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PHYTOESTROGEN IN SEVERAL FRUITS AND LEAVES

(Fitoestrogen dalam Beberapa Daun dan Buah)

L. Maha putra¹, Hening Laswati putra²

ABSTRACT

Phytoestrogen is molecularly almost similar with and acts the same as estrogen and is found a lot in several fruits and leaves grown either in tropical or subtropical countries. However, the quantity of molecular contents are not yet known exactly. In menopause and andropause, people need substitution of estrogens as a replacement therapy of sex hormone, due to the significant hormone decline and impacted disturbance of several organ functions and thus progressively causing severe organ dysfunctions. The objective of this study was to know the estrogen content by analyzing extractions of pegaga, green clover leaves, tomato and papaya fruit of which certain communities have overviewed contents of the leaves and fruit. The samples which were collected for this purpose used 10 times replication in four different groups: the pegaga, green clover leaves, tomato and papaya fruit. All these groups were divided into two (2) subgroups based on the process or subspecies. All samples were made as an infusion 1:4 w/v, and then extracted after centrifugation 1000xg for 15mnts, with 1:5 petroleum ether v/v). After it was evaporated, each extraction then was kept dry-frozen at -20° C until the analysis was performed. Solid phase Radioimmunoassay technique was used to identify the estrogen contents, up to a total of 80 samples. The binding percentage of each sample was then interpolated on a logit-log paper to find out the real concentrations.¹⁴ The lowest estrogen level was found in fresh pegaga leaves extract (Mean+SD) was 47.9+5.5 pg/g, but in dry leaves extract the level was increased twice, about 96.1+11.2 pg/g. Meanwhile the estrogen level in fresh green clover leaves extract was 538.0+30.5 pg/g, more than ten times higher compared to fresh pegaga level, but twice lower than the estrogen level compared to dry green clover leaves extract, which was 1068.0+97.2 pg/g. In the fruit group, the fibrin part of tomatoes had more or less the same estrogen content compared with Thai papaya subspecies, 1037.0+37.7 pg/g and 1175.0+67.7 pg/g, respectively. On the other hand, it was noted that the inner part/fibrin part of tomato had a higher estrogen level of four (4) times compared to the outer part which was 315.0+18.4 pg/g. While it was noted that local Java papaya besides being not so sweet, the estrogen level was also not as high or the same as that found in fresh green clover which is 530.1+50.7 pg/g and 538.0+30.5 pg/g. Based on this study so far, it can be concluded that semanggi/green clover, tomato and papaya could be suggested as a replacement therapy for certain people who are considered to have reduced estrogen content, except that pegaga leaves are not recommended. The last mentioned plant besides that it is difficult to obtain, its estrogen content is also very low.

Key words: Phytoestrogen, pegaga, green clover, tomato and papaya

ABSTRAK

Fitoestrogen secara molekuler hampir sama dan beraktifitas sama seperti estrogen dan ditemukan di jenis tanaman dan buah-buahan baik tropis maupun subtropis. Namun, jumlah kandungan molekulnya belum diketahui secara pasti. Perempuan yang mengalami menopause dan andropause diperlukan suling hormon seks, karena umumnya kandungan hormon tersebut di mereka menurun secara bermakna. Hal tersebut dapat berdampak gangguan aktifitas fungsi organ serta dapat berkembang menjadi gangguan fungsi yang lebih berat. Tujuan penelitian ini adalah untuk mengetahui kandungan estrogen yang berasal dari sari daun pegaga, semanggi, buah tomat dan pepaya secara analisis, sehingga dampak terhadap masyarakat yang berpendapat pemahaman akan manfaat daun dan buah tersebut dapat dikukuhkan. Pengumpulan sampel dilakukan dengan cara cuplikan bertujuan (*purposive sampling*), masing-masing 20 kali ulangan di empat (4) kelompok, yaitu: kelompok daun: pegaga, dan semanggi, serta buah: tomat dan pepaya. Setiap kelompok dibagi menjadi sub-kelompok berdasarkan proses atau subspecies. Semua sampel dibuat infusa (1:4/w/v), setelah itu disarikan dengan petroleum eter (1:5/v/v) yang sebelumnya dipusingkan 1000 xg selama 15 menit. Selesai disarikan, sampel disimpan kering beku -20° C hingga penentuan kadar hormon dilakukan. Kadar estrogen ditentukan dengan metode Solid Phase Radioimmunoassay dengan jumlah keseluruhan 80 sampel, dan dengan memakai kertas logit-log untuk menggambar grafik baku, lalu dengan persentasi interpolasi ikatan setiap sampel didapatkan kadar hormon. Kadar hormon estrogen terendah ditemukan di sari daun pegaga segar dengan (rerata±SD) 47,9±5,5 pg/g dan kadarnya meningkat dua kali lipat di sari daun pegaga kering yaitu 96,1±11,2 pg/g. Sementara kadar estrogen di sari semanggi segar adalah 538,0±30,5 pg/g dibandingkan dengan sari pegaga segar. Kandungan ini sepuluh kali lipat lebih tinggi, tetapi kurang lebih dua kali lebih rendah kadar estrogennya bila dibandingkan dengan sari daun semanggi kering yaitu 1068,0±97,2 pg/g. Di kelompok buah-buahan bagian lendir tomat tampak kurang lebih sama kandungan estrogennya dengan jenis pepaya Thailand yaitu masing-masing 1037,0±37,7 pg/g dan 1175,0±67,7 pg/g. Sebagai catatan bahwa bagian dalam atau bagian yang berlendir buah tomat memiliki kadar estrogen kurang lebih empat kali lipat lebih tinggi dibandingkan dengan bagian luar yaitu 315,0±18,4 pg/g. Di lain pihak pepaya Jawa lokal di samping tidak manis dan kandungan estrogennya tidak begitu tinggi atau sama dengan yang didapatkan di daun semanggi segar masing-masing yaitu 530,1±50,7 pg/g dan 538,0±30,5 pg/g. Daun semanggi, buah tomat, dan pepaya dapat disarankan sebagai sulingan pengobatan hormon untuk orang tertentu yang memerlukan, tetapi daun pegaga tidak dianjurkan karena sulit didapatkan, selain itu kandungan hormon estrogennya juga rendah.

Kata kunci: Fitoestrogen, pegaga, semangi, tomat, dan pepaya

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INTRODUCTION

Tropical countries such as Indonesia have lots of fruits and various kinds of leaves. Most of the fruits taste sweet, are palatable and may contribute to a good healthy condition when consumed in sufficient quantities. However, several of those plants can not be consumed too much due to contraindication in certain body conditions. Meanwhile, most of the leaves found in Indonesia or in the world are unpalatable, and some of those tend to taste too bitter but still have some good effects to health. Semanggi/Green clover/*Marcelea crenata* Presl can grow anywhere in Indonesia as long as the optimal condition is fulfilled such as wet to watery soil, and pegaga/*Centella asiatica* can be grown in wet soil. In certain provinces, both kinds of leaves are famous, such as in Aceh province. Most of the local communities are quite familiar to these leaves, processed as local vegetable salad style, and in Bali Province most of the elderly people use this as a traditional medicine infusion to decrease fever and increase intake of palatable food. Estrogen deficiency is the most importance factor in menopausal women, because this will affect the balance of bone remodelling process.¹ Even in a recent study using rats prepared as a menopausal condition, fed by 120 mg pegaga extract orally each day for 4 weeks showed increasing effects of estrogen receptor beta ($ER\beta$). That means that pegaga leave extract contains estrogen hormones like Phytoestrogen, but unfortunately the concentration was not identified.² The expression of estrogen receptor beta is much more found in primary or secondary sex organs but estrogen receptor alpha is also found. In the case of activity, estrogen receptor alpha expression is aroused due to increase of serum estrogens (organic chemistry) which can be followed by intracellular transcription and translation process The process then effects either the cell proliferation or hyperplasia. Malignant tumours arising in organs, appear without any control of estrogen receptor beta, because this could inhibit the activity of $ER\alpha$.³ Estrogens are agonists to $ER\alpha$ as well as known antagonists to $ER\beta$, meanwhile phytoestrogen tends to be more bound to $ER\beta$.⁴ In mice, the highest estrogen receptors are found during the oestrus phase and the lowest found in dioestrus phase where during this period estrogens are minimum.⁵ Meanwhile semanggi leaves are also quite famous in East Java Province, mainly in Surabaya, these leaves are consumed as a local style salad serve along with local rice crackers and spice. The consumers are mostly women who were in the menopause stage and these customers used to eat it traditionally from generation to generation. Although there is no detailed information about its ingredient at that time, except that there is

a dominant chlorophyll content. As it is known that chlorophyll found in the green clover and pegaga has a good activity due to its contribution to bind Ferro ion to increase the haemoglobin quality.⁶ The therapy effect of these leaves may be transferred due to existence of experience orally in the inter or intra community living mainly in East Java, they said when consume semanggi their health seemed to be better. Therefore, up to now it is a traditional custom which is still going on without any obstacle except for a smaller stock. From the recent study it was reported that menopausal women who had consumed 500 mg extrbloact green clover twice a day for at least 4 weeks may increase the concentration of their blood serum estrogens significantly ($p < 0.05$) compared to the control group of menopausal women.⁷ The experiment was also done in the animal laboratory (using menopause mice), showing that in mice which had been given green clover extract orally for at least 3 weeks the blood serum estrogen concentrations and estrogen receptors ($ER\alpha$) also have been increased. This finding could explained the reality, that by consuming green clover as an alternative medicine could reduce or ease undesired menopause symptoms as well as hot flushes, headache, night sweating, fear and excitement. The other later impact of decreasing estrogen concentration in menopause women is the condition of osteoporosis, lots of women were more prone to fractures compared to men.⁸ In the genital tract, as a direct impact of the decrease of estrogens may cause the disturbance of sexual behaviour in marital women. This is due to vaginal epithelial atrophy, dryness and reducing of collagen compartment and which then influenced the vaginal contractility. In the skin it is known that estrogens is a contributing factor for fibroblast growth and the stimulation of collagen, this is the main factor for caring and maintaining skin, to make the skin smoother, moist and elastic. Meanwhile, white hair either in menopause or andropause people is caused due to decreasing melanin content which is catalyzed by tyrosine enzyme, however the activity of this enzyme can be stimulated by using estrogens, therefore estrogen is believed also as an anti white hair.⁹⁻¹¹ On the other hand, papaya and tomato are also classified as tropical fruits, and well known as well as have good demand around the world. This is due to its palatable and taste, especially for papaya which is served with tomato juice and adding a little bit of syrup is quite common and often consumed in the tropical countries as well as in Indonesia. Both of these fruits which are later mentioned, the ingredients and other contents are not yet much known. However, of both fruits, only the beta carotene is prominently consisting in both and of course rich with fiber that is good for the gastro-intestinal tract either for its function or cellular health. Meanwhile, vitamin C,

B1 (thiamine), B2 (riboflavin) and mineral as well as potassium, and calcium also consist in both fruits. Papaya is also commonly used as an appetizer, to cure stomach illness, recover haemorrhage at menorrhage, and release gastrointestinal disturbance and difficulty in defecation. Moreover, due to its lycopene content in tomato they are believed to reduce pulmonary and prostate cancer risk. Mucous/fibrin like substance which commonly lay in the middle part of the tomato fruit is believed as an anticoagulant, so this can reduce the prevalence of congestive stroke and its tomatine content will also have an effect as a local anti inflammation. So the overall medical effect of tomato could be felt in the haemostatic function of a healthy liver.¹²

All those fruits and leaves described above, have lots of ingredients and are believed to be able to maintain health as well as have a prevention effect to several diseases and dysfunctional organ problems, but only up to now, green clover has been known to contain estrogens. However, the other three plants which have been mentioned above are not yet known whether they could be used as an alternative in traditional medicine especially based on their estrogen content and concentration that may reduce menopause symptoms in women. The main aim of this study was to know the estrogens content in several fruits and leaves by analyzing them to make more sure of the quality for the alternative choice for the consumer as a preventive medicine.

METHODS

There were two (2) kinds of leaves using two (2) different kinds of processes and two (2) kinds of fruits, consisting of two (2) parts. Each kind was used as a sample for analyzing the estrogen concentrations by radioimmunoassay technique. The 1st group were pegaga leaves consisting of 20 replicates. For fresh leaves 10 times replication were made and also 10 times for the dry leaves as well. The 2nd group was green clover/*Marsilea crenata* Presl the same procedure was applied, 10 times replication for the fresh leaves and for the dry leaves. The 3rd group were mature tomatoes consisted of 10 times replication for the outer part and also for the inner part/fibrin like substance. The 4th group were mature Papaya, consisted of 10 times replication for the local type as well as for the Thailand subspecies.

The procedure in preparing the samples

All crude samples were weighted electrically at just 1g for the fresh as well as for the dried samples according to their respective groups, then mixed homogeneously in a ceramic bowl after adding 4mLs physiologic NaCl. The homogenization was

repeated again and then poured into a glass tube and vortexed for 1 minute. The centrifugation was done at 1000G for 10 minutes, the filtrate was poured into an extraction glass tube, and then petroleum benzene was added with that classified matter into a polar extractor with 1:5 (v/v), then vortexed for 5 minutes. All mixtures were then put into a freezer for 15 minutes, only the poured filtrate into glass assay tube was used, but not the frozen pellet at the bottom. The filtrate in the assay tube was then evaporated by gently blowing air into an assay tube placed into a water bath of 38° C. The extract was then diluted with 1mL serum estrogen within 0 pg/mL concentration/Bo as a ready sample.

The procedure in analyzing the sample:

The estrogen concentrations can be detected quantitatively by using Radioimmunoassay technique; using I125 as a tracer bound to the legend/unknown hormone. The principle reaction of this technique is the existence of the occurred competitive molecular binding between the legend and radio-legend into a specific AB fraction of IgG anti hormone estrogens coated in the inner lining of the polypropylene assay tube. Meanwhile the C-fraction IgG of anti-hormone linked with the inner part of polypropylene as a coated inner assay tube. The higher the estrogens concentration in the sample that will strictly impact will also cause a much lower radio illumination caught in the gamma-counter read as counts-per-minute/CPM

The concentration can then be calculated to find out the first binding percentage (%) by dividing the net CPM sample with the net CPM binding in the 0 pg/mL/BO with 100% multiplication. Secondly by an integration binding-% sample to interpolate the linier standard curve the concentration can then could be detected visually. Therefore, the concentration of the estrogen hormone was contrary to the CPM shown on the gamma-counter screen.¹³

The binding percentage of each sample was then interpolated on a logit-log paper to find out the real concentrations.¹⁴ The data of the estrogen concentrations in pg/g, were presented as a statistically description and the mean differences were analyzed using one tail Student-t test.¹⁵

RESULT AND DISCUSSION

From the ten times replication samples used in pegaga leaves to analyze the estrogens content by using Radioimmunoassay (RIA) technique, the lowest concentration of phytoestrogen were found as shown in the 4th sub-group. In the fresh pegaga the concentration was (Mean+SD) 47.9+5.5 pg/g, but the content of the dried pegaga was almost

increased twice to 96.1+11.2 pg/g ($p < 0.05$) (table 1). Only in the recent study² in menopausal rats, it was reported that this happening could be caused by stimulating expression of ER beta and collagen. Following this study, it was reported that in menopause rats, pegaga leaves extract can be used to maintain vaginal elasticity. The substance which containing phytoestrogen could stimulate expression of ER beta, and is believed to be able inhibiting the development of carcinoma. This is due to its activity to neutralize the proliferation effect of ER@.³

On the other hand, also from the ten times replication using green clover leaves extract analyzed using RIA technique it was found that fresh leaves contained 538.0+30.5 pg/g compared to dry leaves extract 1068+.7.2 pg/g ($p < 0.05$) (see Table 1). The dry leaves extract contained almost twice of phytoestrogen concentration compared to the fresh leaves. In the experiment using menopause mice consuming this extract, it seemed to be able to increase osteocalcin as a bone modelling indicator and also ER@. In menopausal women, consuming this green clover extract for 4 weeks, it was shown that the effect of increasing blood serum estrogens and IGF1 was significantly.⁸ This study indicated that estrogens in menopausal women consuming green clover flattened in lower concentration than the cycling women. The latter were able to maintain their bone density and relief their menopause symptoms. The decrease of the sex hormones in menopause or in andropause will result in an increase of blood cholesterol level, which also gives an effect to heart failure as well as to decline its immunity.¹⁶ If both of these leaves were compared, in reality it can be seen that in the the estrogen content of green clover is much more increased or tend to be mostly 10 times higher. It is confirmable that the traditional East Java older people who consume green clover (semanggi) believed this substance can relieve the menopause or andropause symptoms, as far as it is consumed regularly and the quantity of intake is enough.

Table 1. The phytoestrogen content in pegaga and green clover leaves (pg/g) (Mean±SD)

	1 st group (pegaga)	2 nd group (green clover)
Fresh	47.9±5.5a	538.0±30.5b
Dried	96.1±11.2c	1068.0±97.2d

The superscript difference either in one column or row was significantly different < 0.05 .

From the fruit groups, the 3rd group consisting of mature tomatoes with 10 times replication in the inner part/fibrin like substance the estrogens content (Mean+SD) showed 1037.0+37.7 pg/g, which was three (3) times higher compared to the same replication of the outer part, that was only 315.0+18.4 pg/g ($p < 0.05$). (Table 2). While the

4th group which consisted of two (2) subgroups, the estrogen content in extracted local mature papaya (Mean±SD) showed 530.1+50.7 pg/g, which was almost as twice lower compared to the Thai subspecies is 1175.0+67.7pg/g ($p < 0.05$).

Considering the estrogen contents in all these groups mentioned above, leaves and fruits could be used as replacement therapy mainly in menopause and andropause people against a progressive process of osteoporosis. From another study it is reported that phytoestrogen acts as ordinary estrogens to stimulate and increase osteocalcine as a precursor in bone remodeling.^{8,10} Estrogens present in the blood serum and its dependance on the adsorbing capacity in the intestine the phytoestrogen also can function as anti malignancy, making the skin much smoother and keep its elasticity which is due to fibroblast growth, and keeping vaginal epithelial cells to grow to inhibit dryness, which is all supported by estrogen.^{2,9,17}

Table 2. The Phytoestrogen content in Tomato and Papaya Fruit (pg/g) (Mean±SD)

	3 rd group (Tomato)	4 th group (Papaya)
Local	-	530.1+50.7a
Thai	-	1175.0+67.7b
Inner part/fibrin	1037.0+37.7c	-
Outer part	315.0+18.4d	-

The difference of superscript in one column was significantly different $p < 0.05$

CONCLUSION

All these groups, from the 1st to the 4th, contained estrogens. The lowest estrogen content was present in fresh pegaga leaves, and the highest estrogen content was found in the mature Thai papaya subspecies. The inner part of tomato/fibrin like substance contained high enough estrogens, the same as was presented in Thai papaya subspecies. Based on this study, all leaves and fruits samples were able to be used as a replacement therapy, depending on the intestine absorption capacity, regularity and quantity consumed daily. Regarding these research results, a suggestion for a future study is needed in order to know the biomarker response by analyzing the same substances in an animal or a human laboratory.

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