



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203





Human Journals

Research Article

November 2017 Vol.:10, Issue:4

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## Effectiveness Test Combination Ethanol Extracts of Fenugreek Seed (*Trigonella foenum-graecum* L.) and Cayenne Fruit (*Capsicum annum* L.) in Hair Growth Activity

			
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<b>Submission:</b>	31 October 2017		
<b>Accepted:</b>	7 November 2017		
<b>Published:</b>	30 November 2017		



HUMAN JOURNALS

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**Keywords:** *Trigonella foenum-graecum* L., *Capsicum annum* L., extract fenugreek seed and cayenne Fruit, trigonelline, capsaicin, test activity, hair growth.

### ABSTRACT

Hairs undergo a process of growing up and growing long-ago loss and then a change of new hair. Hair growth is expected to take place optimal premises to produce healthy hair and avoid hair loss and baldness is a disorder in which a lot of hair occurs in both men and women. This is generally the case caused by physiological conditions, emotional or physical stress, nutritional deficiencies, hormonal disorders and drugs. Fenugreek seed and cayenne fruit can be used for hair grower. The purpose of this study was to obtain a combination of extracts of fenugreek seeds and Cayenne fruit as a hair grower. The results of determination showed that plant is cayenne fruit (*Capsicum annum* L.) and Fenugreek seed (*Trigonella Foenum-graecum* L.). Both extracts obtained by maceration using 70% ethanol. The other identity that meets the requirements of the ethanol extracts of fenugreek seed with 2.05% trigonelline and cayenne fruit with identity of capsaicin 0.41%. Comparison combination ethanol extract of cayenne fruit and fenugreek seeds used were concentration 0.05%: 5% (combination 1); 0.1%: 10% (combination 2) and 0.2%: 20% (combination 3). Extract combination activity test using the method performed by Tanaka, observations were made 28 days using male rabbits of *New Zealand White* race. The results show that all combination of having activity growth hair. The best combination ethanol extract cayenne fruit with concentration 0.1% and fenugreek seed with concentration 10% have activity growth hair 2.735cm.

## INTRODUCTION

Hair is a part that comes out of the skin and scalp, which has basic functions as a protector, sensory organs, maintaining the stability of body temperature and as a sign of social status. Hair can grow and grow long, this happens because the cells of the matrix region or the hair bulbs are continuously splitting. Hair grows into adulthood and grows longer than hair loss and then there is a new hair change. Hair growth is expected to take place optimal premises to produce healthy hair and avoid hair loss and baldness is a disorder in which a lot of hair occurs in both men and women. This is generally the case caused by physiological conditions, emotional and physical stress, nutritional deficiencies, hormonal disorders and medications.

Hair grower preparations especially synthetic have been developed to cope with hair loss and baldness. Commonly used synthetic materials such as minoxidil, which can cause side effects such as allergies in the skin to headaches.

Natural or herbal ingredients which reportedly can be used to overcome the loss, for example, aloe vera, hibiscus, ginseng, ginkgo biloba, gotu kola, green tea, beans, fenugreek, fruit cayenne and celery<sup>1,2</sup>. Fenugreek seed (*Trigonella foenum-graecum* L.) with concentration of 10% extract ethanol was applied to rabbit has effectiveness as grower optimal hair<sup>3</sup>. Fenugreek seed contains alkaloids, including trigonelline, gentianine and carpaine, steroidal saponin: disogenin, glitogenin and tipogenin that have effect a precursor of estrogen/hormone sex. Phytoestrogens from fenugreek seed allegedly could resolve loss hair and improve growth hair<sup>4</sup>. Fenugreek is a potent dihydrotestosterone (DHT) blocker and a rich source the role of multivitamin B important stimulates growth hair. Inhibition of DHT synthesis helps preventing hair loss and resolving baldness<sup>5</sup>.

Cayenne Fruit (*Capsicum annum* L.) with concentration extract a maximum of 0.5% have effectiveness for hair grower<sup>6</sup>. Cayenne fruit contains capsaicinoid (capsaicin and dihydrocapsaicin), carotenoids (capsanthin, alpha-carotene, violaxanthin and acid ester fat), flavonoids (isoflavones, apiiin and luteolin-7-O-glucoside), steroid saponins (capsicsidin) and oil Essential (2-methoxy-3-isobutyl pear Azin and N-(13-methyl tetradecylastamid / capsiamid)<sup>7,8</sup>. Allegedly capsaicin and isoflavones these acts to improve growth hair. Capsaicin activates vanilloids-1 receptor; thereby improve the release of CGRP

(*Calcitonin gene related peptide*) from nerve neuron, and CGRP increase production of IGF-I (*Insulin-like Growth Factor*). IGF-I has an important role in growth hair<sup>9,10</sup>.

## **MATERIALS AND METHODS**

### **Collection and identification of plant**

Collection ingredients plant fenugreek seed and cayenne fruit obtained from area of Solo, Central Java. Determination of plant fenugreek seed and cayenne fruit do in Bogor-based, field Botany, Center Research Biology LIPI, Cibinong. The samples were identified and deposited by Dr. Joeni Setijo Rahajoe, Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences. The voucher herbarium specimen number is 1476/IPH.1.01/If.07/VI/2016.

### **Making of the Extract Fenugreek Seed**

Fenugreek seed powder dissolved in 75 parts 70% ethanol for 2 days with a ratio of 1:10, stirred every 6 hours then filtered use flannel. Filtrate moved to vessel closed (filtrate A), then settled for one night. Residue soaked back use  $\frac{1}{4}$  part ethanol 70% during one day with stirred every 6 hours then filtered use flannel so obtained filtrate B. The filtrate A and B together then be settled for overnight, the filtrate was concentrated with *rotary evaporator* on temperature up to 50°C solvent yawn almost perfect, and extract thickened with way heated in on water bath until solvent yawn perfect. The yield obtained weighed and recorded.

### **Making of the Extract Cayenne Fruit**

Cayenne fruit powder dissolved in 75 parts 70% ethanol for 2 days with a ratio of 1:10, stirred every 6 hours then filtered use flannel. Filtrate moved to vessel closed (filtrate A), then settled for one night. Residue soaked back use  $\frac{1}{4}$  part ethanol 70% during one day with stirred every 6 hours then filtered use flannel so obtained filtrate B. The filtrate A and B together then be settled for overnight, the filtrate concentrated with *rotary evaporator* on temperature up to 50°C solvent yawn almost perfect, and extract thickened with way heated in on water bath until solvent yawn perfect. The yield obtained weighed and recorded.

## Capsaicin Assay<sup>11</sup>

### Preparation of standard solution of capsaicin

Accurately weighed about 10 mg of capsaicin standard was transferred to a 10 ml standard flask volumetric. It was dissolved in 2 mL of methanol and the contents of the flask were sonicated in an ultrasonic bath for 5 min for dissolution of capsaicin complete, the contents were then diluted up to the mark with methanol. Pipette 1 mL of solution was transferred to a 10 mL standard volumetric flask, the contents then diluted up to the mark with methanol.

### Preparation of the cayenne fruit solution

About 0.1 gram Fenugreek seed extract was accurately weighed and transferred to 25 mL stoppered conical flask. Then 10 mL of methanol was added to it and the flask was sonicated in an ultrasonic bath for 15 min. The contents were then diluted up to the mark with methanol. The solution was then finally filtered using nylon 0:45 µm filters (Millipore) before the analysis.

### Chromatographic conditions and Procedure

Capsaicin quantitative analysis was carried out by reversed phase HPLC method, with a C18 column and methanol-water (20:80) as mobile phase, flow rate 1,0 ml/minute and using a UV detector at 235 nm. Retention time and area under curve of each sample was recorded.

About 20.0mL of each sample of *capsicum annuum* solutions were injected into the chromatographic system. Than 20.0 mL of each standard of capsaicin solutions were injected into the chromatographic system. Record the chromatogram, measure the peak response of which the retention time is the same as the standard.

$$\% = \frac{L_u}{L_p} \times \frac{C_p \times V}{W} \times 100\%$$

Lu=sample peak area; Lp=standard peak area; Cp=standard concentration; V=sample volume (mL); W=weight (gram)

## Trigonelline Assay<sup>12</sup>

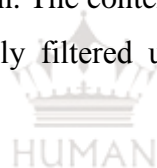
### Preparation of standard solution of trigonelline (1000 µg / mL)

Accurately weighed about 63.36 mg of trigonelline hydrochloride equivalent to 50.0 mg of trigonelline standard was transferred to a 50 mL standard volumetric flask. It was dissolved in 20 ml of methanol and the contents of the flask were sonicated in an ultrasonic bath for 5 min for complete dissolution of trigonelline. The contents were then diluted up to the mark with methanol to obtain a stock solution of trigonelline with a concentration of 1000.0 µg / mL.

Working standard solution was prepared by serial dilution of the standard stock solution.

### Preparation of the fenugreek seed solutions

About 0.1 g Fenugreek seed extract was accurately weighed and transferred to 50.0 mL stoppered conical flask. About 10.0 mL of methanol was added to it and the flask was sonicated in an ultrasonic bath for 15 min. The contents were then diluted up to the mark with methanol. The solution was then finally filtered using nylon 0.45 µm filters (Millipore) before the analysis.



### Chromatographic conditions and Procedure

Samples were eluted using the mobile phase of methanol: distilled water (95: 5, v / v), adjusted to pH 3.5 with hydrochloric acid and delivered at a flow rate of 1.0 ml/min. Detection was Carried out at 267 nm. About 20.0mL of each fenugreek seed solutions were injected into the chromatographic system. Then 20.0mL of each standard solutions of trigonelline with a concentration of 6.25µg/mL to 125µg/mL were injected into the chromatographic system. The peak areas were recorded for each injected concentration of trigonelline solution. The peak areas were recorded and the mean peak area, standard deviation (SD) and percent relative standard deviation (% R. SD) for each concentration of trigonelline was calculated.

**Test effectiveness of hair grower of ethanol extract combination fenugreek seed and cayenne fruit.**

Using rabbit on this research had the ethical approval by Health Research Ethics Committee, School of Medicine Sebelas Maret University, Dr. Moewardi General Hospital. The number of this approval is 316/IV/HREC/2016. Before given treatment rabbit adapted more one week ago so that no become stressed. Sample was applied each day 2 times morning and the afternoon with 2 drops on each part day first smearing considered day-to-0. Observation do for 28days, with take six strands hair rabbit every 7 days once, counted on the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and 28<sup>th</sup>. Hair taken with way cut straightened and pasted on tape then be measured with period sliding. On day to - 28 do shaving hair each area then weighed weight hair. The combinations are made as follows as shown in Table 1.

**Table 1: Combination of ethanol extract of fenugreek seed and cayenne fruit**

Sr. No.	Material	Concentration (%)		
		Combination 1	Combination 2	Combination 3
1	Fenugreek Seed	5	10	20
2	Cayenne Fruit	0.05	0.1	0.2

The combination of ethanol extract of fenugreek seeds and cayenne fruit is applied to the rabbit's skin with part of Region I is applied with a combination of 1; Region II is applied with a combination of 2; Region III is applied with a combination of 3; Region IV not applied (normal control); Region V is applied with minoxidil (positive control) and Region VI is applied with ethanol (negative control).

**RESULT AND DISCUSSION**

**Determination of plant and Extraction**

Identification of the seeds of fenugreek and cayenne fruit is done in Herbarium Bogoriense, Biology Research Center Indonesian Institute of Sciences (LIPI) Jl. Raya Jakarta-Bogor KM.46, Cibinong. The results pointed that the determination of plants are cayenne fruit (*Capsicum annum* L) and fenugreek seed (*Trigonella foenum-graceum* L).

Fenugreek Seed Extract and cayenne fruit extract obtained from maceration process using ethanol 70%. The subsequent concentration of the extract using *a rotary evaporator*. The

yield resulting from fenugreek seeds weighing 330 grams or 16.50% and cayenne fruit weighing 58.5 grams or 11.7%.

**Table 2: Extract have character organoleptic as following**

Sr. No.	Parameter	Fenugreek seed	Cayenne Fruit
1	Form	Thick	Thick
2	Color	Chocolate Black	Red
3	Flavors	Somewhat bitter	Spicy

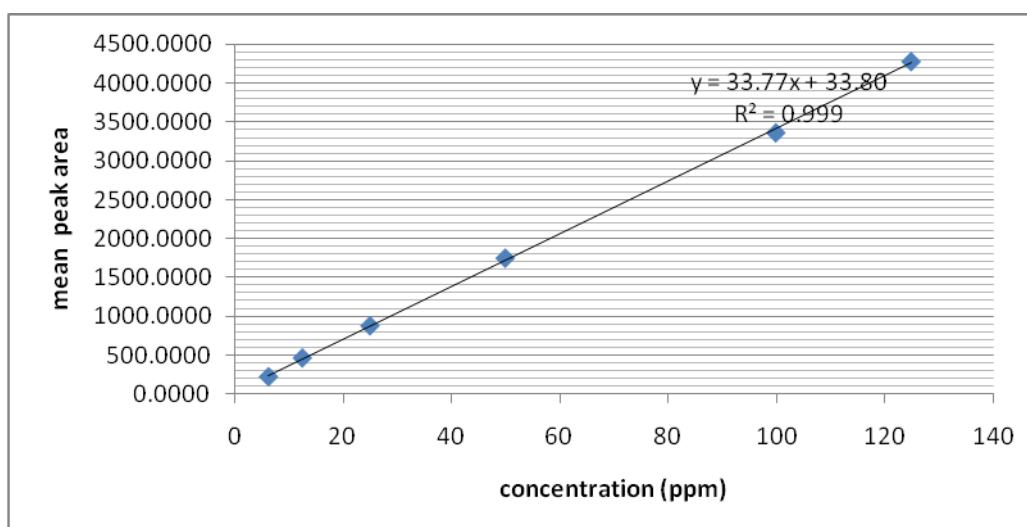
**Examination Identity Extract**

**Levels of Trigonelline**

Results Testing of HPLC was obtained levels of trigonelline is 2.05% and has meet requirements of the 2<sup>nd</sup> edition of Indonesia Medicinal Plant Extracts Monograph. According to the 2<sup>nd</sup> edition of Indonesia Medicinal Plant Extracts Monograph, trigonelline content is not less than 0.9%.

**Table 3: Regression Standard**

Sr. No	Concentration of trigonelline (ppm)	Mean Peak Area
1	6.25	224.7335
2	12.5	466.3575
3	25	880.5215
4	50	1751.0040
5	100	3364.2783



**Table 4: Trigonelline levels**

Sr. No.	Sample	Peak Area	Trigonelline levels (%)	Trigonelline Levels average (%)
1	Repeat one sample 820 ppm	575,391	1.96	2.05
2	Repeat two sample 820 ppm	626,866	2.14	

**Levels of capsaicin**

The results of testing HPLC are obtained levels of capsaicin at 0.41% and have meet requirements of Herbal Pharmacopoeia of Indonesia. According to Farmakaoepe Herbal Indonesia capsaicin content is not less than 0.29%.

**Table 5: Capsaicin levels**

Weight Sample (g)	Peak Area Sample	Peak Area Standards	Concentration of standard (ppm)	Volume of sample (ml)	Levels of capsaicin (%)
0.1062	755872	4351052	100	25	0.41

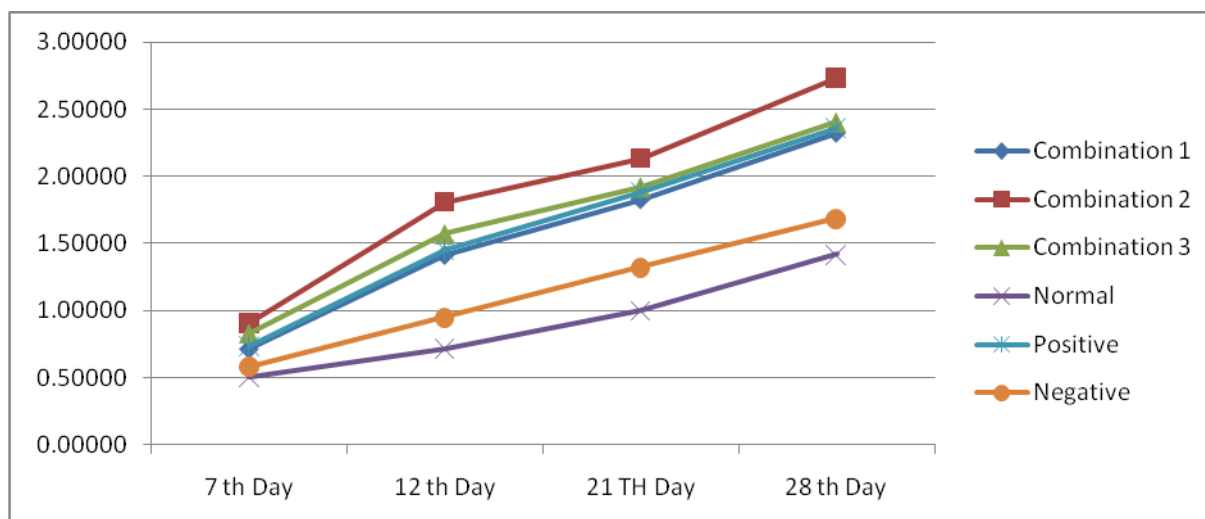
Capsaicin has action to improve growth hair. Capsaicin activates vanilloids-1 receptor, thereby improve the release of CGRP (*Calcitonin gene related peptide*) from nerve neuron, and CGRP increases production of IGF-I (*Insulin-like Growth Factor*). IGF-I has an important role in growing hair.<sup>1,6</sup>

**Test Activities of Hair Growth of Combination Ethanol Extract Cayenne Fruit and Fenugreek Seed**

**Table 6: Rabbit hair growth rate given a combination of ethanol extract cayenne fruit and fenugreek seed**

Treatment	Average length (cm) ± SD			
	Day 7	Day 14	Day 21	Day 28
Combination 1	0.711 ± 0.03	1,411 ± 0.40	1.825 ± 0.5	2,320 ± 0:35
Combination 2	0.903 ± 0.19	1809 ± 0.24	2134 ± 0.2	2,735 ± 0.21
Combination 3	0.826 ± 0.12	1,569 ± 0.22	1.919 ± 0.26	2,404 ± 0.28
Normal control	0.501 ± 0.05	0.713 ± 0.04	0.998 ± 0.17	1.419 ± 0.15
Positive control	0.735 ± 0.02	1,453 ± 0.46	1.885 ± 0.40	2.360 ± 0.37
Negative control	0.582 ± 0.07	0.949 ± 0:05	1321 ± 0.24	1.686 ± 0.32





**Figure No. 1: Graph rabbit hair growth rate by a combination of ethanol extract of cayenne fruit and fenugreek seeds from day 7 through day 28**

The average length of hair each week is processed statistically, which is on the 7<sup>th</sup> day with test *Shapiro-Wilk* showed that the average hair length is normally distributed and with test *Levene* show that average long hair homogeneous. Based on *ANOVA* test show a significant difference from the average of rabbit hair length by a combination of ethanol extract of cayenne fruit and fenugreek seeds and by *Mann Whitney* show there is a significant difference between control positive, combination extract all concentrations compared to normal controls and negative controls. It showed statistically that all combinations have activity growth hair which is better than normal and negative controls on day 7<sup>th</sup>.

On day 14<sup>th</sup> with test *Shapiro-Wilk* showed that the average hair length is not normally distributed and with test *Levene* showed that average long hair in homogeneous. Based on *Kruskal Wallis* test showed the significant difference from the average of rabbit hair length by a combination of extracts and by *Mann Whitney* showed there are significant differences between the combination of all concentrations compared to normal control and negative control. It showed statistically that all combination has an activity of growth hair which is better than normal and negative control on day 14<sup>th</sup>.

On day 21<sup>st</sup> with test *Shapiro-Wilk* showed that the average hair length normally distributed and with test *Levene* showed that average long homogeneous hair. Based on test *ANOVA* showed there is a significant difference from the average hair length rabbits given extracts combination and by *Mann Whitney* test there is a significant difference between combination

extract all concentrations were compared with normal controls and negative controls. This shows statistically that all combination having activity growth better hair than normal and negative control on day 21<sup>st</sup>.

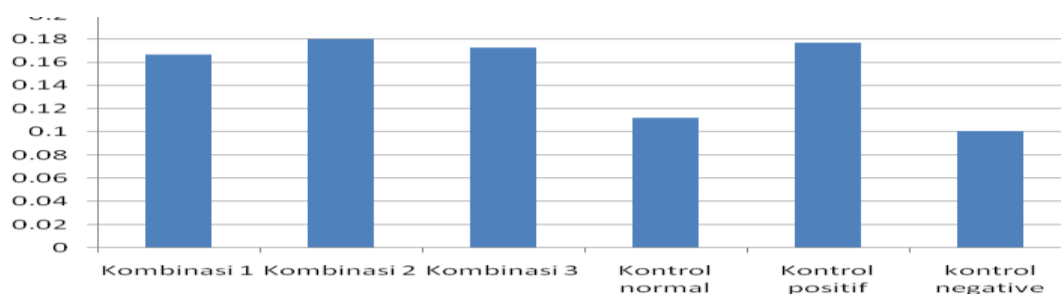
On day 28<sup>th</sup> with test *Shapiro-Wilk* showed that the average hair length normally distributed and with test *Levene* showed that average long hair homogeneous. Based on *anova* significant difference from the average of rabbit hair length by a combination of extracts and by *Mann Whitney* showed there are significant differences between the combination of the ethanol extract of cayenne fruit: fenugreek seed concentration of 0.05%: 5% (mean hair length 2.320 cm), a combination of ethanol extract of cayenne fruit: fenugreek seed concentration of 0.1%: 10% (mean hair length 2.735 cm) , a combination of ethanol extract of cayenne fruit: fenugreek seed concentration of 0.2%: 20% (mean hair length 2.404 cm) compared with normal controls and negative controls. Statistics showed a combination of 0.1% cayenne fruit extract: 10% fenugreek seeds extract have a better activity than its growth combination extract of 0.05% cayenne fruit extract: 5% fenugreek seeds extract but no difference with combination extract 0.2% cayenne fruit extract: 20% fenugreek seeds extract.

### Hair Weight



**Table 7: Hair weight of the 28<sup>th</sup> day of rabbit that was given a combination extract**

Sr. No.	Rabbit	Weight Hair (gram)					
		Combination 1	Combination 2	Combination 3	Normal control	Control positive	Control negative
1	1	0.1672	0.1747	0.1717	0.1056	0.1619	0.0988
2	2	0.1617	0.1782	0.1762	0.1045	0.1784	0.0998
3	3	0.1682	0.1786	0.1792	0.1187	0.1762	0.1018
4	4	0.1687	0.1872	0.1618	0.1198	0.1898	0.1023
	Average	0.1665	0.1797	0.1722	0.1122	0.1766	0.1007



**Figure No 2: Weight chart rabbit hair day 28<sup>th</sup> were given a combination extracts**

Hair weight of day-to-28 is statistically processed, which is on the 28<sup>th</sup> day with test *Shapiro-Wilk* showed that the hair weight is normally distributed and with test *Levene* show that weight hair in homogeneous. Based on test *Anova* there was a significant difference from the weight of rabbit hair given the combination of ethanol extract of cayenne fruit: fenugreek seed and based on *Mann Whitney* test there was a significant difference between the combination of 0.05% cayenne fruit ethanol extract:5% fenugreek seed ethanol extract (hair weight 0.1665), a combination of 0.1% cayenne fruit ethanol extract: 10% fenugreek seeds ethanol extract (average weight of hair 2.735 cm), a combination of 0.2% cayenne fruit ethanol extract:20% fenugreek seed ethanol extract (average hair weight 0.1722) compared with normal control and negative control. This shows the combination of 0.1% cayenne fruit ethanol extract: 10% fenugreek seeds ethanol extract has a large mean weight of hair.

## CONCLUSION

In the present study the observation of 28 days, the combination of the ethanol extract of cayenne fruit and fenugreek seed has hair growth activity; with the best combination of 2 which had an average hair length of the total 2.735 cm and average hair weight 0.1722 gram.

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