

# Natural Phytoestrogen Contents in Several Fruits; the Future Replacement Hormone Therapy in Menopause Women

(Kandungan Fitoestrogen Alami pada Beberapa Buah Buahan; Suatu Sulih Terapi Hormon di Masa Depan pada Wanita Menopause)

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## ABSTRAK

*Fitoestrogen secara melekul hampir sama dan mempunyai aktifitas sama seperti estrogen dan ditemukan pada jenis tanaman dan buah buahan baik di Negara tropis maupun subtropics. Tetapi jumlah kandungan melekulnya belum diketahui secara pasti. Pada orang-orang menopause dan andropause diperlukan sulih hormone seks karena mereka umumnya mengalami penurunan hormone tersebut secara berarti yang bisa berdampak pada gangguan aktifitas fungsi organ dan bisa berkembang menjadi gangguan fungsi organ yang lebih berat. Tujuan dari penelitian ini untuk mengalisis kandungan estrogen ekstrak buah pisang, melon merah, jeruk manis, durian dan advokat, yang dampaknya terhadap komunitas masyarakat mempunyai opsi pemahaman akan kandungan hormone estrogen pada berbagai jenis buah buahan tersebut. Pengumpulan sample dilakukan dengan cara purposive sampling dengan masing-masing 10 kali ulangan pada 5 kelompok yaitu, kelompok pisang, melon merah, jeruk manis, durian dan advokat. Semua sample dibuat infusa (1:4/w/v) setelah itu di ekstrak dengan petroleum eter (1:5/v/v) di mana sebelumnya dilakukan sentrifugasi 1000xg selama 15 menit. Setelah ekstraksi, sample disimpan kering beku -20° C hingga penentuan kadar hormone dilakukan. Radioimmunoassay fase padat telah dilakukan untuk menentukan kadar hormone estrogen dengan jumlah keseluruhan 70 sampel termasuk 3 subkelompok pisang, dan dengan memakai kertas logit-log untuk linierisasi grafik standar lalu dengan interpolasi persentase ikatan masing-masing sample maka didapat kadar hormone. Kadar hormone estrogen tidak dapat ditemukan pada pisang baik susu, ambon dan keprok (rata-rata ± SD) 0 pg/g. Sedangkan kadar hormone estrogen pada buah advokat adalah 244,4 ± 13,3 pg/g, untuk kadar estrogen ekstrak isi buah durian montong adalah 918,0 ± 37,0 pg/ml, yang tampak hampir 4 kali lipat lebih tinggi dari kadar estrogen ekstrak isi buah avokat. Kadar estrogen pada buah melon merah adalah (Rataan ± Sd) adalah 1254,0 ± 48,1 pg/ml, yang tampak paling tinggi diantara semua jenis buah yang dilakukan analisis kadar hormone estrogenya. Sedangkan ke 3 jenis pisang susu, keprok dan pisang ambon serta jeruk manis tidak dapat dipakai sebagai sumber estrogen alami pada ibu menopause karena kadarnya 0 pg/ml.*

**Kata kunci:** fitoestrogen, buah pisang, melon merah, durian, jeruk manis dan advokat

## INTRODUCTION

Tropical country as well as Indonesia has lot kinds of fruit, most of fruit taste are sweet, palatable and could be contribute 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 leaf can be 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 of food. Estrogen deficiency is most importance factor in menopause women because of the imbalance bone remodeling process (Fitzpatrick, 2003). Even recent study in rat made it menopause and provided orally everyday 120 mg extract pegaga for 4 weeks effected increasing of estrogen receptor beta(ERβ), that

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mean pegaga leaf 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 $\alpha$  (George, *et al.*, 1997). As well as known estrogen is agonist to ER $\alpha$  and antagonist to ER $\beta$ , meanwhile phytoestrogen tend more bind to ER $\beta$  (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 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 chlorofile content. As well as known the chlorophyl that found in green clover and pegaga has a good activity contribute to bind ferr0 ion to increase hemoglobin quality (Greenspan and Gardner, 2004). The therapy effect of these leaf 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 500 mg 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@) also have been increased. This finding could 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 impact 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, however 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 red melon and durian also classified into a tropical fruits. Melon with fresh taste is due to water content up to 95%, vitamin content as well as vitamin A 640 mg, B 0.03 mg and vitamin C 34mg/100g can be used as digestive decongestion . Melon also containing Adenosine as known as anticoagulant in blood stream so acted in preventing congestive stroke and coronary heart failure. It is also due to it heavy carotene group content (Karotenoid) melon be able as preventive pulmonary carcinoma, anti uric acid potent also one of those its action if consume mix with lemon. Durian is king of fruit also known as controversial fruit is due to its smell lots of people sympathy and several of the antipathy. Durian containing with carbohydrate, protein, fatty acid and mineral, that is not good consumed by overweight, diabetes , high blood serum cholesterol people. Meanwhile sweet orange and avocado known as common and healthy fruit spread out in South East Asia and Middle America. but both of these fruits have known and have a good demand round the world is due to its palatable and taste specially for sweet orange serve as juice and avocado by adding a little bit syrup and chocolate serve as avocado juice is quite 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 (Sari, 2011).

From all those fruits 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 no any known the estrogen content. The main aim of this study was to analysis the estrogen content in several fruits to make more alternatively choice to the consumer as a preventive or replacement medicine.

## MATERIALS AND METHODS

This research was designed as observational analytic study with purposive sampling, which were 5 kind of fruits and only banana as a 1<sup>st</sup> group consisted with 3 sub-group (kepok, susu, and ambon). For the 2<sup>nd</sup> group was red melon, the 3<sup>rd</sup> group was orange, the 4<sup>th</sup> group was durian and for the last 5<sup>th</sup> group was avocado. For all of each group was consisted with 10 times replicate used as a samples and were analyzed the estrogen concentration using radioimmunoassay technique.

In preparing sample: All crude samples were balanced electrically just 1g as well as fresh accordingly group, then homogenized in ceramic bowl and added with 4 mL NaCl physiologic again repeated homogenized and then pawed into glass tube and vortex for 1 minute. Centrifugation was done 1000 xg 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 filtrates 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 <sup>125</sup>I-E2 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 0 pg/ml/BO multiply with 100%. Secondly by integrate binding-% sample to interpolate linier 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). concentrations (Chard, 1990). The data as an estrogen concentration in pg/g, was presented as statistic descriptive (Steel and Torrie, 1998).

## RESULT AND DISCUSSION

From ten time replication samples that used in 3 sub-group of banana to analyze estrogen content using Radioimmunoassay (RIA) technique, found that no phytoestrogen (E2) concentration showed into those 3 sub-groups, there was (Mean + Sd) 0 pg/g (Table 1). It is therefore, if we look at to the custom of old Javaness people that consume banana mainly steam kapok banana it doesn't make sense in compensation of imbalance estrogen hormone concentration during post-menopause women, may be banana the only tasted sweet, smoother, much more digest and as vitamin C, B-Complex and serotonin as neurotransmitter in brain activity (Sari, 2011). Meanwhile red melon of course with fresh and sweet taste also as a mineral resource as well as sodium, calcium and much more potassium and vitamin A 640 mg/100 g fruit (Sari, 2011) and the importance thing is the high concentration of estrogen was (Mean ± Sd) 1,254.0 ± 48.1 pg/g there was the highest estrogen/phytoestrogen concentration among those fruits that analysis (Tabel 1). This estrogen concentration is much more equivalent with dried green clover extract (semanggi) leaf 1068 ng/g, but only 538.0 ng/g the estrogen content for fresh green clover extract that quite common vegetable local salad consume by old women people in East Java (Laswati, 2007). As long as normal activity and physiologically absorption in order in-vivo, this fruit can be used as replacement therapy in menopause women. The substance there are containing phytoestrogen could be stimulate expression of ER beta and realized should able to inhibit development of carcinoma, is due to its activity to neutralized proliferation effect ER@ (George, *et al.*, 1997). The experiment was done in menopause women that consume dried green clover extract one a day can be stimulate increasing of estrogen concentration in women and estrogen receptor (ERα) in menopause mice (Laswati, 2007). The other experiment also showed that estrogen receptor (ERβ) also increased in rat that consume pegaga leaf extract (Raden, 2010). The dry leaf extract containing almost twice phytoestrogen then fresh leafs. 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 in menopause women there is known the estrogen flatted in lower concentration but non in cycling women, so be able to maintain bone density and relief of menopause

symptoms. Decreasing of sex hormone in menopause or in andropause impacted increasing of blood cholesterol level so to be effect to heart failure and also be able to decline of immunity (**Biben**, 2006). Although no yet any experiment in-vivo for these kinds of fruits most probably to be have much more the same affect in menopause women or some can be more responsive effect as far as could be consumed regularly and quantitatively enough.

**Table 1.** The Phytoestrogen Content in Several Banana and red Melon (pg/g) (Mean ± Sd)

	1 <sup>st</sup> group (Banana)	2 <sup>nd</sup> group (red Melon)
"Ambon"	0.0 ± 0.0	1,254.0 ± 48.1
Replicate	10	10
"Susu"	0.0 ± 0.0	
Replicate	10	
"Kepok"	0.0 ± 0.0	
Replicate	10	

From the 3 fruit groups later on there were 3<sup>rd</sup> group was a sweet orange showed the estrogen content (Mean + Sd) with 10 times was 0 pg/g, same concentration as all 3 subspecies banana those were analyzed. That mean orange fruit could not be expected as a replacement medicine as well as semanggi, red melon, red clover for menopause women (*Tabel 2*). However sweet orange still has another a good contribution in immunology system in body protection is due to high vitamin C content and its high fructose make consumer felt longer full or not hungry than food containing glucose (**Sari**, 2011). Meanwhile the 4<sup>th</sup> and 5<sup>th</sup> group were durian and avocado respectively the estrogen concentration (Mean ± Sd) were 918.0 ± 37.0 pg/g for durian and 244.4 ± 13.4 pg/g for avocado (*Table 2*). This finding showed that durian has almost 3.5 times higher estrogen concentration compared to avocado. More over the consumer prefer to choice avocado to relief of thirsty than durian is due to durian too sweet, too much cholesterol, glycerol and controversial odor, but avocado felt more contribute healthy life due to be able to contribute make smoother and elasticity the skin so look much younger in the ages because of its Vitamin A vitamin E content. Avocado also believed and really containing with high non saturated fatty acid so has double action are 1<sup>st</sup> to reduce blood serum LDL be able preventing of stroke and coronary failures 2<sup>nd</sup>-ly is due its oleic acid content that known as quite firm anti-free radical agent be able to prevent live cell make faster damage. The other hand although the estrogen concentration avocado is lower than durian more over than red melon but avocado has a complete mineral composition

as well as ferry (Fe) and cuprum (Cu) these mineral good and very useful in regeneration of blood cell, while mineral mangle (Mn) and Zing(Zn) are needed in rhythm cardiac contraction and keeping in blood pressure (**Sari**, 2011).

Considered to estrogen content to all these fruit groups were mention above that be able as replacement therapy mainly in menopause and andropause people against a progressive process of osteoporosis. As well as reported that phytoestrogen is acted as ordinary estrogen to stimulate and increase osteocalcin as precursor in bone remodeling (**Laswati**, 2007b; **Basiad**, 2003). Estrogen present in blood serum its depend on the adsorbing capacity in the intestine the phytoestrogen also can be acted as anti malignancy, make the skin much smoother and kept the elasticity is due to mainly action of estrogen, vitamin A and Vitamin E be able to stimulate fibroblast growth, and kept vaginal epithelial cell grow to inhibit dryness (**Schering**, 2002; **Speroff et al.**, 1998; **Raden**, 2010).

**Table 2.** The Phytoestrogen content in Sweet Orange, Durian and Avocado Fruit (pg/g) (Mean+Sd)

3 <sup>rd</sup> group (Sweet Orange)	4 <sup>th</sup> group (Durian Mnt)	5 <sup>th</sup> group (Avocado)
0.0 ± 0.0 (n = 10)	918.0 ± 37.0 (n = 10)	244.4 ± 13.4 (n = 10)

## CONCLUSION

All these groups 2, 4, and 5 containing of estrogen. The lowest estrogen content or non detectable present in those 3 subtype; banana and sweet orange, The highest estrogen concentration found in red melon. Meanwhile durian has estrogen concentration in moderate concentration and avocado has lower estrogen concentration compared to all those fruit consisting of estrogen. The only group 2 (red melon) and group 5 (avocado) be able as replacement therapy, but not for durian even the estrogen content also quite enough. This impact will be depend on intestine absorption capacity, regularity and quantitatively consume a day. As a suggestion needed a future study to analyze biomarker response in laboratory animal or human.

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