

Preliminary Phitochemical Analysis of Guava Leaves (*Psidium guajava L.*) as Antidiarrheal in Calves

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Abstract. Guava plants (*Psidium guajava L.*) are fruit plants originating from South America and can thrive in the territory of Indonesia. Now the biggest center for guava cultivation is spread in DKI Jakarta, West Java, Central Java, East Java, Yogyakarta, Bali, West Nusa Tenggara, Sumatra and Kalimantan. In addition to taking the fruit, guava leaves are also used as a traditional medicine for the treatment of diarrhea in human and animals. This study aims to identify active compounds in guava leaves. There are several secondary metabolites with pharmacological effects as anti diarrhea such as flavonoid, alkaloid, tannins and essential oils. The leaves sample used in this study were taken from farmers in the West Denpasar area, Bali. Extraction of guava leaf leaves was done using 96% ethanol, and phytochemical analysis was carried out to detect the presence of active compounds. The data obtained is presented and analyzed descriptively qualitatively. The results showed that the ethanol extract of guava leaves contained active compound included steroid, triterpenoid, phenolics, alkaloids, flavonoids, saponins and tannins. It can be concluded that guava leaf extract contains active compounds that are potential antidiarrheal.

Keywords: antidiarrheal, guava leaves extract, phytochemical

I. INTRODUCTION

Indonesia is a tropical country that has a large collection of plants with medicinal potential, which has been used as a traditional medicine for generations. The use of phytopharmaca tends to increase with the issue of back to nature and the financial crisis which results in weak purchasing ability [1]. This condition is supported by the understanding of people who consider traditional medicine to be safer without side effects and also easier to obtain without a prescription [2]. Traditional medicine is widely used by the middle to lower classes, especially in efforts to prevent, promote and rehabilitate. At present, one of the plants that is widely cultivated and has medicinal properties is a guava [3].

Guava plants were first grown in South America. The distribution of this plant has been found worldwide, especially tropical countries including Indonesia [4]. In Indonesia, the largest guava cultivation center covers the area of DKI Jakarta, West Java, Central Java, East Java, Yogyakarta, Bali, West Nusa Tenggara, Sumatra and Kalimantan. As it is known, these tropical plants are planted for fruit, but the leaves can be used for medicine [5]. Review article mentions this plant shows a variety of biological activities such as antidiarrheal, antimicrobial, antioxidant, hepatoprotective, anti-allergic, anti-

spasmodic, anti-diabetic, anti-inflammatory and antitussive activity [6].

Guava fruit contains many nutrients, vitamins and antioxidants compound. It has been reported that there are 100 mg ascorbic acid in 100 g of ripe guava fruit. Both active ingredients are reported to play a role in immunostimulants [7]. While the leaves are reported to contain flavonoids, alkaloid, triterpenoid, tannins and essential oils [8]. Flavonoids especially quercetin, alkaloids and tannins act as antidiarrhea [9]. Essential oils have the potential as antibacterial [10]. Other studies revealed guava leaf extracts can increase the number of platelets [11] and accelerate the healing of open wounds in mice [12-13]. Ascorbic acid, flavonoids and tannins are reported to have a potential to increase platelets in patients with dengue fever [14].

In this study phytochemical screening was carried out to determine the active compounds of guava leaves taken from the West Denpasar sub-district, Bali. This extract will be used for research as antidiareous candidates in Balinese calves. Calf diarrhea is a disease commonly reported in neonatal to young animals [15]. Some causes of diarrhea in calves include metabolic disorders, poor quality nutrition, infectious agents and non-infectious agents [16]. Infectious agents include enteropathogenic bacteria [17] viruses [15][18] and protozoa [19]. Diarrhea causes

economic losses for farmers, in the form of medical expenses, care workers, disruption of growth and risk of death. The morbidity is very high reaching 62% with mortality case 39% [16].

Handling of calf diarrhea is generally based on clinical symptoms. The technology for controlling diarrhea in livestock has experienced development such as vaccines and synthetic drugs. But the treatment of diarrhea using herbal medicine such as guava leaves is potential to be developed, considering this plants also acts as an immunomodulator and the farmer can easily be cultivated around the enclosure garden.

II. RESEARCH METHOD

Preparation of guava leaf extract

Dried simplicia of fresh guava leaves was blended into powder. The powder weighed 1500 gram, was then processed by maceration method with 96% ethanol for a week at room temperature. The macerated are then filtered with a filter paper. The supernatant was evaporated in a rotary evaporator at temperature 40 °C. Ethanol extract of guava leaves can be stored until further examination. Phytochemical screening was carried out to identify the class of active compounds contained in the extract.

Phytochemical analysis

The phytochemical screening procedure was conducted according to Harborne (1987). This included examination of steroids and terpenoids, phenolic, tannins, flavonoids, alkaloids, and saponin [20].

Steroids and terpenoids test; guava leaf extract was mixed with anhydrous acetic acid and sulfuric acid (Liebermann Burchard) each of 2 drops. The blue discoloration will indicate the presence of steroids and if the colour change to purple, will indicate the presence of terpenoids.

Phenol test; guava leaf extract was added by 2 drops of FeCl₃ solution, blue to black color showed phenol content in the extract.

Tannin test; guava leaf extract was added by 2 drops of solution of FeCl₃ and H₂N₄ into the test tube. The positive results of the tannin on the extract will show a yellowish color.

Flavonoids test (Wilstater Reagent); 1 ml of guava leaf extract was added by 2 drops of concentrated HCl and added 0.2 milligram Mg powder, the positive reaction will show a yellow discoloration. Flavonoids test (NaOH Reagent); 1 ml of guava leaf extract was added by added 2 drops of 10% NaOH reagent, a positive reaction will show an orange or red color change.

Alkaloids test (Mayer reagent); 1 ml of guava leaf extract was added by 2 drops of chloroform and 2 drops of

meyer reagent, a positive reaction of the alkaloid showed the formation of white deposits.

Saponin test; guava leaf extract was added by 2-3 drops of distilled water then shake the test tube slowly, the positive results of saponin content are indicated by the presence of foam in the test tube. Addition of HCl solution aims to stabilize the foam.

Data analysis

The data obtained is presented and analyzed by descriptively qualitatively.

III. RESULT AND DISCUSSION

The results of screening test of guava leaf extract showed that guava leaf contained secondary metabolites included steroids, terpenoids, flavonoids, alkaloids, tannins, phenols and saponins (Table I).

TABLE 1
 PHITOCHEMICAL SCREENING OF GUAVA LEAFS EXTRACT

No	Type analysis	Reagent	Discoloration	Test result
1	Steroid/ Terpenoids	Sample + Anhydrous acetic acid + sulfuric acid (Liebermann Burchard)	Blue Purple	(+) Steroid (+) Terpenoid
2	Fenolics	Sample + FeCl ₃	Dark blue	(+) Fenolic
3	Tannins	Sample + FeCl ₃ + H ₂ N ₄	Yellow	(+) Tannin
4	Flavonoids	Sample + Mg/HCl	Yellow	(+) Flavonoid
5	Flavonoids	Sample + NaOH 10%	Red	(+) Flavonoid
6	Alkaloids	Sample + kloroform + meyer	White sediment	(+) Alkaloid
7	Saponins	Sample + aquades + HCl	Stable foam	(+) Saponin

Steroids and terpenoids tests using the Liebermann-Bouchard method showed positive results, marked by changes in color to blue-green (steroids) and red-purple discoloration (terpenoids) This is due to the ability of terpenoid and steroid compounds to result colors by sulfuric acid in anhydride acetic acid solvents [21].

Examination of phenol compounds on guava leaf extract showed positive results. Phenolic compounds that react with FeCl₃ cause discoloration to purple, blue and deep black. This is due to the reaction between FeCl₃ and the -OH aromatic group on phenol compounds [22].

Examination of tannin compounds by adding guava leaf extract with a solution of FeCl₃ and H₂N₄ (hydrazine) showed a positive result marked discoloration to yellowish [10]. Tanin in celery leaves is reported as an antioxidant

that can reduce total cholesterol and LDL cholesterol in hyperglycemic mice. The mechanism of tannin in reducing total cholesterol levels by inhibiting LDL oxidation [23]. Tannin has scavenger activity of hydrogen peroxide (H₂O₂), thereby reducing the formation of free radicals and lipid peroxidation [24].

Flavonoid test used two methods, namely Wilstater reagent and 10% NaOH reagent. Both methods showed positive results of guava leaf extract containing flavonoids. Wilstater reagents were carried out by adding concentrated Mg and HCl to the sample. The addition of concentrated HCl was used to hydrolyze flavonoids into their aglycones by hydrolyzing O-glycosyl. Glycosyl will be replaced by H⁺ from acid because of its electrophilic nature. Reductions with concentrated Mg and HCl can produce complex compounds that are red or orange in flavonol, flavanone, flavanonol and xanton. Based on previous study phytochemical screening of alcoholic extract root bark of guava. Flavonoids have been reported to have free radical scavenger properties. Flavonoids existing in the extracts could play an important role and are phenolic compounds widely distributed in the plant kingdom and have several pharmacological properties such as spasmolytic and antidiarrhoeal activities. Flavonoids are secondary metabolites that act as exogenous antioxidants [25][26].

Alkaloid test with Mayer reagent showed positive results marked white crystal sediment. In this reaction a bond is replaced where nitrogen which has a free electron pair on the alkaloid forms a covalent coordinate bond with a K⁺ ion from potassium tetraiodobismutate to produce a precipitate of potassium-alkaloid complex. Other studies reveal alkaloids as powerful antioxidants that are good for protecting cells from damage caused by free radicals and toxicity [22].

The saponin test showed a positive result because of the foam formed after the shaking of the sample and distilled water, with the addition of HCl foam solution stabilized [20]. Saponins have glycosyl as a polar group and steroids as nonpolar groups, which are surface active and will form micelles when shaken with water [27]. The study report that saponins have antioxidant and antibacterial effects [22][25][28].

Multiple pathogens are known to contribute to the development of calf diarrhea. Other factors that influence the severity of the disease include the environment and management practices. This multifactorial nature makes calf diarrhea difficult to control [15]. The morbidity and mortality are very high in newborn cows and young animals. This causes high economic losses due to calf deaths, high medical costs and stunted growth [29]. Non-infectious calf diarrhea is triggered by a bad environment, cold temperatures, a changing feed program, overcrowding facility or other mechanical causes [16]. Infectious

diarrhea may be caused by bacterial, viral and protozoan infections [15-16].

The results of this study, analysis of revealed that guava leaf extract contained phytochemicals that are known to have pharmacological activity. Flavonoids are hydroxylated polyphenolic compounds that have a response to microbial infections [15]. Tannin is a polyphenol compound with anti-bacterial activity, which is capable of binding a lot of proline protein [30]. Both of these activities are able to form complex bonds with intracellular and extracellular proteins and lyse bacterial cell walls. Other reports mention terpenoids, saponins and essential oils also effectively inhibit bacteria [10][31]. Secondary metabolites in guava leaf extract showed immunostimulatory [24-25][28] activity such as; increasing platelet [11][14] in protecting grouper liver from viral nervous necrosis (VNN) infection [18].

The in vitro antimicrobial activity of guava leaf extract is indicated by the ability to inhibit gram-positive bacteria (*Bacillus aureus* and *Staphylococcus aureus*) [12], *Escheresia coli* [10], *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, *Streptococcus pneumoniae* and *Klebsiella pneumonia* [31], *Aspergillus niger* and *Candida albicans* [30], and viral nervous necrosis [18]. All of these abilities of guava leaves extract have the potential to be developed as anti-diarrhea in calves.

IV. CONCLUSION

The phytochemical analysis the ethanol extract of guava leaves contained active compound including steroid/triterpenoid, phenolics, alkaloids, flavonoids, saponins and tannins. Thus the guava leaves extract has the potential to treatment of diarrhea in calves.

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