

# ***Pueraria mirifica* Tuber Fermented Juice Products: Sensory Evaluation and Test for Estrogenic Potency**

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## **Abstract**

Three formulas of *Pueraria mirifica* tuber fermented with *Saccharomyces cerevisiae* TISTR No. 5339 for one month were prepared. After fermentation, each formula of *P. mirifica* fermented juice was tested for sensory evaluation by 20 male and 15 female testers. It was found that the first choice of *P. mirifica* fermented juice which was chosen by male and female testers was formula 1. Three months later, each formula was tested again by 20 male and 15 female testers and it was found that both sexes of testers preferred formulas 1 and 3. Each formula was tested for the estrogenic potency in bilaterally ovariectomized immature rats. The results revealed that the potency ranking of estrogenic activities of PM fermented juice were formula 3, 1 and 2, respectively.

## **INTRODUCTION**

*Pueraria mirifica* Airy Shaw & Suvatabandhu or white kwao kreur, *P. mirifica* is a leguminous plant that grows in groups in the deciduous forest especially in northern Thailand. The plant part used is tuber. It is believed that *P. mirifica* pronounces a rejuvenating effect in both aged men and women, producing soft and youthful skin, turning white or gray hair black, inducing the regrowth of hair, improving the complexion and memory. Preparations of the plant may also induce breast soreness and development, and induce menstruation.

The *P. mirifica* tuber has demonstrated potent estrogenic effects (Pope et al., 1958; Smitasiri et al., 1986). It contains many kinds of substances such as miroestrol, deoxymiroestrol, puerarin, mirificin coumestrol, beta-sitosterol, genistin, genistein, daidzin, daidzein, butenin, pueramirifin, lithium, phosphorus, sodium, starch, protein, sugar, and fiber (Smitasiri, 2002).

The estrogenic effect of *P. mirifica* tuber has been found in the ethanolic and aqueous extracts (Sornsrivichai et al., 1987; Vanasont, 1989) and the antioxidative effect has been found in the ethanolic extract (Sang-Arun et al., 2001). Due to the potency of the plant, the regular consumption of *P. mirifica* is recommended by the Ministry of Public Health of Thailand.

The aim of this project was to produce fermented *P. mirifica* tuber juice and to study the sensory evaluations, and estrogenic potency in bilaterally ovariectomized rats.

## **MATERIALS AND METHODS**

### **Production of Fermented Juice**

*P. mirifica* tubers were collected from the deciduous forest in Amphur Dok Kham Tai, Phayao Province in March 2002.

#### **1. Preparation of Starter.**

1. Begin with 75 g dried *P. mirifica* tuber powder or 900 g fresh *P. mirifica* tuber (depending on desired final preparation).
2. Mix the plant material with distilled water then boil for 15 minutes and filter through aseptic gauze.
3. Adjust the filtrate by adding citric acid until pH reaches to 4.5.
4. Adjust the filtrate by adding sugar until the total soluble solids reach 10°Brix.

5. Pour 100 ml of filtrate into aseptic volumetric flask, which contains 0.2 g ammonium sulphate. Close the flask with sterilized cotton wool and store at room temperature until cool.
6. Inoculate 2 loops of *Saccharomyces cerevisiae* TISTR No. 5339 (aseptic technique).
7. Shake at 200 rpm for 2 days.

## **2. Fermentation.**

1. By adding sugar, adjust the remaining filtrate from no.4 (in preparation of starter) until the total soluble solids reach 22°Brix.
2. Pour into an aseptic container that contains 15 g ammonium sulphate. The container should have 10% headspace. Close the container with sterilized cotton wool and store at room temperature until it cool.
3. Inoculate the starter with aseptic technique and store at room temperature.
4. Check the pH, acidity and total soluble solids everyday (the sampling methods are aseptic technique) for 1 month (total soluble solid about 9-10°Brix).
5. Stop the fermentation process.

## **3. Storage of *P. mirifica* Fermented Juice.**

1. Transfer the supernatant part to the pot.
2. Heat until 60°C for 30 minutes, add egg white from 4 eggs and stir well.
3. Filter through aseptic gauze and store the filtrate in the aseptic container until precipitated (about 2-3 days).
4. Transfer the clear liquid part to the bottles with aseptic technique.
5. Divide the liquid into 2 parts, immediately test the first and store the second one to test after three months.

## **4. Preparation of *P. mirifica* Tuber Fermented Juice Product Formula.**

Formula 1: 75 g dried *P. mirifica* tuber powder in distilled water for a total volume of 15 liters.

Formula 2: 37.5 g dried *P. mirifica* tuber powder mixed with 450 g fresh *P. mirifica* tuber, in with distilled water for a total volume of 15 liters.

Formula 3: 900 g fresh *P. mirifica* tuber, added with distilled water until 15 liters.

## **Sensory Evaluation of Fermented Juice**

Sensory evaluation of fermented juice formula was carried out two times by both male and female testers using The Hedonic scale test. The first time, the evaluation was managed by 20 male and 15 female testers. After three months storage the evaluation was repeated by 20 male and 15 female testers.

## **Test of Estrogenic Potency**

In this experiment, 28 immature female Wistar rats at the age of 3 weeks (National Laboratory Animal Center, Mahidol University at Saraya, Nakhon Pathom) were utilized. The rats were reared in an air-conditioned room ( $24 \pm 1^\circ\text{C}$ ) and the light was controlled (12 hour light 12 hour dark). Each immature rat was anaesthetized by nembutal injection intraperitoneally. Both ovaries were removed. Fourteen days after bilaterally ovariectomy, the rats were divided into four groups (7 rats/group): the first group was the control group receiving 2 ml of distilled water orally, once daily in the morning during 8.00-9.00 a.m. for 7 consecutive days. The second, third and fourth groups did the same as the control group but receiving 3-month PM tuber fermented juice formula 1, 2 and 3 respectively instead of distilled water.

On day 8, each rat was sacrificed, weighed and the uterus of each rat was removed and weighed by using 4-digitally electronic balances. Statistical analysis was made using ANOVA and least significant difference.

## **RESULTS AND DISCUSSION**

Table 1 shows the sensory evaluation of fermented *P. mirifica* tuber juice products carried out by 20 male and 15 female human testers. It was found that the first choice of

fermented *P. mirifica* juice, which was chosen by male and female testers was formula 1. Three months later, each formula was tested again by 20 male and 15 female testers and it was found that both sexes of testers preferred formulas 1 and 3. All products were tested for the estrogenic potency. The potency ranking of estrogenic activities were formulas 3, 1 and 2, respectively (Table 2). The fermented *P. mirifica* tuber juice formulas 1 and 3 were the favorite juices among both sexes of testers and these formulas produced the estrogenic activities in mice.

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#### Tables

Table 1. Sensory evaluation test of fermented PM tuber juice products by male and female testers.

PM tuber fermented juice product	Sensory evaluation ranking			
	Immediate test		Three month storage test	
	20 Males	15 Females	20 Males	15 Females
Formula				
1.	68	65	85	87
2.	27	29	35	31
3.	54	53	79	85

Sensory evaluations: appearance, color, odor, taste, sweetness, flavor and percentage of alcohol.

Table 2. Test of estrogenic potency of fermented PM tuber juice products in bilaterally ovariectomized immature rats.

Group (2 ml/day, 7 days)	Number of rats	Body weight (g)	Uterine wet weight (mg)
Distilled water	7	275 ± 15.4	150.7 ± 34.6
PM tuber fermented juice Product			
Formula 1	7	297 ± 34.1	285.2 ± 41.8*
Formula 2	7	293 ± 12.9	261.5 ± 44.8*
Formula 3	7	273 ± 23.3	329.3 ± 64.5**

X ± S.D.; \* P < 0.05; \*\* P < 0.01