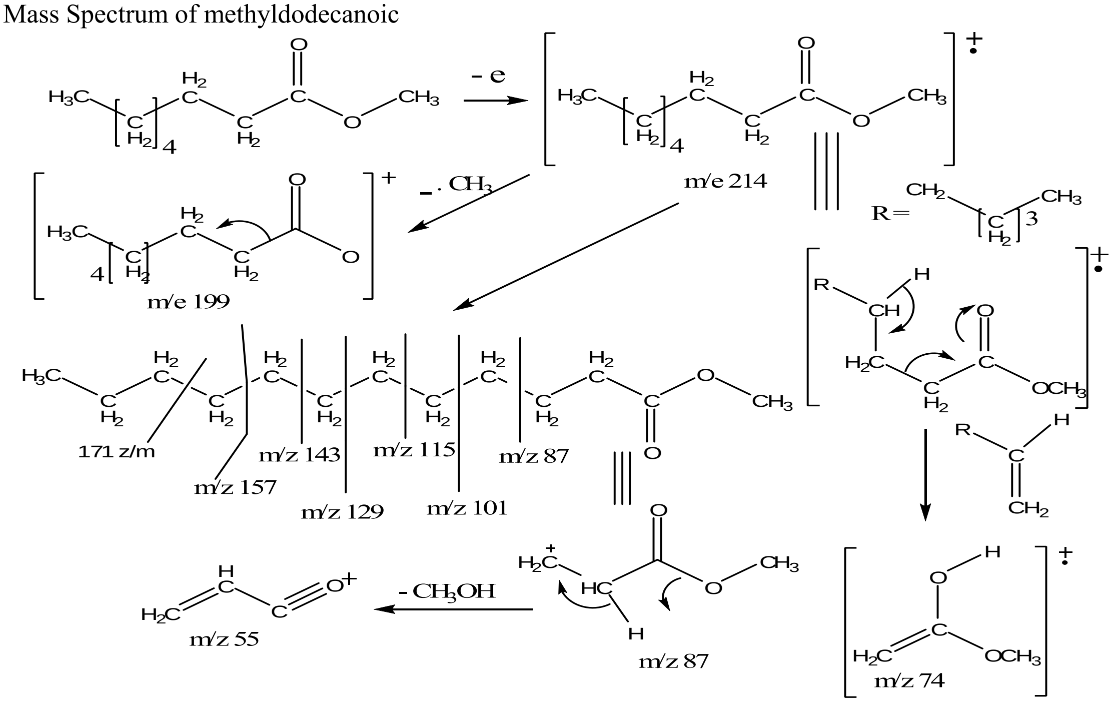

Fig. 6: Fragmentation of methyl dodecanoate

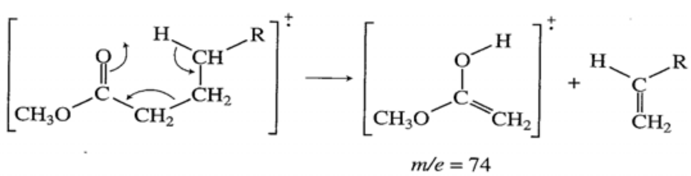

Fig. 7: McLafferty rearrangement reaction of methyl ester

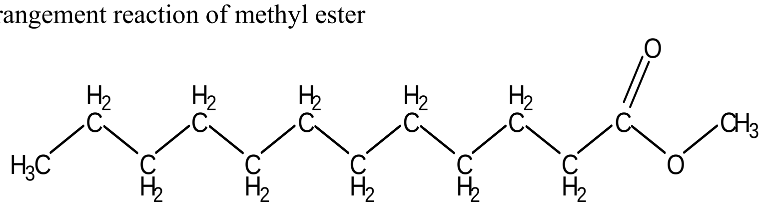

Fig. 8: Structure of methyl dodecanoate

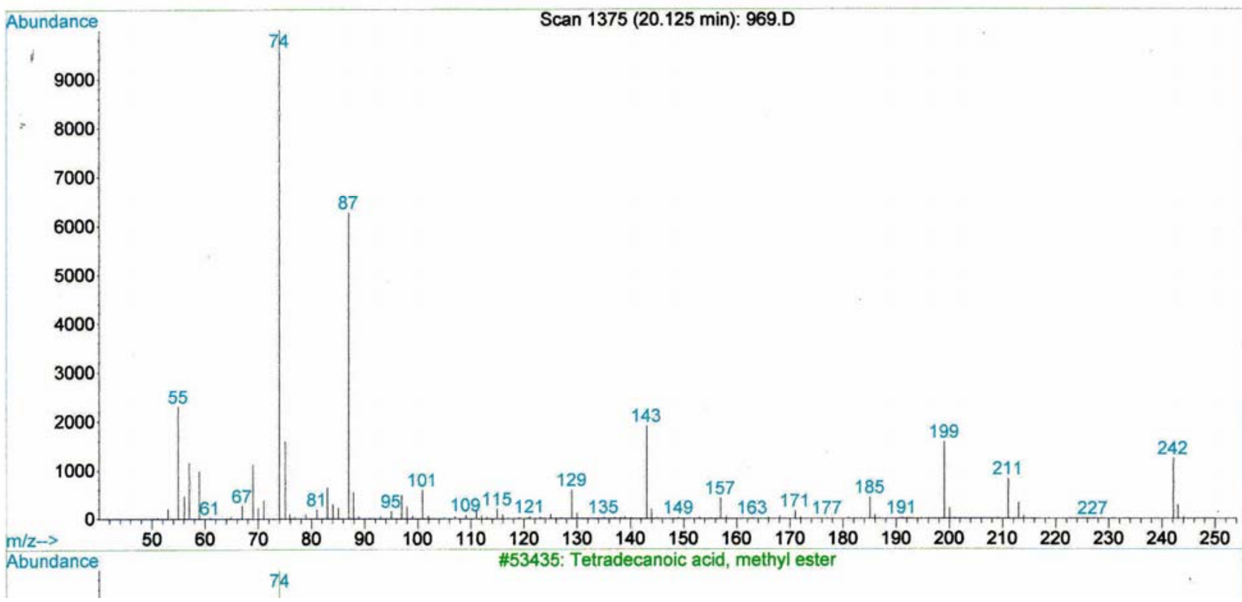

Fig. 9: Mass Spectrum methyl tetradecanoate

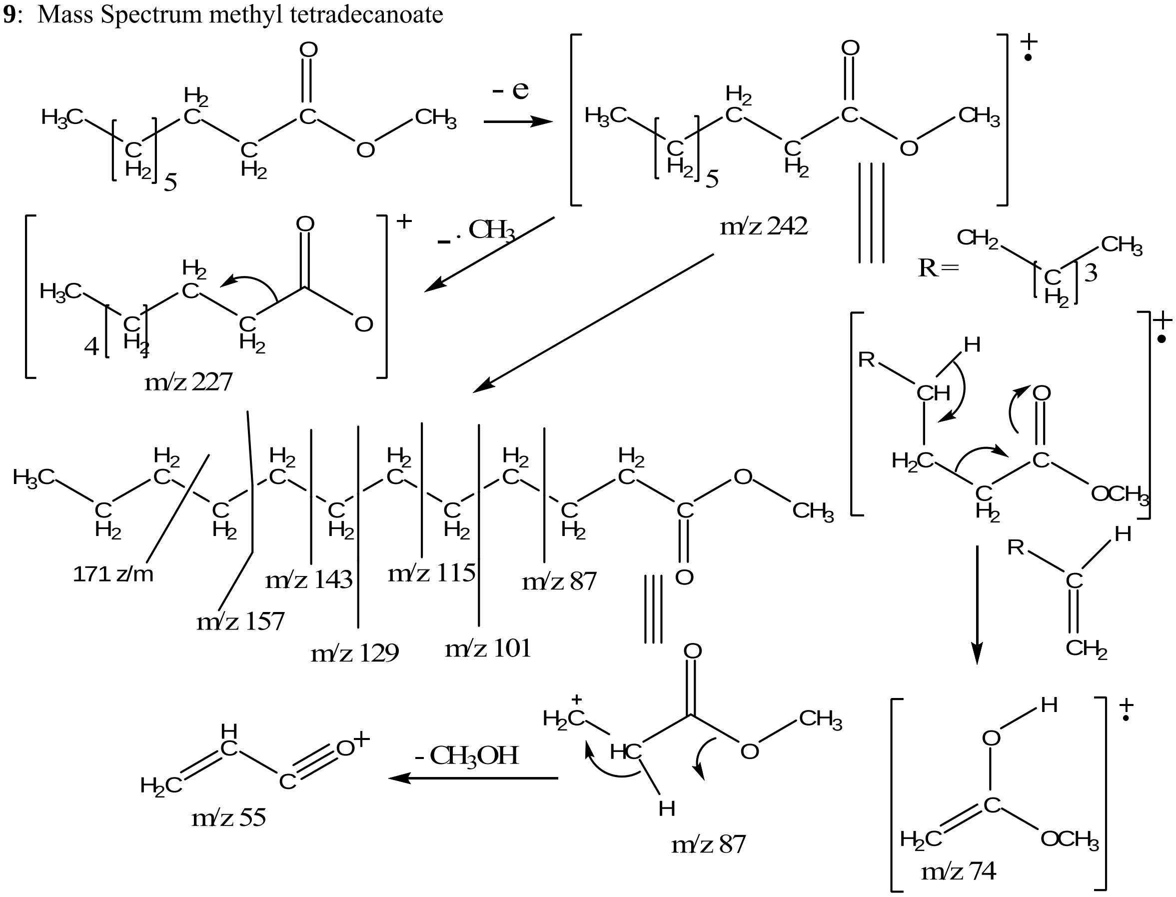

Fig. 10: Fragmentation of methyl tetradecanoate

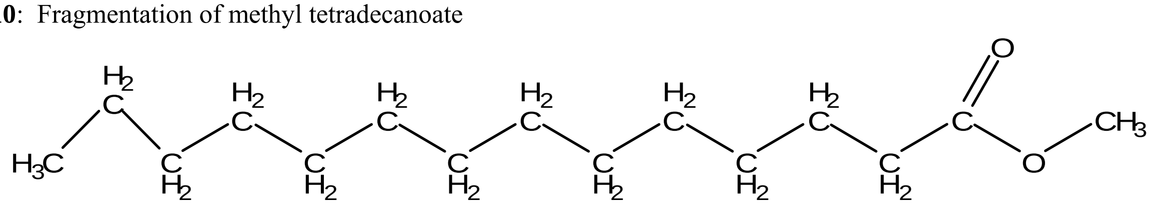

Fig. 11: Structure of methyl tetradecanoate

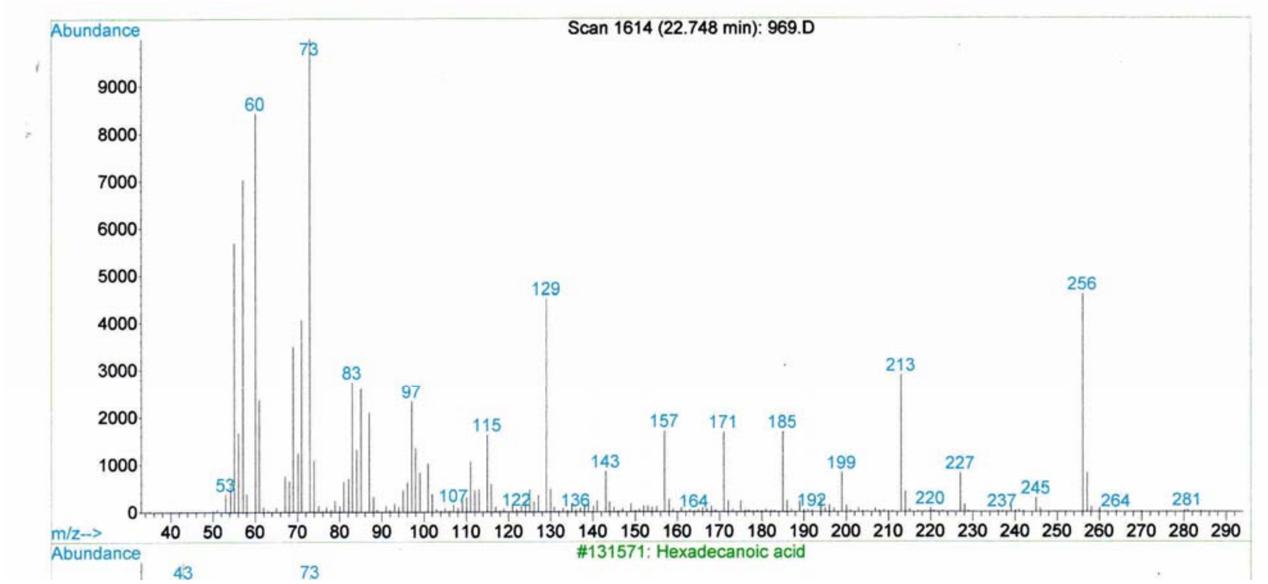

Fig. 12: Mass Spectrum of hexadecanoic acid

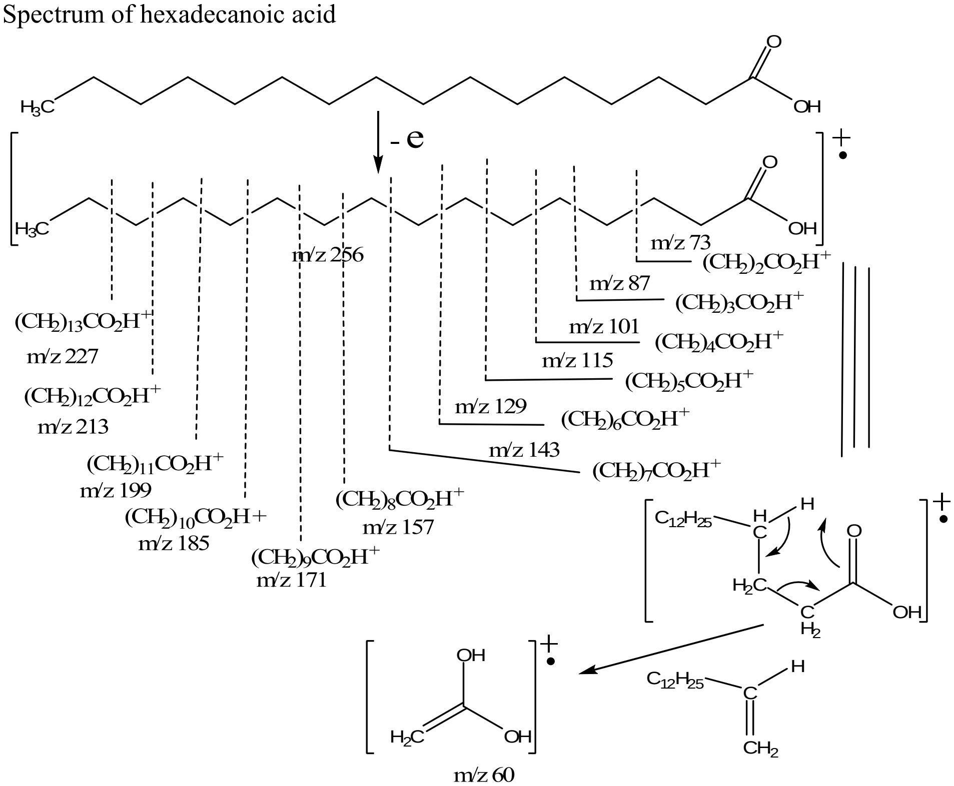

Fig. 13: Fragmentation of hexadecanoic acid

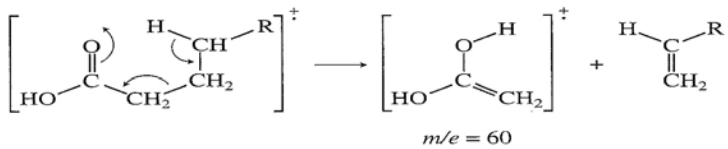

Fig. 14: McLafferty rearrangement reaction of carbocylic acid

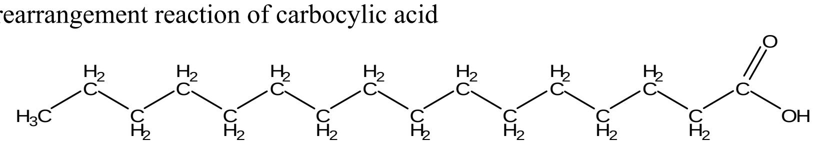

Fig. 15: Hexadecanoic acid

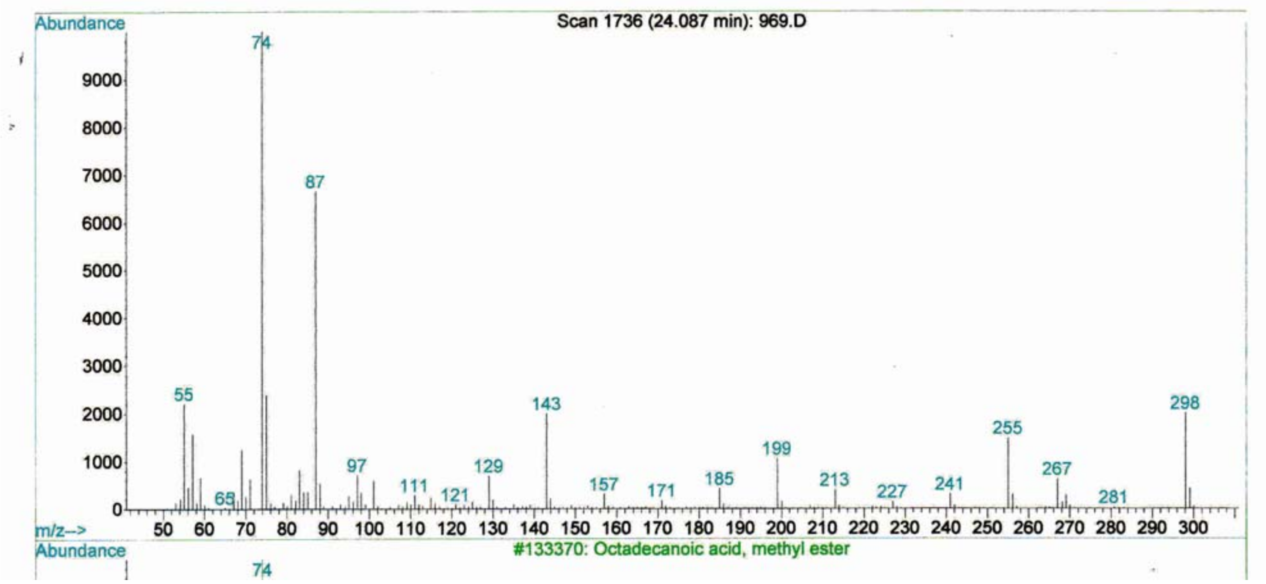

Fig. 16: Mass pectrum of methyl octadecanoate

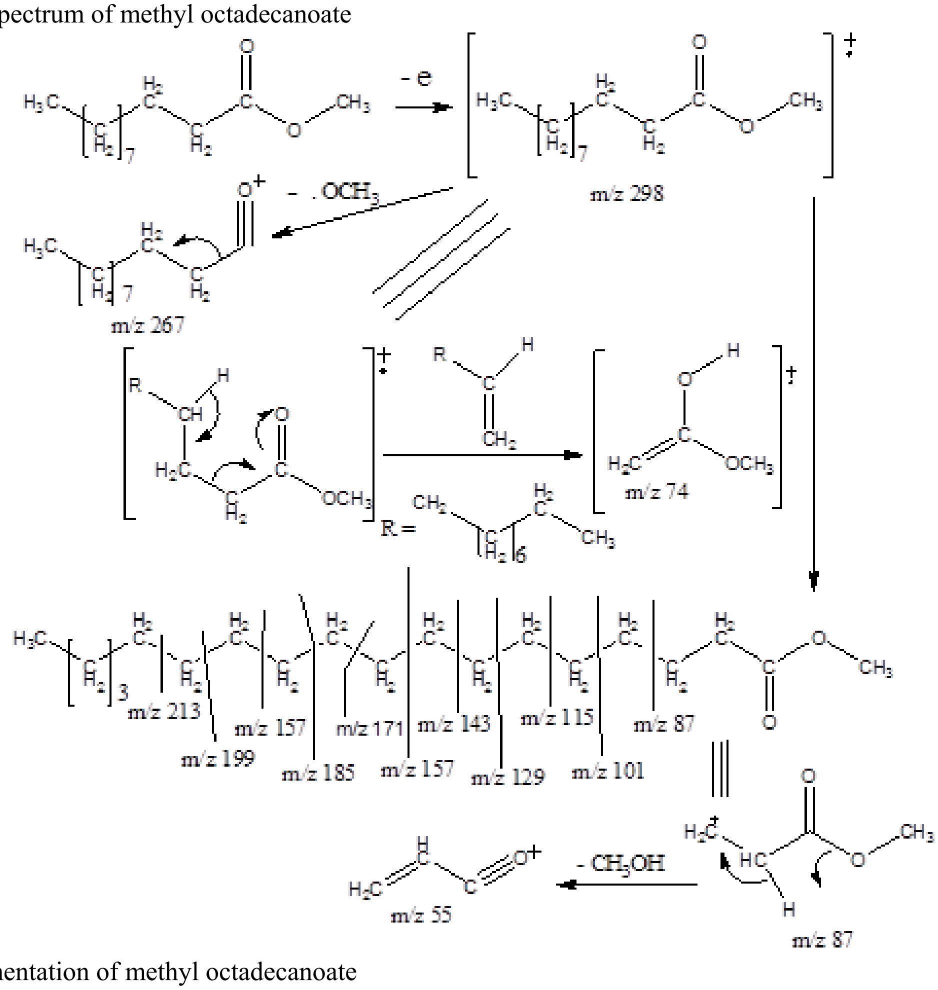

Fig. 17: Fragmentation of methyl octadecanoate

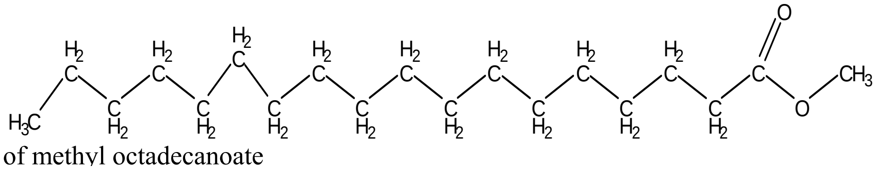

Fig. 18: Structure of methyl octadecanoate

peak clusters with 14 amu differences, existence of basic peak m/z 73 and peak m/z 60. Fragmentation pattern was characterized by peak clusters and 14 amu differences. Fig. 13 shows fragmentation of hexadecanoic acid.

Basic peak occurred on m/e 73 was a representation of propanoic acid ion compound. The peak on m/z 60 was a characteristic of carboxylic acid which a result of Mc Lafferty rearrangement with *β-Cleavage* reaction (fig. 14). Based on GC-MS analysis, the compound that has retention time 22,748 was hexadecanoic acid. The structure described in fig. 15.

Methyl octadecanoate

GC-MS analysis showed that compound with retention time 24,087 has MS spectrum as described in fig. 16. The spectrum showed that molecular ion m/z 298 and basic peak is m/z 74 with peak pattern of m/z 14 amu difference.

According to MS spectrum characteristic, suspected compound with retention time 24,087 was methyl octadecanoate, an aliphatic ester. Molecular weight of methyl octadecanoate was 298 g/mol, confirmed by MS spectrum (fig. 16) showing peak of molecular ion (M^+) on m/z 298. In the picture there are several characteristics of methyl ester compound such as small molecular ion peak, occurrence of peak cluster with 14 amu differences. Fig. 17 shows the fragmentation of methyl octadecanoate

Basic peak occurred on m/e 74 which are a representation of methoxyethanol compound as a result of McLafferty rearrangement with *β-Cleavage* reaction (fig. 17). Compound that has retention time 17,689 was methyl octadecanoate with structure described in fig. 18.

DISCUSSION

The quest of traditional medicines for their potential as antifertility agents has become increasing. This encourages research for the development of herbal for this purpose with positive properties such as better compatibility with humans, lesser effects, and more effective than chemical compounds (Kaur *et al*., 2011). The preliminary phytochemical screening conducted by Simbala and Tallei (2010) showed that the fruit extract of *Areca vestiaria* contains tannins, flavonoids, hydroquinones, triterpenoids and saponines. These compounds may have potential as antifertility agents. Kafle *et al*. (2011) reported that alcohol extract of fruit of *Areca catechu*, a sister taxa of *Areca vestiaria*, has an antifertility activity at 300 and 600mg/kg body weight doses. Using GC-MS analysis we found that the bioactive compounds isolated from the fruit extract are pentadecane, methyl-dodecanoate, methyl-tetradecanoate, hexadecanoic acid and methyl-octadecanoate.

Peak on fig. 1 was confirmed with fragmentation of pentadecane molecules according to McLafferty (1993) which is described in fig. 2. Pentadecane is an alkane hydrocarbon (n-alkane) with the chemical formula $C_{15}H_{32}$. Secondary carbocation is more stable compared to primary carbocation (McMurry, 2010), therefore has larger peak. Silverstein and Webster (1997) declared that the peak of straight chain hydrocarbon will always appear even though in low intensity. Fragmentation pattern was characterized by cluster of peaks with 14 amu differences. The largest fragment occurred on cation with C3 and C4 atoms. Pentadecane (0.23%) was found in *Melia azedarach* (Sen and Batra, 2012) and *Azadirachta indica* and has been used as pesticide (Siddiqui *et al*., 2004)

The peak of MS spectrum in fig. 5 was confirmed by molecule fragmentation of methyl dodecanoate or methyl laurate ($C_{13}H_{26}O_2$) according to McLafferty and Tureček (1993). This is described in fig. 6. Pavia *et al*. (2008) wrote that the peak of molecular ion of aliphatic ester will always occur even though in small intensity. Fragmentation pattern characterized by peak cluster and each has 14 amu differences. Basic peak with methyl ester character was m/z 74 (Silverstein and Webster, 1997). *Abrus precatorius* seed contains methyl dodecanoate (6%) (Obeta *et al*., 2014) and has antifertility activity on swiss male albion mice (Abu *et al*., 2011).

Referring to fig. 7, Pavia *et al*. (2008) wrote that peak of molecular ion (M^+) of aliphatic ester used to occur even though in low intensity. Fragmentation pattern were characterized by peak clusters and has 14 amu differences. Basic peak that were characteristic of straight chain methyl ester was m/z 74 (Silverstein and Webster, 1997). Peak on fig. 9 was confirmed by molecule fragmentation of methyl tetradecanoate (methyl myristate) according to McLafferty and Tureček (1993).

Referring to fig. 12, Pavia *et al*. (2008) declared that the peak of molecular ion (M^+) of aliphatic ester occurs even though in low intensity. Basic peak in fig. 12 that is a characteristic of straight chain methyl ester is m/z 73 (Silverstein and Webster, 1997). Peak in MS spectrum was confirmed as hexadecanoic acid (palmitic acid) according to McLafferty and Tureček (1993). This is described in fig. 13. Mc Lafferty rearrangement with *β-Cleavage* reaction (fig. 14) results in the peak on m/z 60 as a characteristic of carboxylic acid (Pavia *et al*., 2008). Tetradecanoic acid (methyl-tetradecanoate/methyl myristate) and hexadecanoic acid (palmitic acid) are found in the formulation of *Mucuna pruriens* seed, *Murraya koenigii* leaf, *Saraca asoca* bark and Lanthiram bark powder, medicinal plants with antifertility activity (Meenakshi and Salavathy, 2015)

Referring to fig. 16, Pavia *et al*. (2008) wrote that the peak of molecular ion will always occur even though in low intensity. Fragmentation pattern is characterized by peak clusters and 14 amu difference. Basic peak of straight chain methyl ester was m/z 74 (Silverstein and Webster, 1997). Peaks in MS spectrum confirmed by molecule fragmentation of methyl octadecanoate (methyl stearate) according to McLafferty and Tureček (1993). This is described in fig 17. *Psidium guajava* contains methyl octadecanoate (22.18%) (Nisha *et al*., 2011) and traditionally it is used for antifertility (Muruganandan *et al*., 2001).

CONCLUSION

Large numbers of medicinal plants are constantly being screened for their pharmacological value such as anti-fertility, among other is *Areca vestiaria* (pinang Yaki). Our GC-MS analysis revealed the presence of total five compounds in pinang Yaki. These ingredients may be the bioactive substances for antifertility, therefore the activity of each compound is advised for investigation. The five compounds are pentadecane, methyl-dodecanoate (methyl laurate), methyl-tetradecanoate (methyl myristate), hexadecanoic acid (palmitic acid) and methyl-octadecanoate (methyl stearate).

REFERENCES

Abu SM, Hossian A and Anwarul I (2011). Antifertility studies on ethanolic extract of *Abrus precatorius* seed on swiss male albino mice. *Int. J. Pharm. Sci. Res.,* **3**(1): 288-292.

Badan Pusat Statistik (2016). Perkiraan Penduduk Beberapa Negara, 2000-2014. Accessed from http://www.bps.go.id/linkTableDinamis/view/id/960

Kafle S, Shanbhag T, Shenoy S, Amuthan A, Prabhu K, Mohan S, Somayaji SN, Shrestha J (2011). Antifertility effect of *Areca catechu* in male albino rats. *Int. J. Pharm. Sci. Rev. Res.,* **10**(1): 79-82.

Kaur R, Sharma A, Kumar R and Kharb R (2011). Rising trends towards herbal contraceptives. Scholars Research Library. *J. Nat. Prod. Plant Resour.,* **1**(4): 5-12.

McLafferty FW and Tureček F (1993). Interpretation of Mass Spectra 4[Th] Edition. University Science Books, Mill Valley, California, USA, p.371.

McMurry J (2010). Fundamentals of Organic Chemistry, 7[th] Edition. Cengage Learning, USA, p.672.

Meenakshi SA and Kalavathy S (2015). Analysis of bioactive compounds in antiinfertility formulation using GC MA and FTIR techniques. *Int. J. Res. Biochem. Biophys.,* **5**(2): 20-24.

Muruganandan S, Srinivasan K, Tandan SK, Jawahar L, Suresh C and RaviprakashV (2001). Antiinflammatory and analgesic activities of some medicinal plants. *J. Med. Aromatic Plant Sci.,* **22/23**(4A/1A): 56-58.

Nisha K, Darshana M, Madhu G and Bhupendra MK (2011). GC-MSAnalysis and anti-microbial activity of PsidiumGuajava (leaves) grown in Malva region of India. *Int. J. Drug Dev. & Res.,* **3**(4): 237-245.

Obeta JC, Agu CV, Njoku OU, Okonkwo CC and Anaduaka EG (2014). Potentials of non-edible *Abrus precatorius* seed oil towards biodiesel production. *African J. Biotech*., **13**(44): 4226-4235.

Pavia DL, Lampman GM, Kriz GS and Vyvyan JA (2008). Introduction to Spectroscopy. Cengage Learning, USA, p.752.

Sen A and Batra A (2012). Chemical composition of methanol extract of the leaves of *Melia azedarach* L. *Asian. J. Pharma. Clin. Res.,* **5**(3): 42-45.

Siddiqui BS, Rasheed M, Ilyas F, Gulzar T, Tariq RM and Naqvi SNH (2004). Analysis of Insecticidal *Azadirachta indica* A. Juss. Fractions. *Z. Naturforsch* **59**: 104-112.

Silverstein FR, Webster FX (1997). Spectrometric Identification of Organic Compounds 6th Edition. John Wiley & Son, Inc., New York, p.212.

Simbala HEI and Tallei TE (2010). Ethnobotanical, proximate, and phytochemical studies of *Areca vestiaria*, Giseke (Pinang Yaki). Proceeding of International Conference on Medicinal Plants ICOMP 2010. Surabaya, Indonesia, pp.9-14.

WHO (1988). Penuntun Laboratorium WHO untuk Pemeriksaan Semen Manusia dan Interaksi Semen-getah Serviks. Balai Penerbit FK-UI, Jakarta, Indonesia, pp.18-24.