DETERMINATION OF ACTIVE SUBSTANCES IN TORBANGUN PLANT (*Coleus amboinicus* Lour) BY USING GAS CHROMATOGRAPHY-MASS SPECTROPHOTOGRAPHY (GC-MS) AND POTENTIAL COMPONENT ANALYSIS (PCA) FOR INCREASING WOMEN MILK PRODUCTION

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Abstract

Active substance is having an effect reaction in the living plant or animal tissue exposed to it and eliciting a response from living tissue. Many substances find in Torbangun plant (Coleus amboinicus Lour) and the plant has active substances that effected for increased breast milk production. Consumption of Torbangun leaves can be increase breast milk production up to 65%. The aim of present research for determination of active substances in Torbangun Plant by Using GC-MS and PCA program for Increasing Women Milk production. Torbangun leaves as sample were obtained from Senen Market in Jakarta, Indonesia. An experiment was conducted at Chemical Natural Resource and Engineering Laboratory, Universiti Malaysia Pahang. The active substances experiment carried out by using GC-MS and PCA. The result of active substances in Torbangun leave powder found 46 compounds. The compounds carried out through processing with PCA program. The compounds were phenol (PHE), gamma sitosterol (GS), campesterol (CS), Isocholesteryl methyl ether(IME), Octadecanoic acid (OA), Stigmasterol (SS), Benzoic acid (BZA), 1,2-Benzenediol(BZD), phenol, 3-methoxy-2,4,5-trimethyl (PMT) and alpha-amyrin (AA). This paper for study effect of active substances in Torbangun leaves that stimulate women milk production.

Keywords: *Torbangun (Coleus amboinicus Lour), active substance, GC-MS, PCA and Milk production.*

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INTRODUCTION

Torbangun *(Colues amboinicus* Lour) has been used by the Bataknese women breeding in North Sumatera, Indonesia as a lactagogue for hundreds of years. An investigation into this traditional usage found that it increased milk volume produced by 65%, compared to 20% for fenugreek seeds. They believed that the consumption of Torbangun leaves for one month after birth could increased women milk production (Damanik *et al.,* 2006). Torbangun plant is not well documented, and science evidence is limited to establish lactagogue as active substance for milk lactation stimulant. The present paper reports to determination of active substances in Torbangun plant (*Coleus amboinicus* Lour) by using Gas Chromatography-Mass Spectrophotography (GC- MS) and Potential Component Analysis (PCA).

Milk production or lactation is breast milk produced by mammary glands located in the breast tissue. Several hormones regulate the development of the mammary glands as well as the initiation and maintenance of lactation (Julie, 2008). Lactation is a wonderful way to care for your baby and perfect food for babies. It has all the right nutrients in just the right amounts (Sarah, 2007). Lactation is widely recognized as the healthiest feeding option for mothers and babies. Among the many positive outcomes associated with breastfeeding are the reduced risk of breast and ovarian cancers in the mother as well as a reduction in acute and chronic infections, leukemia, and diabetes in the breastfed child. Beneficial outcomes associated with breastfeeding extend beyond the reduction of illness to encompass enhanced mother/child bonding, increased child spacing, increased intelligence scores in the child, and shared regulation of the child's food intake (Taveras *et al*., 2004 and Gartner *et al*., 2005).

Torbangun leaves also have antimicrobial effects against *Candida albicans* and Streptococcus mutant. The plant has the effect to cure stomatitis and also fungicidal (Hole *et. al*, 2008). Torbangun leaf as antiseptic has high activity against worm infection (Vasquez *et al.,* 2000). Thus, this leaf could be used as against asthma diseases and (Jain and Lata 1996). The leaves also contain potassium which cleans blood, prevents of infections, decreases pain, emerge relax fell and furrows membrane mucous. Hot climate could elevate stress cause anorexia, milk secretion and body weigth to decrease (Mepham 1987).

The leaves of the indigenous Torbangun plant possess a distinct aroma and are used in many food preparations to enhance flavor. Apart from this, they also exhibit medicinal properties (Hole *et al*, 2008). This compound is being known to exhibit antimicrobial, antiviral, anti-inflammatory, and antioxidant activities, which make it a valuable product for the pharmaceutical, food and cosmetic industries. The plant of Torbangun is a bushy shrub being cultivated throughout India and is being reported to contain certain essential oils, e.g. thyme, carvacrol and cis-caryophylline, which possess antibacterial, antifungal, antiseptic, antioxidant and antiviral properties. The extracts are being widely used for the treatment of asthma, bronchitis, chronic coughs, sores, burns and insect stings and urinary diseases. They are also used in mouthwashes, tooth pastes, soaps, creams, lotions, ointments, throat lozenges and cold remedies. Our preliminary investigations on the accumulation of essential oils by tissue cultures of *Coleus amboinicus* showed that the regenerated plants retained the biosynthetic capacity of the parent plant in the production of thyme and carvacrol (Hole *et al..* 2008). Torbangun plant has scented leaves and these are often rubbed into the hair and body after bathing (Morton, 1992). In the Amazon, the leaves are mixed with sugar and used as an intoxicant (Jain and Lata, 1996), while in Tonga and Martinique the leaves are used in the cleaning of textiles to perfume them (Prudent et al., 1995). Torbangun plant is also used as insect repellants (Omolo et al., 2004).

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METHODS

Plant material

Torbangun plant (*Coleus amboinicus* Lour) bought at Senen Market in Jakarta, Indonesia. The sample identified and authenticated by Indonesia Institute of Sciences, Research Center for Biology No. 1033/IPH. 1.02/If.8/VIII/2010. The chemicals utilized were distilled water, ethanol and acetone solution.

Equipment

Glassware that are petri-dish, reaction tube, baker glass, volumetric flash, volumetric glass, erlenmeyer, balance bottle, volumetric pipette and graduate pipette, funnel, soxhlet, rotary evaporator and GC-MS unit.

Methods

(a) Preparation of sample extract

Fresh leave of Torbangun plants were being blended with used distilled water and then being dried using by freeze dryer to turn them into powders. Twenty grams powder of Torbangun leaves were being mixed with 100 ml of various solvent (distilled water, ethanol and acetone solution). The plant extracts were being prepared by using soxhlet apparatus being collected for 8 hours and stored in a vial (Alade and Irobi, 1993). A part of the plant extracts was being prepared by using Rotary Evaporator equipment until dried and collected with added ethanol solution and stored in a vial (Adam, 1995).

(b) GC-MS Analysis

The GC-MS analysis of the Torbangun leave extract was being performed using a Claruss 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary coloum (5% Diphenyl 95% dimethyl poly siloxane) (30 nm x 0.25 mmID x 0.25 µmdf) and mass detector turbo mass gold of the company which was being operated in EI method. Helium was being carried gas at a flow rate of 1 ml/min, the injector was operated at 200^{O}C and the oven temperature was being programmed at 60^{O}C for 15 min, then gradually increased to 280^{O}C at 3 minutes.

RESULTS AND DISCUSSION

The result of Gas Chromatography Mass Spectrophotometer (GC-MS) analysis in Torbangun leave powder found 48 compounds. The compounds carried out through processing with principal component analysis (PCA) statistical.

(a) Principal Component Analysis (PCA)

A principal component analysis is often being performed before a regression, to avoid using correlated variables. PCA was being used to classify samples and find variables and all compounds of Torbangun leaves that would contribute to the differentiation. Factor analysis was being used to understand the correlations between variables instead as the dimension of the variables were small. One group of compounds was being separated with another groups and this group compounds that were responsible as active substances in Torbangun plant. The compounds were phenol (PHE), gamma sitosterol (GS), campesterol (CS), Isocholesteryl methyl ether (IME), Octadecanoic acid (OA), Stigmasterol (SS), Benzoic acid (BZA), 1,2-Benzenediol (BZD), phenol, 3-methoxy-2,4,5-trimethyl (PMT) and alpha-amyrin (AA). The correlation circle of variables is being shown in Figure 1.

The varimax methods were being used to produce orthogonal transformations to the reduced factor so as to better identify the high and low correlation. The correlation circle showed a projection of the initial variables in the factor's space (Figure 2.).

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Table I. Result of compounds in Torbangun leave powder by using Gas Chromatography Mass Spectrophotometer (GC-MS).

No.	Compound	Quality (%)	Abreviation	Peak Area
1	Phenol, 2-methyl-5-(1-methylethyl)	94	PM	8.091
2	2-Methoxy-4-vinylphenol	96	MV	0.126
3	Cyclohexasiloxane, dodecamethyl-	91	CCHX	0.130
4	Caryophyllene	99	CYP	0.439
5	Alpha.-Caryophyllene	98	ACP	0.608
6	Cycloheptasiloxane	90	CHSX	0.183
7	Cyclohexene	97	CH1	0.071
8	Phenol, 2,4-bis(1,1-dimethylethyl)	96	PHE.2	0.248
9	Tetradecanoic acid	97	TDA	0.115
10	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	90	BCD	0.643
11	Pentadecanoic acid,	98	PTDA	0.592
12	n-Hexadecanoic acid	99	NHA	2.414
13	Hexadecanoic acid, ethyl ester	96	HA	1.061
14	Phytol	95	PTOL	3.976
15	9,12,15-Octadecatrienoic acid,	99	OTA	4.205
16	1-Phenanthrenecarboxaldehyde,	90	PTCA	2.639
17	1-Phenanthrenecarboxylic acid,	96	PTCA	3.356
18	1,2-Benzenedicarboxylic acid,	91	BZCA	0.795
19	2,6,10,14,18,22-Tetracosahexaene,	99	TCH	3.513
20	Vitamin E	99	VITE	0.418
21	Gamma.-Tocopherol	99	GT	0.229
22	3-Methyl-4-isopropylphenol	94	MI	13.587
23	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-,	95	DTM	0.456
24	Cyclopentanol, 1-(1-methylene-2-propenyl)-	92	CCP	0.169
25	1H-Indole-3-carboxaldehyde	96	ICD	0.196
26	Heptadecanoic acid	98	HAD	0.722
27	1-Octadecene	97	OTDC	0.420
28	Nonanoic acid,	97	NNA	3.323
29	Decanedioic acid,	94	DCA	0.504
30	1,2-Benzenediol	94	BZD	0.084
31	2,5-Cyclohexadiene	96	CH	0.029
32	Benzeneacetic acid	90	BZAA	0.21
33	Indole-5-aldehyde	97	IDA	0.191
34	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	95	HPM	0.095
35	4-Hexadecen-6-yne,	94	HXC	1.505
36	7,10,13-Hexadecatrienoic acid,	93	HXA	3.449
37	Gamma.-Sitosterol	99	GS	3.361
38	alpha.-Amyrin	92	AA	3.506
39	beta.-Tocopherol	90	BT	60.427
40	di-.alpha.-Tocopherol	92	DAT	0.144
41	Benzoic acid	90	BZA	3.456
42	Phenol, 3-methoxy-2,4,5-trimethyl-	91	PMT	0.36
43	Stigmasterol	99	SS	4.927
44	Octadecanoic acid,	98	OA	0.743
45	Isocholesteryl methyl ether	96	IME	2.085
46	Campesterol	97	CS	1.625

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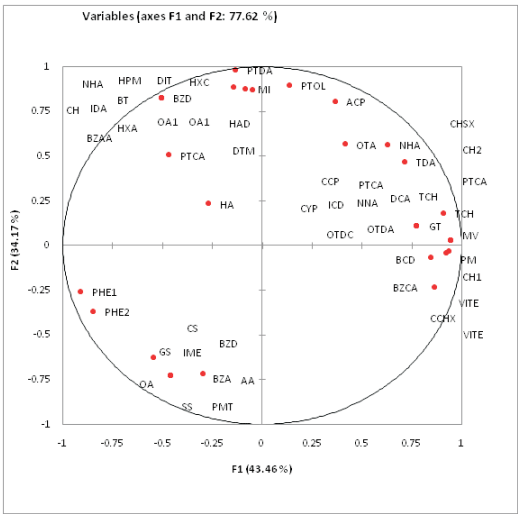

Figure 1. Correlation circle of compounds in Torbangun leave extract composition

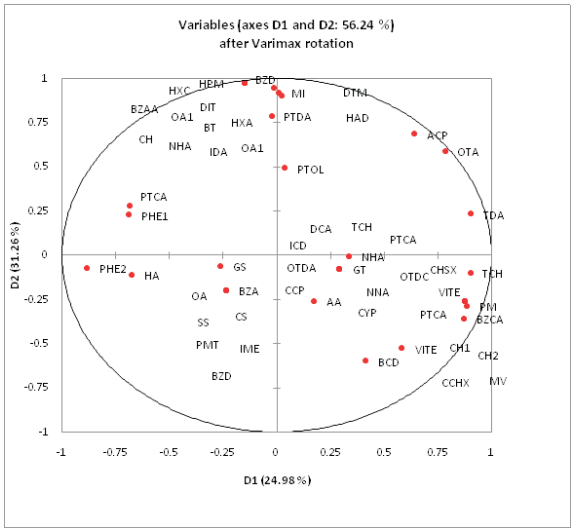

Figure 2. Correlation circle after varimax rotation of compounds in Torbangun leave composition.

Discussion

Recovery of active substances for mammary gland

The phytosterol regulatory element binding protein family of transcription factors is recognized as regulating fatty acid and cholesterol biosynthesis. Protein kinase play a major role in the lactational switch. (Steven *et al.,* 2007). The mechanism of active substances in Torbangun plant such as phytosterol is vital to cellular function, as components of membranes and a precursor to fat-soluble vitamins and steroid hormones (Fahy *et al.,* 2005) and play a vital role in cell signaling in several ways. Steroid hormones of the ovary and placenta were regulated and important stimulators of mammary gland development (Laron *et al.,* 1989). Although, phytosterol also stimulate apoptosis (physiological mechanism of cell loss that depends on both pre-existing proteins and de novo protein synthesis) in breast and prostate cancer cells (Rao and Janezic, 1992)and(Holtz and Fink, 1998). Steroid metabolism in lactating with elevated milk production (Milo *et al.,* 2005) and stimulators of mammary gland development (Lamote *et al*., 2004). In high-producing, lactating, follicle growth rate may be similar to lower producing cows, but circulating estradiol would increase at a slower rate due to elevated steroid metabolism (Milo *et al.,* 2005). In the lactating condition has increased metabolism of steroid hormones as milk production increases (Neville *et al.,* 2001).

Another substances such as alpha amyrin its antimicrobial, antidepressant, anti-inflammatory and gastroprotective activities (Keterere *et al*., 2003; Oliveira *et al.,* 2004; Oliveira *et al.,* 2005; Aragao *et al.,* 2006; Lima-Junior *et al.,* 2006). á,â-amyrin exhibits anti-inflammatory and antioxidant effects. Both inflammation and oxidant stress play a pathogenic role in a rat model of acute pancreatitis (Caroline *et al.,* 2010). Whereas, benzoic acid its antibacterial activity benzoic acid is also able to modify bacterial populations of the gastrointestinal tract of the piglets and to enhance their performance after weaning (Kluge *et al*., 2006; Guggenbuhl *et al.,* 2007; Torrallardona *et al.,* 2007). Benzoic acid, its salts and esters are used as preservatives in cosmetic products ((European Commission, 2005).

Phenolic and flavonoid have an indirect effect on health because they are metabolized by the same pathways as various xenobiotics or endogenous hormones (Zhu, 2002). the antioxidant properties of polyphenols and their

probable role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases. Furthermore, polyphenols, which constitute the active substances found in many medicinal plants, modulate the activity of a wide range of enzymes and cell receptors (Middleton *et al., 2000).*

Deleterious effect of active substances

Most side effects are from short-term use; however, long-term use can lead to additional side effects. These may include an increase in appetite, difficulty sleeping (insomnia), changes in mood and behavior, flushing (redness) of the face, and short-term weight gain due to increased water retention. These side effects usually resolve after a few days once the steroids have been stopped (Daniel, 2009). Whereas, long-term use or excessive doses of steroids (Barrett, 1995 and Yamamoto et al., 2006). These effects include harmful changes in cholesterol levels (increased low-density lipoprotein and decreased high-density lipoprotein), acne, high blood pressure, liver damage and left ventricle of the heart (De Piccoli *et al*., 1991). Deleterious of phenolic is highly irritating to the skin, eyes, and mucous membranes in humans after acute inhalation or dermal exposures and quite toxic to humans via oral exposure. Whereas, chronic case have been reported that anorexia, progressive weight loss, diarrhea, vertigo, salivation, a dark coloration of the urine, and blood and liver effects (ATSDR, 1998).

CONCLUSION

Torbangun plant (*Coleus amboinicus* Lour) has phytosterol such as steroid compounds as active substances effected on increasing milk production and and important stimulators of mammary gland development

Torbangun plant as medicinal plant contained phenolic compound and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases.

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