NATURAL PHYTOESTROGEN CONTENTS IN SEVERAL FRUIT AND LEAVES: THE FUTURE REPLACEMENT HORMONE THERAPY IN MENOPAUSE WOMEN

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

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ABSTRACT

Phytoestrogen is molecularly almost similar with and act same as estrogen and found lot in several fruits and leaves 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 leaves, 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 leaves, tomato and papaya fruit. All these groups divided into 2 subgroups based on process of subspecies. All samples were made infuse (infus 1:4(w/v)) then extracted after was spinning 1000xg for 15mins, with 1.5 petroleum ether(w/v). After evaporated each extract then kept dry-frozen -20°C until analysis to be performed. Solid phase Radioimmunoassay technique was used to identify the estrogen content. The lowest estrogen level presented in fresh pegaga leaves extract (Mean±Sd) was 47.9±5.5 pg/g and twice increasing the level in dry leaves extract was 96.1±11.2pg/g. Meanwhile the estrogen level in fresh green clover leaves 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 leaves 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 melekal hampir sama dan mempunyai aktifitas sama seperti estrogen dan ditemukan pada jenis tanaman dan buah buahan terutama di negara tropis. Tetapi jumlah kandungan melekal hormon belum diketahui. Pada orang-orang menopause dan andropause diperlukan suluh hormon seks karena meraka umumnya mengalami penurunan hormon tersebut secara berarti yang bisa berdampak pada fungsi organ. Tujuan dari penelitian ini untuk menganalisis kandungan estrogen ekstrak daun pegaga, semanggi.

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INTRODUCTION

Tropical country like Indonesia has lot kinds of fruit and leaves. Most of those fruit taste sweet, palatable and contributable 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 leaves 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/Trifolium 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 leaves 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(ERβ), 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. Appcar without any control of estrogen receptor beta, malignant tumor supposing arise in organ, because ER beta could be inhibit activity of ERon...
(George, 1997). As well as known estrogen is agonist to ERα and antagonist to ERβ, meanwhile phytoestrogen tend more bind to ERβ (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 leaf also quiet 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 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 ferrous ion to increase hemoglobin quality (Greenspan and Gardner, 2004). The therapy effect of these leaves 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 menstruate women had consumed 500mg extract green clover twice a day for at least 3 weeks the blood serum estrogen concentration and estrogen receptor (ERα) 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, moistness 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 (Sproff et al, 1998; Baziad 2003; Al Baghdadi and Eswies 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
MATERIALS AND METHODS

This study was classified into an observational analysis with purposive sampling. There were 2 kinds of leaves, consistent in 2 different kinds of process and 2 kinds of fruits, each of 2 parts, which were used as samples and were analyzed. The estrogen concentration was determined using radioimmunoassay technique. The 1st group was pega leaves, which consisted of 20 replicates, 10 times for fresh leaves and 10 times replicate for dry leaves. The 2nd group was green clover/Marsilea crenata Presl, which also had 10 replicates for fresh leaves and 10 times dry leaves. The 3rd group was mature tomato, consisted of 10 times replicate for outer part and inner part/fibrin like substance also ten replicate. The 4th group was mature Papaya, consisted of 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 papped into glass tube and vortex for 1 minute. Centrifugation was done 10000g for 10 mnts, and the filtrate was papped 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 papped 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 samples, by using Radioimmunoassay technique, there was £\textsubscript{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 leaves to analyze estrogen content using Radioimmunoassay (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 leaves as local salad style and in Bali Province most the peoples they made this leave serve as infusion (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 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 leaves contained 538.0±30.5 pg/g compared to extract dry leaves 1068±7.2 pg/g (p<0.05) (Table 1). The dry leaves extract containing almost twice phytoestrogen then fresh leaves. 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