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# Study of Bitter Leaf Beer Formulations and Bitter Hops Substitution

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## ABSTRACT

The objective of this research was to study the possibility of making good quality craft beer by using *Vernonia Amygdalina* to substitute bitter hops. According to its bitter taste, it was fondly called as Bitter leaf. Anti-nutritional phytochemicals within the plants are responsible for the bitter taste. Bioactive compounds such as alkaloids, saponins, terpenes, flavonoids, phenolic acids, steroids, coumarins, and etc., are responsible for its ethnobotanical uses. From previous researches, Bitter Leaf was reported as a well-known as medical plant with several uses. Therefore, in this study, Bitter Leaf was used as one of the supplement ingredients in our brewing. In this research, final Bitter Leaf beer formulation has been chosen from consumer preference done by sensory evaluation. There are many differences varieties of bitter herbs used to substitute bitter hops. Since the anti-nutritional properties and phytochemical compounds which are functioning as anti-microbial and anti-bacterial agents. Both are the most important properties that could be use instead of bitter hops. However, one last important property that Bitter Leaf could not be substituting bitter hops completely is the foam stabilization. In conclusion, Bitter Leaf could be substituted bitter hops since its characteristic and properties are similar to bitter hops. By the way of contrast, the flavor of beer might be

different from commercial beers which brewed by using bitter hops. To guarantee the beer's shelf life, a modern preservation is needed.

**Keywords:** *Vernonia amygdalina*, Bitter Leaf, Substitution, Bitter hops, Bioactive compounds

## INTRODUCTION

In this era of globalization, the trend of studying herbs are popular since various types of herbs are providing different benefits such as medicinal and supplemental purposes. Medical benefits that are provided from herbs to human health could prevent and treat various diseases. Due to health concerned, people trend to live their life healthier by focusing on the consumption of healthy food such as vegetables, fruits or other nutritional food especially herbs which are promoting good health to them. Therefore, the world came up with the discoveries and studies of herbs that are able to offer lots of surprising health benefits of human. According to the studies, the most famous one is *Vernonia amygdalina*, or known as Bitter Leaf, and "Nan Chao Woei" in Thai language which was adapted from Chinese language. [1]

*V. amygdalina* was known as Bitter Leaf since its bitterness, and under the specification of Vernonia in short. *V. amygdalina* is a kind of herbs under the Kingdom of Platae, order of Asterales, under the family of Asteraceae, tribe of Vernonia, and genus of Vernonia. *V. amygdalina* is originated from African countries [2][3]. Previous researches reported that *V. amygdalina* is well known as a medical plant with several uses, including in treating diabetes and fever reduction, etc. [2][4].

The attributes from *V. amygdalina* to human health in medicinal properties could provide health benefits to consumer [2]. Moreover, its bitter taste could go well with the taste of beer. Due to the hops are costly and unsustainable, using *V. amygdalina* could be used for the studies of substituting bitter hops. Its characteristics are similar to bitter hops, and it will be used as a supplement ingredient in the brewing. In expectation, the beverage after formulated will be a kind of herbs beer which providing health benefits to consumer and reducing the usage of bitter hops in the same time.

# MATERIALS AND METHODS

## Materials

*V. amygdalina* or Bitter Leaf was purchased from a Chinese Pharmacy in Bangkok Province, Thailand. The type of malt barley grain used was Pale Ale Malt from Weyermann®, yeast (Saccharomyces cerevisiae) from Red Star® and hops (Cascade) with 8.1% of alpha acid from Brew-By-Me.

## Brewing processes

3 liters of water were boiled, milled malts or malt extracts were added to the boiled solution and adjusts the ºBrix until 12 ºBrix. Then, malting process was started by heating the solution at 40 ºC for 40 minutes. Bitter hops (Cascade) was added and the solution was heated up to 75 ºC for 20 minutes. After boiling for an hour, stop heating and aroma hops (Cascade) was added. Whirlpool was done to let the residual of hops formed a cone in the middle of wort. The wort was transferred into a sterile tank and cooled down to room temperature. Weighed amount of Bitter Leaves were added then followed by activated yeast. In this period, the fermentation was started. The value of ºBrix was checked everyday until it was constant. The filtration was done, and filtered beer has been carbonated and stored in cold room.

## Beer Formulations

The experiment has been separated into three formulations by varying the amount of Bitter Leaf, bitter hops and aroma hops to improve the formulation.

**Table 1.** First batch beer formulation used for primary consumer's sensory evaluation.

| Formula | Ingredients | | | | | |
|---|---|---|---|---|---|---|
| | Water (L) | Bitter Leaf (g) | Malt Extract | Bitter Hops (g) | Aroma Hops (g) | Yeast (g) |
| Control | 3 | - | Until 12 ºBrix | 5 | 5 | 3 |
| 1 | 3 | 1 | '' | 5 | 5 | 3 |
| 2 | 3 | 2 | '' | 5 | 5 | 3 |
| 3 | 3 | 3 | '' | 5 | 5 | 3 |
| 4 | 3 | 4 | '' | 5 | 5 | 3 |

Remarks: ": Adjusting the ºBrix with malt extracts until 12 ºBrix; -: none of Bitter Leaf added.

From the consumer's sensory evaluation, the results were used to improve the formulation in second batch of formulation. The bitterness provided from bitter hops were reduced from 5 g to 4 g. The amount of Bitter Leaf used in this formulation was varied into 1, 2, and 3g.

**Table 2.** Second batch of beer formulation improved from primary consumer's sensory evaluation.

| Formula | Ingredients | | | | | |
|---------|-------------|--|--|--|--|--|
| | Water (L) | Bitter Leaf (g) | Malt Extract | Bitter Hops (g) | Aroma Hops (g) | Yeast (g) |
| Control | 3 | - | Until 12 ºBrix | 4 | 5 | 3 |
| 1 | 3 | 1 | " | 4 | 5 | 3 |
| 2 | 3 | 2 | " | 4 | 5 | 3 |
| 3 | 3 | 3 | " | 4 | 5 | 3 |

Remarks: ": Adjusting the ºBrix with malt extracts until 12 ºBrix; -: none of Bitter Leaf added.

The amount of bitter hops used in third formulation was reduced from 4 g to 3 g. The variation of Bitter Leaves was 3 g and 4 g and aroma hops was reduced from 5 g to 4 g. In this batch, formulation was varied into two different batches which brewed by using malt extracts and milled malt grains.

**Table 3.** Beer formulation brewed by malt extracts.

| Formula | Ingredients | | | | | |
|---------|-------------|--|--|--|--|--|
| | Water (L) | Bitter Leaf (g) | Malt Extract | Bitter Hops (g) | Aroma Hops (g) | Yeast (g) |
| Control | 3 | - | Until 12 ºBrix | 3 | 4 | 3 |

Remark: -: none of Bitter Leaf added.

**Table 3**. Beer formulation brewed by malt extracts. (Cont.)

| Formula | Ingredients | | | | | |
|---------|-------------|------------|-------------|-------------|------------|-----------|
| | Water (L) | Bitter Leaf (g) | Malt Extract | Bitter Hops (g) | Aroma Hops (g) | Yeast (g) |
| 1 | 3 | 1 | Until 12 ºBrix | 3 | 4 | 3 |
| 2 | 3 | 2 | ʻʼ | 3 | 4 | 3 |

Remarks: ʻʼ: Adjusting the ºBrix with malt extracts until 12 ºBrix;
   -: none of Bitter Leaf added.

**Table 4**. Beer formulation brewed by using milled malt.

| Formula | Ingredients | | | | | |
|---------|-------------|------------|-------------|-------------|------------|-----------|
| | Water (L) | Bitter Leaf (g) | Malt Extract | Bitter Hops (g) | Aroma Hops (g) | Yeast (g) |
| Control | 3 | - | Until 12 ºBrix | 3 | 4 | 3 |
| 1 | 3 | 1 | ʻʼ | 3 | 4 | 3 |
| 2 | 3 | 2 | ʻʼ | 3 | 4 | 3 |

Remarks: ʻʼ: Adjusting the ºBrix with malt extracts until 12 ºBrix;
   -: none of Bitter Leaf added.

The fourth formulation was varied by cut and smashed Bitter Leaf and brewed by using milled malt.

**Table 5**. Beer formulation for the formulation using cut Bitter Leaf.

| Formula | Ingredients | | | | | |
|---------|-------------|------------|-------------|-------------|------------|-----------|
| | Water (L) | Bitter Leaf (g) | Malt Extract | Bitter Hops (g) | Aroma Hops (g) | Yeast (g) |
| 1 | 3 | 1 | Until 12 ºBrix | 3 | 4 | 3 |

**Table 6**. Beer formulation for the formulation using smashed Bitter Leaf.

| Formula | Ingredients | | | | | |
|---------|-------------|---------------------|---------------|----------------------|--------------------|-----------|
| | Water (L) | Bitter Leaf (g) | Malt Extract | Bitter Hops (g) | Aroma Hops (g) | Yeast (g) |
| 1 | 3 | 1 | Until 12 ºBrix | 3 | 4 | 3 |

## Sensory analysis

Sensory tests were done to obtain the final formulation of the beer. Samples used for sensory tests were the formulation 1 from the third batch which brewed by milled malt and formulation using smashed Bitter Leaf brewed by milled malt from fourth formulation. 9-point Hedonic Scale sensory evaluation was used and the attributes evaluated were foam stability, colour, clarity, aroma, bitterness, alcohol content, complexity, after taste and overall impression. The sensory analysis was done by 10 trained panelists.

## Consumer acceptance test

Consumer acceptance test was carried out to determine the acceptability of consumer towards the final formulation product. The final product was evaluated by 10 trained panelists.

## RESULTS AND DISCUSSIONS

**The study of beer formulation of Bitter Leaf Beer**

According to table 1., 2., 3., 4., 5., 6., there were 4 batches of formulation showed. The improvement of formulations was adjusted according to the results obtained from consumer's sensory evaluation. The improvement was adjusting by varying amount of Bitter Leaf, bitter hops and aroma hops used. First and second batches were preliminary test only. The formulation from third and fourth batches were used for sensory test. The best sensory test samples have been repeated and tested by using 9-Point Hedonic Scale.

**Table 7**. The results of sensory evaluation for beer brewed by malt extract.

| Attributes | Average scores ± SD | | |
|---|---|---|---|
| | Control | 1 | 2 |
| Foam stability | 5.4 ± 0.55[b] | 5.4 ± 0.55[a] | 5.2 ± 0.84[b] |
| Color | 5.6 ± 0.55[b] | 5.6 ± 1.14[a] | 5.0 ± 1.22[c] |
| Clarity | 5.6 ± 1.14[bc] | 5.6 ± 0.55[a] | 4.8 ± 0.84[c] |
| Aroma | 5.4 ± 0.55[cd] | 4.4 ± 2.30[a] | 5.2 ± 0.45[cd] |
| Complexity | 4.4 ± 0.55[b] | 3.8 ± 2.59[a] | 4.8 ± 0.84[b] |
| Bitterness | 4.2 ± 1.64[b] | 5.2 ± 1.48[a] | 5.2 ± 0.84[b] |
| After Taste | 3.4 ± 1.52[b] | 4.4 ± 2.51[a] | 4.4 ± 1.14[b] |
| Alcohol content | 5.4 ± 1.14[bc] | 5.2 ± 1.64[a] | 5.0 ± 0.71[c] |
| Overall liking | 5.2 ± 1.30[c] | 4.8 ± 0.84[ab] | 4.6 ± 0.55[cb] |

**Remark**: mean ± SD, Means with the same letter are not significantly different ($p \leq 0.05$)

**Table 8**. The results of sensory evaluation for beer brewed by malt extract.

| Attributes | Average scores ± SD | | |
|---|---|---|---|
| | Control | 1 | 2 |
| Foam stability | 6.4 ± 0.55[a] | 6.4 ± 0.55[b] | 6.6 ± 0.89[a] |
| Color | 7.0 ± 0.71[a] | 7.0 ± 0.00[bc] | 6.6 ± 0.89[ab] |
| Clarity | 6.8 ± 0.84[a] | 7.2 ± 0.45[bc] | 6.4 ± 1.14[ab] |
| Aroma | 8.4 ± 0.55[ab] | 7.6 ± 0.89[d] | 6.6 ± 1.14[bc] |
| Complexity | 7.6 ± 1.14[a] | 7.4 ± 0.55[b] | 7.6 ± 0.89[a] |
| Bitterness | 7.8 ± 0.84[a] | 7.2 ± 1.09[b] | 7.4 ± 0.89[a] |
| After Taste | 6.8 ± 0.89[a] | 7.4 ± 0.89[b] | 7.8 ± 0.45[a] |
| Alcohol content | 7.6 ± 0.89[a] | 7.6 ± 0.55[c] | 6.2 ± 0.84[b] |
| Overall liking | 7.0 ± 0.00[a] | 7.6 ± 0.55[c] | 6.2 ± 0.84[b] |

**Remark**: mean ± SD, Means with the same letter are not significantly different ($p \leq 0.05$)

**Table 9.** The results of sensory evaluation of beer formulated in fourth batch.

| Attributes | Average scores ± SD | |
|---|---|---|
| | **1** | **2** |
| Foam stability [ns] | 6.0 ± 1.15 | 5.2 ± 1.93 |
| Color [ns] | 6.4 ± 1.35 | 6.5 ± 0.97 |
| Clarity [ns] | 5.3 ± 2.11 | 5.5 ± 1.58 |
| Aroma [ns] | 5.1 ± 2.68 | 5.7 ± 1.95 |
| Complexity [ns] | 5.8 ± 1.87 | 6.2 ± 1.39 |
| Bitterness [ns] | 4.0 ± 2.40 | 6.2 ± 1.39 |
| After Taste[*] | 4.8 ± 1.87 | 6.5 ± 1.08 |
| Alcohol content [ns] | 4.9 ± 2.18 | 5.5 ± 1.43 |
| Overall liking[*] | 6.0 ± 1.05 | 7.0 ± 0.82 |

**Remark:** 1: Formulation brewed by using cut Bitter Leaf; 2: Formulation brewed by using smashed Bitter Leaf; attributes with * means the two mean are significantly different ($p \leq 0.05$), ns means the two mean are not significant different ($p \geq 0.05$).

**Results of sensory evaluation of Bitter Leaf beer formulation**

There were two batches of formulation which classified by beer brewed using cut Bitter Leaf and smashed Bitter Leaf. According to the results, the formulation of using cut Bitter Leaf would contain less aroma compared to smashed Bitter Leaf. Therefore, this experiment was studied to find out the appropriate formulation for making Bitter Leaf beer before studying the possibility of bitter hops substitution.
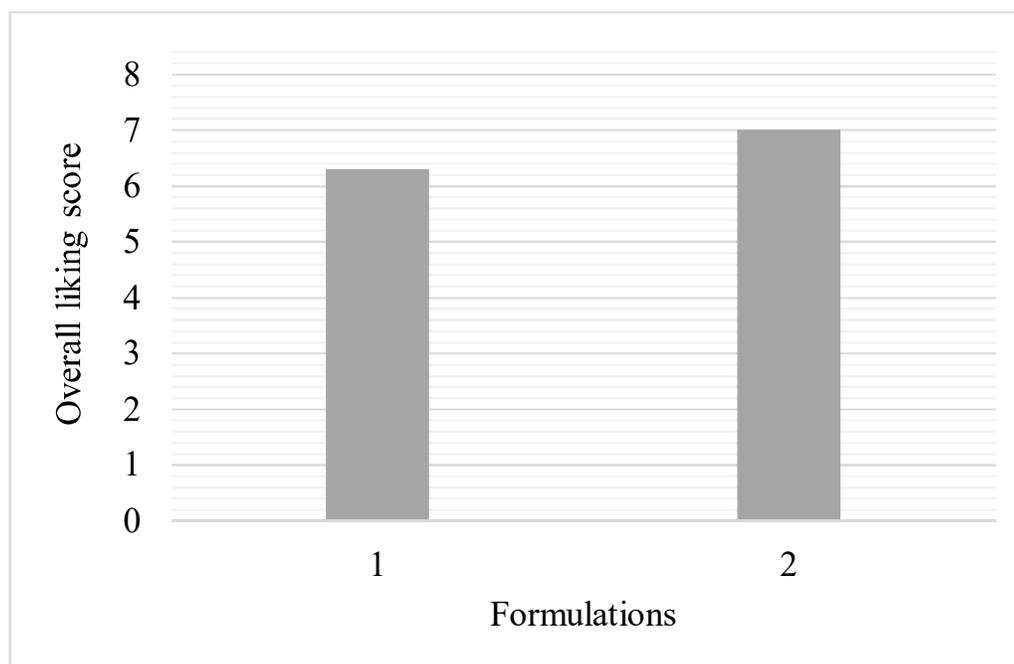
**Figure 1.** The sensory test chart for overall impression of two formulations.

According to the result obtained from figure 1., the formulation 2 which brewed by using smashed Bitter Leaf with milled malt obtained the highest score of overall impression which was 7.0. The formulation was brewed by using 1 g of Bitter Leaf. From sensory evaluation, small amount of Bitter Leaf used in brewing provided a better taste of aroma of beer compared to the formulation which used more than 1 g of Bitter Leaf.

**Table 10.** Mean scores of each attributes and overall impression done by 10

trained panelists.

| Attributes | Mean Score |
| --- | --- |
| Foam stability | 5.2 |
| Colour | 6.5 |
| Clarity | 5.5 |
| Aroma | 5.7 |
| Complexity | 6.2 |
| Bitterness | 6.2 |
| After taste | 6.5 |
| Alcohol content | 5.5 |
| Overall impression | 7.0 |

From the results showed in table 10., trained panelists were preferred the formulation of beer brewed by using smashed Bitter Leaf. Thus, the formulation brewed by smashed Bitter Leaf was used as final beer formulation. The overall impression of consumers toward the develop beer product obtained a mean of 7.0 which was moderately good score.

**Bitter hops substitution by using Bitter Leaf**

According to previous researches, *V. amygdalina* contained bioactive compounds such as tannins, flavonoids, alkaloids, and phenolic compound [5]. Due to its characteristic and bitter taste, its flavor is suitable for substituting hops. Bitter taste from *V. amygdalina* could be used for hops substitution but the amount of leaves added need to be in an appropriate amount to prevent the over-bitterness and off-flavor of beer. In this research, the beer was brewed by using both *V. amygdalina* and hops. According to the formulation, the bitterness provided from the addition of Bitter Leaf could reduce the amount of hops used. The anti-microbial properties of *V. amygdalina* could improve the shelf life of beer as hops did. Therefore, to substitute the hops by using *V. amygdalina* is possible but the flavor might be different, and the sterilization steps need to be done very well since hops is an important ingredient which

conducted to the shelf life and stability of the beer. The foam stability of the beer still needs to obtain from hops.

## CONCLUSION

In conclusion, the beer formulation using *V. amygdalina* (Bitter Leaf) as an supplement ingredient was successfully developed. From the results obtained from sensory tests of beer brewed by using smashed Bitter Leaf has been chosen as the best formula with the best aroma and taste. The scores evaluated by using 9-Point Hedonic Scale was ran by SAS Program and the results showed the significant different than others. The results of attributes obtained by 10 trained panelists for sensory evaluation were included foam stability (5.2 ± 1.93), colour (6.5 ± 0.97), clarity (5.5±1.58), aroma (5.7 ± 1.95), bitterness (6.2 ± 1.39), alcohol content (5.5 ± 1.43), complexity (6.2 ± 1.39), after taste (6.5 ± 1.08), overall liking (7.0 ± 1.05). The mean score obtained was 7.0 which was greater than other formulations. The uses of *V. amygdalina* could be used for hops substituition since the properties and characteristic which are similar to hops. By the way of contrast, the flavor of beer might be different from commercial beers which brewed by using bitter hops.

## ACKNOWLEDGEMENTS

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