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Effects of 70% Ethanol Extract of *Foeniculum vulgare* and *Coleus amboinicus* as a Potential Diarrhea Treatment Using Intestine Transit Method

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ABSTRACT: Diarrhea is a condition where the frequency of defecation increases more than three times a day. In underdeveloped nations like Indonesia, diarrhea is a sickness that frequently affects people. Diarrhea can be fatal, causing dehydration and even death if not treated properly. Using alternative treatments like fennel seeds and cumin leaves is one way to treat diarrhea. Fennel seeds and cumin leaves include secondary tannin metabolites that are effective antidiarrheal agents. Test the antidiarrheal effect of *Foeniculum vulgare* ethanol extract (FVEE) and *Coleus amboinicus* ethanol extract (CAEE) using the intestinal transit method on mice was carried out on 9 groups, each group consisting of 4 mice: negative control, Loperamide control, Diapet® control, FVEE and CAEE at doses of 100, 200 and 400 mg/kg. To induce diarrhea through oral administration, castor oil was used in the experiments. Four hours following the start of the induction, the treatment group received. They received Norit an hour later, and the mice were sacrificed 20 minutes after receiving Norit. The intestines were taken out of the mice after they had been dissected, starting from the rectum to the pylorus. Measured the length of the intestine overall and the portion that passed through the Norit marker. Calculations were made to determine how much of the gut went by a marker compared to the entire intestine. The results of the study showed that FVEE has an antidiarrheal effect at a level of 100 mg/kg and CAEE has an antidiarrheal effect at a dose of 200 mg/kg.

KEYWORDS: fennel seeds; cumin leaves; antidiarrhea; intestinal transit; ethanol extract

1. INTRODUCTION

A digestive illness known as diarrhea is characterized by changes in bowel motions, frequency, and consistency of feces, which become more watery and liquid or pasty. Diarrhea can appear suddenly or gradually. Numerous factors, including contaminated water and food, drug use, endocrine disorders, and infections, contribute to this condition. Diarrhea can be divided into three categories based on how long it lasts: acute (1–13 days), persistent (14 days or more), and chronic (more than 30 days). It can also be divided into four categories based on the physiological mechanisms involved: osmotic, secretory, inflammatory, and motor. The majority of the aetiologies involve one or more of these mechanisms in their complex pathophysiology [1]. It can range from a moderate condition that causes cramping in the stomach and loose stools to a severe syndrome that causes a lot of bloody or watery stools and can cause organ failure, dehydration, abnormal electrolytes, and even death [2]. In developed countries, the prevalence of chronic diarrhea is thought to be between 1% and 5% of the adult population. This condition is characterized by an increase in defecation frequency and an irregularity in stool consistency three or more times in a 24-hour period, which results in water and electrolyte loss [3]. Beyond acute morbidity and death, there is rising attention in the connection between persistent stunting, early diarrhea, and cognitive development, as well as in the potential downstream benefits of effective prevention and treatment [2].

Fennel, also known as *Foeniculum vulgare* Mill. (Apiaceae family), is a popular perennial plant with a fragrant scent. Originally from Southern Europe and the Mediterranean area, it is now widely farmed in both temperate and tropical climates around the world. Extracts of *F. vulgare* and some of its constituents have

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shown antioxidant, antibacterial, antifungal, estrogenic, and antituberculosis activities and *Foeniculum vulgare* is used for the treatment of diarrhea in Mexican traditional medicine [4]. Fennel as known as Adas in Indonesian. A traditional medicine has made considerable use of *Foeniculum vulgare* to treat a variety of diseases. Fennel is utilized in a number of alternative and balancing traditional medical systems, including Ayurveda, Unani, Siddha, Indian, and Iranian systems. Its stem, fruit, leaves, seeds, and entire plant are all utilized medicinally in various ways to treat a range of sick diseases. Traditional uses of *F. vulgare* include treating stomach aches, arthritis, conjunctivitis, constipation, diarrhea, dieresis, fever, flatulence, gastralgia, gastritis, insomnia, irritable bowel syndrome, mouth ulcers, stomachaches, respiratory conditions, skin conditions, and other infectious diseases [5].

Coleus amboinicus, a well-known traditional plant with widespread cultivation in Africa, Asia, Australia, and South America, is used on a daily basis as a food supplement or for therapeutic purposes [6]. The species name, amboinicus, is derived from Ambon Island in Indonesia, where it was reportedly discovered. Ambon is one of the Maluku Islands in Indonesia. The plant is used in traditional Indian medicine to cure a variety of ailments, including malaria fever, inflammation, cough, chronic asthma, bronchitis, liver illnesses, renal problems, and gallstones. In Indonesia, it's used to promote lactation after childbirth, as well as an aromatic carminative and anthelmintic. The leaves of the plant are often eaten raw or used as flavoring. In India, the leaves of *C. amboinicus* are consumed with buttermilk, yogurt, etc., during infection-induced diarrhea. Over the years, several studies have been carried out confirming the very wide spectrum of *C. amboinicus* activity. In vivo studies showed that the plant has analgesic and anti-inflammatory activities [7]. This herb has traditionally been used to treat coughs, fevers, indigestion, loss of appetite, throat infections, nasal congestion, lactagogues, constipation, and digestive issues. *C. amboinicus* leaves contain phenolic chemicals, including carvacrol, flavonoid, rosmarinic, caffeic, and chlorogenic acids. This plant contains many flavonoids, including salvigenin, 6-methoxygenkwanin, quercetin, chrysoeriol, luteolin, apigenin, eriodyctol, and taxifolin [8].

In many national healthcare settings, the use of herbal medicine has reportedly increased while many people have returned to using traditional medicine to treat various health issues. More than 80% of people, especially in underdeveloped nations, are thought to depend mostly on traditional remedies for their medical needs [9]. The aim of this study is to evaluate the effectiveness of *Foeniculum vulgare* ethanol extract (FVEE) and *Coleus amboinicus* ethanol extract (CAEE), as a potential natural product for treating diarrhea.

2. MATERIALS AND METHODS

2.1. Extract Preparation

Making *Foeniculum vulgare* ethanol extract (FVEE) and *Coleus amboinicus* ethanol extract (CAEE) using the maceration method. Maceration is carried out using 70% ethanol solvent with a ratio of simplicia and ethanol solvent of 1:3. The ethanol solution is added to the maceration vessel after the simplicia has been added. Avoid direct sunlight, keep the marinade in a cool area for 3 days, and stir the mixture every so often. The filtering process was carried out 3 times. To obtain extract from fennel seeds, the macerated sample is evaporated using a rotary evaporator at a temperature of 50°C until the extract thickens.

2.2. Study Design : Treatment of Animals

Thirty-six male mice (*Mus musculus*) were randomly divided into nine groups: negative control given Na CMC 1%, groups had given Loperamide control at doses of 2 mg/kg, groups had given Diapet® control at doses of 1,2 g/kg and groups had given FVEE and CAEE at doses of 100, 200 and 400 mg/kg (FV100, FV200, FV400, CA100, CA200 and CA400). This study uses an experimental design. Mice will be slaughtered, dissected, and examined in this study using the intestinal transit method, measuring the potency of drugs in the intestines of mice using carbon adsorbent markers. The mice were obtained from PPOMN Badan POM and then acclimatized for 7 days for the purpose of adapting to the new cage. Mice were starved for 18 hours before testing but given water. The goal of fasting was to avoid eating anything that could interfere with the testing process. Then mice were induced using castor oil at dosage 0,5ml/20 g BW to cause diarrhea. The mice were given therapy based on their treatment group four hours after induction. After sixty minutes of administration, all mice were given the carbon adsorbent marker suspension orally, and after twenty minutes of administration, the mice were slaughtered by dislocating the neck, which had already been anesthetized with ether solvent. The mice will then be surgically on. Mouse intestines were carefully separated.

2.3. Determination of Tannin Content

The goal of assessing tannin content was to determine the percentage of tannin content from the simplicia. The tannin content was determined using the permanganometric technique. Determination of tannin content in Fennel seeds and Cumin leaves by weighing simplicia then dissolving it in distilled water, heating it and then after cooling it, the solution is filtered. As an indicator, indigo sulfonic acid was added to the resulting solution to produce a golden yellow color. The solution was then titrated with 0.1 N $KMnO_4$ which was previously standardized until it turned golden yellow. The titrant volume was recorded and duplicated for three times.

2.4. Antidiarrheal Activity Evaluation

Stretched the mouse's intestines after removing the intestines from the animal. Then, measure the length of the intestine that passes where the carbon adsorbent marker (Norit: black marker substance) moves from the pylorus to the rectum, then compare the distance traveled by the carbon adsorbent marker in mice. The average value was calculated and compared with the drug control and negative control groups. After that, statistical analysis was carried out using Anova.

3. RESULTS

3.1. Determination and Extraction

Plants of *Foeniculum vulgare* Mill. and *Coleus amboinicus* Lour. were determined in the Biology Laboratory, FMIPA, University of Indonesia, Depok, West Java. The goal of determining fennel seed and cumin leaves plants is to demonstrate the correctness of the plant type utilized in the research.

The extract obtained from 500 gram of simplicia was 78.70 gram, with a yield of 15.75% for *Foeniculum vulgare* ethanol extract and extract obtained from 500 gram of simplicia was 88.9 gram, with a yield of 17.78% for *Coleus amboinicus* ethanol extract. The extraction technique produced the following yields:

Table 1. Result of Extraction Yield

Sample	Weight of Simplicia (g)	Weight of Extract (g)	Yield (%)
Foeniculum vulgare	500	78.70	15.75
Coleus amboinicus	500	88.9	17.78

3.2. Determination of Tannin Content

The tannin content of fennel seeds is determined by weighing the simplicia, dissolving it in distilled water, boiling and leaving it, and then filtering the solution [10]. As an indicator, indigo sulfonic acid was added to the resulting solution. Titration with 0.1 N $KMnO_4$ was performed until a golden yellow color was produced.

Table 2. Result of Determination of Tannin Level

Extract	Replication	Weight (mg)	Titran Volume (mL)	Blank Volume (mL)	Tannin Level (%)	Average of Tannin Level (%)
Foeniculum vulgare	1	401.1	0.82	0.20	7.35	7.19
	2	401.2	0.82	0.20	7.35	
	3	401.0	0.78	0.20	6.88	
Coleus amboinicus	1	400.2	0.54	0.20	4.04	4.04
	2	400.7	0.54	0.20	4.04	
	3	400.5	0.54	0.20	4.04	

3.3. Antidiarrheal Activity Evaluation

In this antidiarrheal investigation, an ethanol extract of fennel seeds was utilized as a test preparation and was compared to the normal control and control medicinal products of Loperamide and Diapet®. The intestinal transit method was used in this investigation. The intestinal transit method is used to compare the length of the intestine through which the marker moves to the length of the total intestine as a ratio value. The outcomes are as follows:

Table 3. Result of Ratio

Treatment	Ratio of each mice				Average of Ratio
	1	2	3	4	
Negative Control	0.31	0.29	0.27	0.38	0.31
Loperamide	0.14	0.25	0.16	0.19	0.19
Diapet®	0.21	0.15	0.15	0.22	0.18
FV100	0.18	0.13	0.16	0.15	0.16
FV200	0.22	0.14	0.22	0.18	0.19
FV400	0.26	0.25	0.22	0.27	0.25
CA100	0.23	0.21	0.28	0.26	0.25
CA200	0.17	0.20	0.23	0.20	0.20
CA400	0.27	0.16	0.10	0.45	0.25

FV : Foeniculum vulgare
CA : Coleus amboinicus

Based on the data presented in the table, the following results are interpreted graphically:

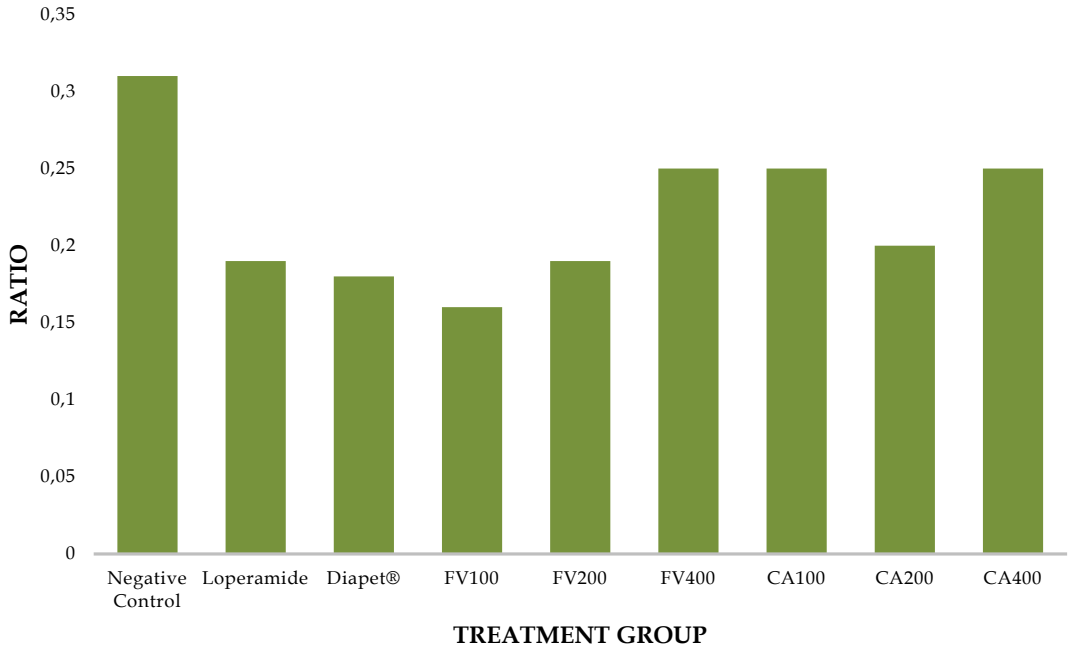

Figure 1. Ratio Length of Intestine

4. DISCUSSION

FVEE was prepared by macerating the seeds in 70% ethanol for three days and stirring occasionally. Stirring causes the cell walls of the simplicial to break down, making it easier to extract secondary metabolites. Macerate is collected by filtration, the filtering procedure was repeated three times. So that additional secondary metabolites in simplicia can be attracted. 70% ethanol was chosen as the solvent because it may attract non-polar, semi-polar, and polar molecules [11]. The maserate obtained is then concentrated using a rotary evaporator until a thick ethanol extract.

Based on the findings of this investigation, the value of tannin content was calculated using the permanganometric titration method. The tannin content at FVEE was 7.19%, while at CAEE it was 4.04%. Tannins are polyphenolic chemicals that occur naturally in plants. Their natural presence has spurred their historical use in a variety of ways. Polyphenols are a diverse group of secondary metabolites that are retained in the vacuoles of vegetal cells as esters or glycosides [12]. Hydrolysable tannins consist of simple phenols with little substitution and nucleophilicity in their native state. The global production of commercial tannins is expected to be 220,000 tonnes per year, with condensed tannins accounting for more than 90% of the market [13]. Tannins are naturally occurring polyphenol chemicals found in a variety of plants and trees, including green tea, coffee, and fruits like pomegranate, persimmon, and grape. Tannin-containing plant species in Asia, particularly China, have been utilized for thousands of years as astringents, anti-diarrheals, anti-hemorrhage agents, anti-carcinogens, and antimicrobials [14].

For thousands of years, several tannin-containing plant species in Asia, particularly China, have been utilized as astringents, anti-diarrheals, and anti-hemorrhage agents, anti-carcinogens, and antimicrobials [14]. The antidiarrheal effect of tannin is related to its activity as an antimicrobial. Plants containing tannins have been proven to be susceptible microorganisms tested including *Staphyl. aureus*, *E. coli, Pseud. aeruginosa, K. pneumoniae*, and *Candida albicans*. The study examined the pharmacological effects of a leaf extract containing tannins, which was utilized as a traditional remedy for gastrointestinal ailments in Southern Nigeria. Leaf extracts containing saponins and/or tannins demonstrated antibacterial activity against *E. coli, Staphylococcus aureus*, and *Streptococcus faecalis* [15].

Overall, the study's findings indicate that the two extracts employed, *Foeniculum vulgare* ethanol extract (FVEE) and *Coleus amboinicus* ethanol extract (CAEE), have potential anti-diarrhea activity, as evidenced by a ratio value that is significantly different from the negative control group. The collected ratio data were subjected to statistical analysis using the normality and homogeneity tests. Subsequently, the One Way ANOVA and Kruskal Wallis tests were employed to determine whether significant differences existed between the control and test groups. The results demonstrate that the dose of the extract with the greatest potential to be implemented as an antidiarrheal is 100 mg/kg BW in FVEE and 200 mg/kg BW in CAEE. These two doses were proved to generate significantly different results from the negative control group that did not receive therapy. Then, at an FVEE dose of 100 mg/kg BW, the ratio value is quite close to optimal when compared to medical treatments. This can reinforce the idea that the extract has an antidiarrheal effect and can be utilized as an alternative treatment based on natural substances.

In healthy individuals, the colon absorbs nearly all water and electrolytes consumed during digestion. An estimated 9-10 L of fluid enters the jejunum. Stool water content typically ranges from 80 to 100 mL, resulting in 99% absorption efficiency. Even a 1% decrease in absorptive efficiency can result in more fluid, less formed, and frequent stools, leading to "diarrhea". Disruptions in mucosal absorption or secretion can be caused by injury to the absorptive mucosa, bacterial toxins, enteric neuronal function, or hormone [16]. Reduced absorptive efficiency in the gut can be caused by rapid transit, malabsorption, maldigestion, or surgical changes that cause small bowel syndrome or remove important sections like the terminal ileum. Many diarrheal illnesses cause disruptions in mucosal function and gastrointestinal transit. Antidiarrheal medications reduce the symptoms of diarrhea by improving stool consistency, frequency, or weight, and aid in gastrointestinal transit [17].

Loperamide is the most often used antidiarrheal medication on the market. Loperamide, an opiate analogue, is a commonly used anti-diarrheal drug that inhibits smooth muscle tone and peristalsis through both cholinergic and non-cholinergic mechanisms. The study found that loperamide-modified prostaglandin E (PGE) increased fluid secretion in the rat intestinal tract, suggesting that its anti-diarrheal properties may be linked to both intestinal motility and secretory processes [18]. Loperamide, a phenylpiperidine opioid, has

similar pharmacological effects as meperidine. It slows intestinal transit by stimulating m-opioid receptors in the myenteric plexus, has antisecretory effects, and blocks intestinal calcium channels. The drug has a wide margin of safety, largely owing to its extremely low bioavailability (0.3%). Side effect of Loperamide At very high plasma concentrations, loperamide interferes with cardiac conduction [19].

5. CONCLUSION

Finally, the permanganometric titration method revealed that tannin was present in both *Foeniculum vulgare* and *Coleus amboinicus* ethanol extract. Based on the findings of this investigation, FVEE and CAEE were shown to have potential as antidiarrheal potential effects, with substantial differences from the negative control group. The tannin component, which has historically been recognised to have an antidiarrheal effect, is assumed to be responsible for the extract's pharmacological activity. However, further investigation is needed into the other chemicals in the *Foeniculum vulgare* and *Coleus amboinicus* extract that may have an anti diarrhea impact, so that the extract's application can be expanded even further in the future.

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