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# Phytochemical test on herbal drinks loloh cemcem at Penglipuran Village, Bali

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**Abstract.** Loloh is a traditional Balinese drink that is believed to be able to overcome certain diseases. The Balinese people make loloh independently for self-consumption or trading in the small-scale home industry. The making of loloh is usually passed down from generation to generation, but until now has not been standardized. Loloh cemcem is one of the traditional drinks from The Penglipuran Village, Bangli Regency, Bali. This study aims to conduct phytochemical tests on loloh cemcem in Bali, especially from Penglipuran Village. Phytochemical tests include levels of alkaloids, steroids, terpenoids, phenolics, saponins, flavonoids, steroid, and tannins. The results of this study indicate that loloh cemcem produced in Penglipuran Village contains flavonoids, terpenoids, tannins, phenols, and alkaloids. But do not contain saponins and steroids. Loloh cemcem has the potential to be an antibacterial, antioxidant and another benefit that still needs further investigation.

## 1. Introduction

Loloh is a traditional Balinese drink that is believed to be able to overcome certain diseases [1]. Various kinds of herbal plants that are effective in treating diseases in Bali are listed in Lontar Usadha. There are around 50,000 Lontar Usadha which record various traditional medical techniques available in Bali [2].

The Balinese people make loloh independently to be consumed alone or traded in a small-scale home industry. Loloh is commonly used to prevent a disease or treat diseases. The making of loloh is usually passed down from generation to generation. Loloh that has been circulating so far has not been standardized and mixed with various ingredients. Some examples of loloh in Bali include: loloh cemcem, loloh sembung, loloh tempuyung, and loloh cinnamon. In general, loloh is made using extracts from plants, namely the roots, stems, bark, seeds, flowers, fruits and leaves [2,3].

Loloh cemcem or kecemcem is one of the traditional drinks from Penglipuran village, Bangli Regency which is made from cemcem leaves, blended with coconut water and some other ingredients [4]. Cemcem leaves with the Latin name *Spondias pinnata* (Linn. f.) Kurz (Family – Anacardiaceae) is a deciduous tree distributed in India, Sri Lanka, and South-East Asian countries [5].

From the observations of the researchers, Loloh Cemcem Penglipuran has been widely sold in shops and supermarkets in the areas of Denpasar, Badung, and Gianyar. This drink believed by the community to treat diabetes, heartburn, and urolithiasis [6] Until now, very little research has proven the effectiveness of the loloh and its active ingredients. Sujarwo and his friends have analyzed the antioxidant effects of cemcem leaves and found that the methanol extract of the leaves has a high antioxidant capacity and polyphenol content [7].



This study aims to determine the chemical composition of loloh cemcem in Bali, especially from the village of Penglipuran. Thus, the data of this study will be used for the next study which tested the effectiveness of loloh cemcem in certain diseases.

## 2. Method

Sample selection was done purposively. The sample of this research was loloh cemcem from Penglipuran Village, Bali. The phytochemical test was carried out on the loloh cemcem. This research has obtained research permission by Komite Etik Penelitian (KEP) Fakultas Kedokteran Universitas Udayana/Rumah Sakit Umum Pusat Sanglah Denpasar.

### 2.1. Identification of alkaloids (*calvenor fitzgerald method*)

A total of  $1 \times 10^5$   $\mu\text{g}$  of loloh cemcem was dissolved with  $5 \times 10^3$   $\mu\text{L}$  chloroform and  $5 \times 10^3$   $\mu\text{L}$  ammonia and the results were divided into two tubes. The first tube was added with 10 drops of sulfuric acid ( $\text{H}_2\text{SO}_4$ ) 2 M. The acid layer was separated, divided into 2 test tubes and each tube was tested using Mayer reagent. A positive (+) alkaloid for white deposits.

### 2.2. Saponin test

Several drops of loloh cemcem were dissolved in hot water as much as  $15 \times 10^3$   $\mu\text{L}$  then heated for 5 minutes. Then filtered and the filtrate was taken as much as  $10 \times 10^3$   $\mu\text{L}$  and put in a test tube. The solution is then beaten. A positive test for the presence of saponins in the solution is characterized by the formation of foam.

### 2.3. Tannin test

A few drops of loloh cemcem are placed in a tube and added with  $\text{FeCl}_3$ , a green to purple color indicates tannin compounds.

### 2.4. Phenolic test

A few drops of loloh cemcem are placed in a tube and added with iron (III) chloride, a green to purple color indicates phenolic compounds.

### 2.5. Flavonoid test

One drop of loloh cemcem was dissolved in  $10 \times 10^3$   $\mu\text{L}$  methanol then divided into four test tubes. Then added NaOH, concentrated  $\text{H}_2\text{SO}_4$ , and concentrated Mg-HCl powder. If there is a change in red color, it will positively contain flavonoids.

### 2.6. Steroid test

One drop of loloh cemcem added  $2 \times 10^3$   $\mu\text{L}$  chloroform then added 5 drops of 6 M.  $\text{H}_2\text{SO}_4$  added. A positive test for steroids in the solution by changing the color of the solution to blue or green.

### 2.7. Triterpenoid test

For the terpenoid test, one drop of loloh cemcem was added  $20 \times 10^3$   $\mu\text{L}$  ethanol,  $2 \times 10^3$   $\mu\text{L}$  chloroform, and  $3 \times 10^3$   $\mu\text{L}$  concentrated  $\text{H}_2\text{SO}_4$ . A positive test for a terpenoid is indicated by the change in color of the solution to red.

## 3. Result

### 3.1. The raw material and process of making loloh cemcem

Loloh cemcem in this study was a pure loloh cemcem (without a mixture of other ingredients) and loloh cemcem with a mixture of several additional ingredients. Loloh cemcem which has been marketed not in pure loloh form but which contains various ingredients. The loloh cemcem raw material consists of: one gallon of water that has been processed with reverse osmosis and ozonization, one kg of cemcem

leaves (up to 7 stems from the stems), one kg of brown sugar, three cayenne pepper seeds, five grams of salt, one young coconut meat, and ½ kg of tamarind.

The manufacturing process begins by joking cemcem leaves with clean water and then grinding it with the machine until it breaks. Next, the cemcem leaves are mixed with other ingredients using gloves, finally given a piece of young coconut meat and put into a bottle. On average, from the preparation of materials to packaging, it takes about 1 hour.

Loloh cemcem does not use preservatives, because that loloh can survive in room temperature for 1 day. If stored in a refrigerator temperature it can last 4-5 days, whereas if frozen it can last up to 4 months. The production of loloh cemcem depends on the availability of raw materials for cemcem leaves. A small portion of raw material is taken from the garden itself and most of the plantations around the villages in Penglipuran. This is because if the cemcem leaves are brought in outside the village, it creates a different aroma and taste. In the range of October to June the tendency of cemcem trees in the region to be thick leafy so that the production of loloh cemcem increased in that period, whereas in the range from July to September the tendency of leaves on the cemcem trees in the region fell so that the production of loloh cemcem declined in that period.



**Figure 1.** Cemcem tree from Penglipuran Village.

### 3.2. Phytochemical test results

The phytochemical test found that loloh cemcem contains a large number of flavonoids and terpenoids besides the content of tannins, phenols, and alkaloids. This results from loloh cemcem that containing a mixture of cayenne pepper seeds, salt, coconut meat, and tamarind. Loloh cemcem with a mixture of some of these ingredients is known do not contain steroids, but pure loloh cemcem apparently contains steroids. Both this sample also do not contain saponin (Table 1 and Table 2).

**Table 1.** Phytochemical analysis loloh cemcem with a mixture of various kinds of ingredients.

| Type of examination | Result | Unit           |
|---------------------|--------|----------------|
| Alkaloids           | +      | White deposits |
| Steroids            | -      | Green Color    |
| Terpenoid           | ++     | Reddish Color  |
| Phenolic            | +      | Green Color    |
| Saponin             | -      | Foam           |
| Flavonoid           | ++     | Reddish Color  |
| Tanin               | +      | Bluish Color   |

+: gives a sediment/color that is sufficient

++: gives a sediment/medium color

+++: gives a large amount of color/prominent

**Table 2.** Phytochemical analysis pure loloh cemcem (without any ingredients).

| Type of examination | Result | Unit           |
|---------------------|--------|----------------|
| Alkaloids           | ++     | White deposits |
| Steroids            | ++     | Green Color    |
| Terpenoid           | +      | Reddish Color  |
| Phenolic            | ++     | Green Color    |
| Saponin             | -      | Foam           |
| Flavonoid           | +      | Reddish Color  |
| Tanin               | ++     | Bluish Color   |

+: gives a sediment/color that is sufficient

++: gives a sediment/medium color

+++: gives a large amount of color/prominent

#### 4. Discussion

Cemcem leaves (*Spondias pinnata* (L.f) Kurz) are included in the genus *Spondias*. Plants included in this genus contain tannins, flavonoids, steroids, triterpenes, saponins, essential oils, amino acids, and polysaccharides [8]. Members of this genus are widely used in traditional medicine to treat various diseases such as abdominal pain, dysentery, diarrhea, anemia, diabetes, dementia, and various infections. People believe that stems, fruits, and leaves of *Spondias pinnata* are useful as traditional medicine, such as the leaves and the fruit can be used as cough medicine and the bark part can be used as a diarrhea medicine [9].

Whereas in Bali, loloh cemcem is believed by the community to cure heartburn, chapped lips, facilitate digestion (bowel movements), increase appetite, to treat diabetes, and urolithiasis [6,10]. Pharmacological studies on different species of *Spondias* show that this plant has a cytotoxic, antioxidant, anthelmintic, antipyretic, analgesic, thrombolytic, hepatoprotective, antimicrobial, antihypertensive, and various other effects [8].

The phytochemical test found that loloh cemcem contains several phytochemical compounds that have an effect on the human body. This study found a large number of flavonoids and terpenoids besides the content of tannins, phenols, and alkaloids from loloh cemcem containing a mixture of other ingredients. While, pure loloh cemcem contains more alkaloids, steroids, phenolics, and tannins. Loloh cemcem with a mixture of some of these ingredients is known to contain no steroids but pure loloh cemcem apparently contains steroids. The difference in results in these samples shows that the produced loloh is not standardized so that the levels of the active ingredients vary. This difference will have an impact on the quality and efficacy of the loloh.

Regardless of the difference in levels in the sample, another study also found the same results that cemcem leaves contains phenol, flavonoids, tannins, alkaloids, and terpenoids that can inhibit the bacterial growth [11] Ariati in 2012 found that cemcem leaf extract had antibacterial potential through analysis of GC-MS spectrophotometry method [10]. Cemcem leaf extract with a concentration of 40% can inhibit the growth of *E. coli* bacteria ATCC 8739 may serve as a potential antibacterial agent [12]. Beside that ethanol extract *S. pinnata* leaf displayed the highest total phenolic content (27.76±1.11 mg GAE/g extract) also show have a potential source of natural antioxidant [13]. This antibacterial effect is sourced from the flavonoid content on loloh cemcem. Flavonoids are well known as antibacterial agents against a wide range of pathogenic microorganism [14].

Cemcem extract also reported potentially developed to an anti-tuberculosis constituent and have ability as anticancer [15,16]. To be able to have an anticancer effect, the active component on loloh cemcem must have the ability to prevent expansion and clonal selection in the human body or inhibit the differentiation of cancer stem cell (CSC) which is known to be the basic for the development of cancer in the body [17]. Loloh cemcem produced in Penglipuran Village contains a mixture of cayenne pepper. Cayenne pepper is known to contain vitamin E, vitamin C and carotenoids (precursor of vitamin A) which functions to regulate various cellular functions such as proliferation, differentiation and cell

death in various cell types. So it is widely used to deal with various health conditions such as poor circulation, weak digestion, heart disease, chronic pain, sore throat, and headaches. Cayenne pepper also known for its cancer fighting property [18,19]. However, its consumption must be avoided in kidney disorders because in large quantities can damage the kidneys so that it can aggravate fibrosis that occurs in the glomerulus and the kidney tubules [20-22]. For this reason, it is necessary to further examine the types of alkaloids, terpenoids, and flavonoids contained in loloh cemcem so that benefits can be used optimally.

## 5. Conclusion

Loloh cemcem produced in Penglipuran Village, Bali contains flavonoids, terpenoids, tannins, phenols, and alkaloids. But do not contain saponins and steroids. Loloh cemcem has the potential to be an antibacterial, antioxidant and another benefit that still needs further investigation.

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