

NATURAL PHYTOESTROGEN CONTENTS IN SEVERALS FRUIT AND LEAFS; THE FUTURE REPLACEMENT HORMONE THERAPY IN MENOPAUSE WOMEN

"kandungan fitoestrogen alami pada beberapa daun dan buah buahan ; suatu sulih terapi hormon di masa depan pada perempuan menopause".

Naskah diterima 26 September 2011, naskah disetujui 29 November 2011

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ABSTRACT

Phytoestrogen is molecularly almost similar with and act same as estrogen and found lot in several fruits and leafs mainly in tropical countries. However, its quantitative the molecular contents is not yet known exactly. Menopause and andropause people needs substitution as a replacement therapy of sex hormone, because they have declined the hormone significantly so impacted disturbance of several organs dysfunction. The objective of this study was to analyze the estrogen content in extract pegaga, green clover leafs, tomato and papaya fruit, so then a positive impact felt in certain community. The samples were collected using purposive sampling with 10 times replicate in four different groups there were pegaga, green clover leafs, tomato and papaya fruit. All these groups divided into 2 subgroups based on process or subspecies. All samples were made infuse/infusa 1:4(w/v) then extracted after was spinning 1000xg f0r 15mnts, with 1:5 petroleum ether(v/v). After evaporated each extract then kept dry-frozen -20oC until analysis to be performed. Solid phase Radioimmunoassay technique was used to identify the estrogen content. The lowest estrogen level presented in fresh pegaga leafs extract (Mean+Sd) was 47.9+5.5 pg/g and twice increasing the level in dry leafs extract was 96.1+11.2pg/g. Meanwhile the estrogen level in fresh green clover leafs extract was 538.0+30.5 pg/g more than ten times higher if compared to fresh pegaga level, but more less lower twice the estrogen level it was compared to dry green clover leafs extract, there was 1068.0+97.2 pg/g. In fruit group appear fibrin part of tomato has more less the same estrogen content with Thai papaya subspecies there were 1037.0+37.7 pg/g and 1175.0+67.7pg/g respectively.

Keywords: Phytoestrogen, pegaga, green clover, tomato and papaya

ABSTRAK

Fitoestrogen secara melekul hampir sama dan mempunyai aktifitas sama seperti estrogen dan ditemukan pada jenis tanaman dan buah buahan terutama di negara tropis. Tetapi jumlah kandungan melekulnya hormon belum diketahui. Pada orang orang menopause dan andropause diperlukan sulih hormone seks karena mereka umumnya mengalami penurunan hormone tersebut secara berarti yang bisa berdampak pada gangguan a fungsi organ. Tujuan dari penelitian ini untuk menganalisis kandungan estrogen ekstrak daun pegaga, semanggi,

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buah tomat dan pepaya sehingga dampak positifnya pada komunitas masyarakat yang memerlukan. Pengumpulan sample dilakukan dengan cara purposive sampling dengan masing masing 20 kali ulangan pada 4- kelompok yaitu , kelompok daun pegaga, daun semanggi , buah tomat dan buah pepaya. Semua sample dibuat infusa(1:4/w/v) setelah itu di ekstrak dengan petroleum eter (1:5 f/v)dimana sebelumnya dilakukan pemutaran 1000xg selama 15 menit. . Radioimmunoassay fase padat dilakukan untuk menentukan kadar hormone estrogen dengan jumlah 80 sampel . Kadar hormone estrogen terendah ditemukan pada ekstrak daun pegaga segar dengan (rata-rata \pm SD) $47,9 \pm 5,5$ pg/g dan kadarnya meningkat dua kali lipat pada ekstrak daun pegaga- kering yaitu $96,1 \pm 11,2$ pg/g. Sementara kadar estrogen pada ekstrak daun semanggi segar adalah $538,0 \pm 30,5$ pg/g dibandingkan dengan ekstrak pegaga segar kandungan ini sepuluh kali lipat lebih tinggi, tetapi kurang lebih dua kali lebih rendah kadar estrogenya bila dibandingkan dengan ekstrak daun semanggi kering yaitu $1068,0 \pm 97,2$ pg/g. Bagian lendir dari tomat kurang lebih sama kandungan estrogenya dengan jenis pepaya Thailand yaitu masing-masing $1037,0 \pm 37,7$ pg/g dan $1175,0 \pm 67,7$ pg/ml.

Kata kunci : fitoestrogen, pegaga, semanggi, tomat, dan pepaya

INTRODUCTION

Tropical country like Indonesia has lot kinds of fruit and leafs. Most of those fruit taste sweet, palatable and contribute to a good body condition of course healthy when be consumed quantitatively enough. But several of those could not be consume to much or much more is due to as a contra indication in certain body condition. Meanwhile most of the leafs are found in Indonesia or in the world are unpalatable and some of those tend to bitter but still have some good effect to our health. Semanggi/ Green clover/ *Marcelea crenata* Presl can grow any where in Indonesia as long as fulfill situation as wet to watery field and pegaga/ *Centella asiatica* can be grow in wet ground. Both of these leafs are famous as in certain Province, let say in Aceh province, most of the local community quit familiar to this leave processed as local salad style vegetable and in Bali Province most of the old people used this infuse/ infusa) as a traditional medicine to decrease fever

and increase palatable food. Estrogen deficiency is most importance factor in menopause women in because of the imbalance bone remodeling process (Fitzpatrick, 2003). Even recent study in rat made it menopause and provided orally everyday 120mg extract pegaga for 4 weeks effected increasing of estrogen receptor beta (ER β), that mean pegaga leave extract is containing estrogen hormone as Phytoestrogen, but unfortunately the concentration was not identified (Raden, 2010). The expression of estrogen receptor beta much more found in primary or secondary sex organ but estrogen receptor alpha also found. In case expression of estrogen receptor alpha arise the activity is due to increasing of estrogen serum (organic chemistry) to be followed with transcription and translation process intracellular. The process then effected either a proliferation or cell hyperplasia. Appear without any control of estrogen receptor beta, malignant tumor supposing arise in organ, because ER beta could be inhibit activity of ER α

(George, 1997). As well as known estrogen is agonist to ER α and antagonist to ER β , meanwhile phytoestrogen tend more bind to ER β (Volkhard et al, 1998). In mice the highest estrogen receptor found during estrus phase and the lowest found in diestrus phase where estrogen concentration is minimum during this period (Mahaputra, 2002). Meanwhile semanggi leave also quit famous in East Java Province mainly in Surabaya, there are consumed as a local salad style reserve along with local rice cracker and spice. The consumer mostly the women who had menopause stadium and this custom has been traditionally from generation to generation. Although no detail information that time about its ingredient in except for dominantly the chlorophyll content. As well as known the chlorophyll that found in green clover and pegaga has a good activity contribute to bind ferro ion to increase hemoglobin quality (Greenspan and Gardner, 2004). The therapy effect of these leafs may be transfer experience by orally inter or intra community mainly in East Java about the health is felt better when consume semanggi. It is therefore up to now this traditional custom is still going on without any obstacle except for a tinier stock. Recently study has been reported that menopause women had consumed 500mg extract green clover twice a day for at least 4weeks could be increase concentration of estrogen blood serum significantly ($p < 0.05$) compared to control group menopause women (Laswati, 2007a). The Experiment was also done in laboratory animal (menopause mice) showed that mice had been given extract green clover orally at

least 3 weeks the blood serum estrogen concentration and estrogen receptor (ER α) also have been increased. This finding could be explained that by consume green clover as an alternative medicine to reduce or ease undesired menopause symptom as well as hot flushes, headache, night sweating, fear and excited could be as a reality. The other later impact of decreasing of estrogen concentration in menopause women is osteoporosis there are a lots women more attached fracture compared to man (Laswati, 2007b). In genital tract as a direct impact of decreasing of estrogen could be disturb sexual behavior in marital women, is due to vaginal epithelial atrophy, dryness and reducing of collagen compartment and then influencing of vaginal contractility. In the skin has known estrogen as a contributor of fibroblast growth and then stimulate collagen as a main factor to care and maintain skin, smoother, moisture and elasticity. Meanwhile White hair either in menopause or andropause people its cause decreasing of melanin content there is catalyzed with tyrosine enzyme, how-ever this enzyme can be stimulate the activity by using estrogen, therefore estrogen realized also as anti white hair (Speroff et al, 1998; Baziad 2003; Al Baghdadi and Ewies 2009). The other hand papaya and tomato also classified into a tropical fruit but well known and have a good demand round the world is due to its palatable and taste specially for papaya and by adding a little bit syrup serve as tomato juice is quit common and interested in tropical countries as well as Indonesia. Both of these fruit that mention later on, no yet much known the ingredient in and the

other content, but both of those fruit the only beta carotene prominently consisted in and of course rich with fiber that good for gastro-intestine tract either its function or cellular health. Meanwhile vitamin C, B1 (thiamine), B2 (riboflavin) and mineral as well as potassium, calcium also consisted to both of these fruit. Papaya also common used as appetite, stomach ill, over hemorrhage at menorrhea, gastro intestinal disturbance and difficulty defecation. Moreover, is due its content lycopene in tomato there are realized could be reduce pulmonary and prostatic cancer risk. Mucous/fibrin like substance there are commonly lay in the middle part of tomato fruit realized as anticoagulant so can be reduce congestive stroke prevalence and its tomatine content also effected as local anti inflammation. So the overall medical effect of tomato could be felt in haemostatic function of healthy liver (Sari, 2011).

From all those fruit and leafs there were described above that having so many ingredients and there are realized be able to maintain health and have a preventing effect to several sickness, but up to now unfortunately just only green clover has been known the estrogen content. However three the other had been mention above not yet know whether that could be as an alternative in traditional medicine mainly on its estrogen content and concentration to reduce menopause symptom in women. The main aim of this study was to analysis the estrogen content in several fruits and leafs to make more alternatively choice to the consumer as a preventive medicine.

MATERIALS AND METHODS

This study was classified into an observational analysis with purposive sampling. There were 2 kind of leafs consisted in 2 different kind of process and 2 kind of fruits consisted of 2 parts kind each were used as a samples and were analyzed the estrogen concentration using radioimmunoassay technique. The 1st group was pegaga leafs consisted of 20 replicates there were 10 times for fresh leafs and 10 times replicate for dry leafs. The 2nd group was green clover/*Marsilea crenata* Presl there also 10 replicates for fresh leafs and 10 times dry leafs. The 3rd group was mature tomato, consisted 10 times replicate for outer part and inner part/fibrin like substance also ten replicate. The 4th group was mature Papaya, consisted 10 times for local one and 10 times replicate for Thailand subspecies.

In preparing sample: All crude samples were balanced electrically just 1g as well as fresh or dried sample accordingly group, then homogenized in ceramic bowl and added with 4mL NaCl physiologic again repeated homogenized and then pawed into glass tube and vortex for 1 minute. Centrifugation was done 1000xg for 10 mnts, and the filtrate was pawed into extraction glass tube then added with petroleum benzene that classified into polar extractor with 1:5 (v/v), then vortex for 5 mnts. All mixture were put into freezer for 15 mnts then only filtrate pawed into glass assay tube meanwhile pellet at the bottom was freezing unused. Those filtrate in assay tube then evaporated by gentle blowing air into assay tube that put into water bath 38°C. The extract then diluted with 1ml estrogen serum with 0 pg/ml

concentration /Bo) as a ready sample.

In Analyzing sample: By using Radioimmunoassay technique, there was I^{125} as a tracer bound to legend/unknown hormone, the estrogen concentration can be detected quantitatively. The principle reaction of this technique is occurred competitive molecular binding between legend and radio-legend into specific AB fraction of IgG of anti hormone estrogen that was coated into inner liner of polypropylene assay tube. Meanwhile C-fraction IgG of anti-hormone linked with inner part of polypropylene as a coated inner assay tube. So much higher the estrogen concentration in the sample that will strictly impacted also to be much lower the radio illuminated that catching into gamma-counter as well as Count-per-minute/CPM. The concentration could be calculated to find out first Binding % by divided of net CPM sample with net CPM Binding in 0pg/ml/BO multiply with 100%. Secondly by integrate binding-% sample to interpolate linear standard curve then the concentration could be detected visualize. It was therefore, the concentration of the estrogen hormone contrary with CPM showed in gamma-counter screen (Mahaputra et al. 1990). The binding percent of each sample then interpolated on logic-log paper to find out the real concentrations (Chard, 1990). The data as estrogen concentration in pg/g, was presented as statistic descriptive and mean differences was analyzed using one tail Student-t test (Steel and Torrie, 1998)

RESULT AND DISCUSSION

From ten time replication samples that used in pegaga leafs to analyze estrogen content using Radioim-

munoassay (RIA) technique, found the lowest concentration of phytoestrogen showed in sub-group 4th there is a fresh pegaga with concentration (Mean+Sd) 47.9 ± 5.5 pg/g and almost increased twice the content in dried pegaga to 96.1 ± 11.2 pg/g ($p < 0.05$) (table 1). As well as known only the local peoples in Aceh Province used this leafs as local salad style and in Bali Province most the peoples they made this leaf serve as infuse (infusa) to reduce stomach warm and stimulate appetite, but they don't have information to the contents in. Only recent study in menopause rats reported that could be stimulate expression of ER beta and collagen. This study reported that in menopause rat, pegaga leafs extract can be used to maintain vaginal elasticity and relief of women menopause symptom (Raden, 2010). The substance there are containing phytoestrogen and could be stimulate expression of ER beta realized should inhibited development of carcinoma, is due to its activity to neutralized proliferation effect ER@ (George, 1997)

The other hand also from ten times replication using green clover leafs extract analyzed using RIA technique found that in fresh leafs contained 538.0 ± 30.5 pg/g compared to extract dry leafs 1068 ± 7.2 pg/g ($p < 0.05$) (Table 1). The dry leafs extract containing almost twice phytoestrogen then fresh leafs. An experiment in menopause mice that consume this extract be able to increase of osteocalcin as a bone modeling indicator and also ER@. In menopause women, by consume this green clover extract for 4 weeks effected to increase estrogen blood serum and IGF1 significantly (Laswati, 2007a). This study indicated that by consume green clover