ANTIBACTERIAL ACTIVITY OFBELUNTAS (Pluchea indica L.) LEAVES EXTRACT USING DIFFERENT EXTRACTION METHODS

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WORD COUNT 2327
TIME SUBMITTED 21-NOV-2022 09:20AM
PAPER ID 92858331

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ABSTRACT

Escherichia coli and *Bacillus subtilis* are bacteria that cause disease in the digestive tract. *Pluchea indica* L. had pharmacological activity of antiseptic power against bacteria that cause digestive tract infections because of contained of antibacterial compounds. The study on the comparison of extraction methods will enable the public to choose a better extraction method to use as an antibacterial agent. This study aims to determine the content of antibacterial compounds in *Pluche indica* L. and to determine the antibacterial ability of *Pluche indica* L. extract using maceration, percolation and soxhletation methods. Based on the research, it is known that *Pluchea indica* L. obtained from maceration, percolation and soxhletation extraction contains flavonoids, alkaloids, saponins and tannins. The highest diameter inhibition of *Escherichia coli* and *Bacillus subtilis* was obtained from *Pluchea indica* L. leaves extract using the Soxhletation method.

INTRODUCTION

Pathogenic bacteria in the gastrointestinal tract are a group of bacteria that can cause gastrointestinal disease (Radji & Maksum, 2011). Digestive tract disease which is diarrhea, can be caused by bacteria and parasites (Zein *et al*, 2012). The bacteria that cause diarrhea is *Esherichia coli*. In addition, *Bacillus subtilis* also has the ability as a pathogen in the digestive tract. Most of the *Escherichia coli* and *Bacillus subtilis* are in the digestive tract as the normal flora (Bettleheim, 2000). These bacteria become pathogenic and caused diarrhea when their numbers increase in the digestive tract (Jawetz, 2005). Various medicinal plants have been believed to have properties to treat certain diseases and as an alternative treatment for certain diseases (Agoes, 2010). One of the plants containing medicinal compounds is *Pluchea indica* L. (Nurhalimah, 2015). *Pluchea indica* L. also have pharmacological activity of antiseptic power against bacteria that cause digestive tract infections (Ismi *et al*, 2010). *Pluchea indica* L. leaves contain alkaloids, tannins, essential oils, sodium, potassium, aluminum, calcium, magnesium, phosphorus and flavonoids (Dalimartha, 1999), and these compounds are believed to be antibacterial compounds.

Most of the research on *Pluchea indica* L. leaves focuses more on antibacterial testing, such as research on the antibacterial testing of *Pluchea indica* L. leaves against *Propionibacterium acne* (Hafsari *et al*, 2015; Suru *et al*, 2019), *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Manu, 2013), *Mycobacterium tuberculosis* (Amilah and Ajiningrum, 2015), and *Escherichia coli* (Mulyadi *et al*, 2016). Research about the use of the extraction method used to obtain extracts is still minimal, even though the type of extraction method can affect the content and biological activity of chemical compounds that are suspected to be antibacterial compounds. In addition, a study on the comparison of extraction methods will allow the public to choose an extraction method that allows better extraction for use as an antibacterial agent. This study aims to determine the antibacterial ability of beluntas

leaves extract (*Pluchea indica* L.) in inhibiting the growth of *Escherichia coli* and *Bacillus subtilis* using three different extraction techniques, maceration, percolation and soxhletation.

MATERIALS AND METHODS

Reagen and chemical compound

Reagen and chemical compound were collected from Department of Chemistry, State University of Surabaya which are Reagen Mayer, reagen Dragendorf, dan reagen Wagner, HCl, Mg powder and $FeCl_3$.

Bakteria and sample

The bacteria used in this study were *Bacillus subtilis* collected from ULP (Research Service Unit) Airlangga University Surabaya, and *Escherichia coli* ATCC 25922 collected from BBLK (Central Health Laboratory) Surabaya. The bacteria then recultured in *Nutrient Agar* media (Merck, Jerman) and stored in an incubator at the temperature of 30°C. *Pluchea indica* L. leaves were collected from MMI (Materia Medika Indonesia).

Ekstraction preparation

The extraction methods used in this research are maceration, percolation and soxhletation methods.

1. Maceration method.
   Dried *Pluchea indica* L. leaves powder (100 g) was extracted using methanol (1000 mL) solvent in a ratio of 1:10 for 10 days at 25-30 °C and then filtered. The extraction was remaceration twice with the new solvent in same volume. The extract was filtered using Whatman filter paper no.1.
2. Percolation method
   Dried *Pluchea indica* L. leaves powder (100 g) was soaked using methanol (1000 mL) solvent in a ratio of 1:10 in a percolator for 24 hours at a temperature of 25-30 ° C. Extraction was repeated twice with the new solvent and the same volume.
3. Soxhletasion method
   Dried beluntas leaves powder (100 g) was soaked using methanol (1000 mL) solvent in a ratio of 1:10 using the Soxhlet method for 10 hours.

The entire filtrate from each extraction method was evaporated using a rotary evaporator under pressure at a temperature of 40 °C. The filtrate were then diluted with sterile aquadest to make a concentration of 1500 ppm, 2000 ppm and 2500 ppm. Aquadest were also as a control.

Qualitative phytochemical assay

A qualitative phytochemical test was carried out to determine the presence of compounds that play a role in the process of inhibiting bacterial growth. These compounds are limited to include flavonoids, alkaloids, saponins and tannins.

1. Flavonoid assay
   Pluchea indica L. leaves filtrate (1 mL) was added with 2N HCl (2 mL) and Mg powder (1 mg) then shaken homogeneously. The sample is said to be positive for containing flavonoid compounds if there is appearence a yellow or red orange color in the tube.
2. Alkaloid assay
   Pluchea indica L. leaves filtrate (1 mL) was put in 3 different test tubes then added with 2N HCl (2 mL). Dragendroff reagent was dropped on tube 1, Mayer's reagent in tube 2, and Wagner reagent in tube 3 three drop each test tube then shaken homogeneously. The sample is said to be positive for containing alkaloid compounds if there is appearence an orange red precipitate in tube 1, greenish white in tube 2 and brownish black in tube 3.
3. Saponin assay
   Pluchea indica L. leaves filtrate (1 mL) was added with hot aquadest (2 mL) then cooled. Then shake vigorously for 10 seconds. The sample is said to be positive for containing saponin compounds if a constant foam is formed for 10 minutes in the test tube.

4. Tannin assay
   Pluchea indica L. (1 mL) added 2 drops of $FeCl_3$ then shaken homogeneously. The sample is said to be positive for containing tannin compounds if there is appearence a blackish green color in the tube.

Bacterial suspension

Nutrient Broth (Merck, Jerman) (3,6 g) dissolved in aquadest (180 mL) then sterilized using autoclave under a pressure of 1 atm, temperature 121 °C for 30 minutes. Each bacteria were inoculated in sterile *Nutrient Broth* aseptically and incubated in an incubator at 30 °C for 24 hours.

Antibacterian activity test

Antibacterial activity test was *in vitro* using the well method with 4 replications. The bacterial suspension (1 mL) was put in a sterile petri dish and added with sterile *Nutrient Agar* media (15 mL) then homogenized (*pour plate method*). Make a well hole using the well tool no.6 aseptically. *Pluchea indica* L. leaves extract with each concentration (30 µL) was inserted into the hole that was made and then incubated in an incubator at 30 ° C for 24 hours.

RESULT AND DISCUSSION

Qualitative phytochemical assay

The phytochemical content that wanted to observed were flavonoids, alkaloids, saponins and tannins. The results were showned in **Table 1**.

Table 1. Qualitative Phytochemical Assay *Pluchea indica* L. Leaves

No.	Compound	Extraction Method		
		Maserasion	Perkolasion	Soxhletasion

1.	**Flavonoid**	+	+	+
2.	**Alkaloid**	+	+	+
3.	**Saponin**	+	+	+
4.	**Tannin**	+	+	+

Keterangan:
+ : positif mengandung senyawa uji
- : negatif mengandung senyawa uji

According to the **Table 1**, it is known that the *Pluchea indica* L. leaves extract using the maceration, percolation and soxhletation method on the flavonoid test tube appeared a yellow color so that it showed positive results containing flavonoids. In the alkaloid test there was an orange red on the Mayer tube, greenish white on the Dragendorf tube and brownish black on the Wagner tube, so that it showed positive results containing alkaloids. In the saponin test, a constant foam was formed for 10 minutes, so it showed a positive result containing saponins and in the tannin test a blackish green color was formed, so that it showed a positive result containing tannins.

Phytochemical qualitative testing is carried out to determine the presence of antibacterial compound contained in *Pluchea indica* L. leaves. These compounds included flavonoids, alkaloids, saponins and tannins. The results of the phytochemical screening of the *Pluchea indica* L. leaves extract (**Table 1**) showed that flavonoids, alkaloids, saponins and tannins were found in the extracts that were extracted by three different methods, which were maceration, percolation and soxhletation. This is the same as the results of previous studies (Khotimah, 2016; Widyawati *et al*, 2011) which stated that beluntas leaves extract positively contained flavonoids, alkaloids, saponins and tannins.

Antibacterial activity test

The aims of this research was to determine the antibacterial ability of *Pluchea indica* L. leaves extract in inhibiting the growth of *Escherichia coli* and *Bacillus subtilis* bacteria. The results of the antibacterial activity test data are presented in **Table 2**.

Tabel 2. Diameter Of Inhibitory Zone

Baktery	Konsentrasion (ppm)	Diameter Of Inhibitory Zone (mm)		
		Maserasion	Perkolasion	Soxhletasion
Eschericia coli	**Kontrol -**	0	0	0
	1500	4,3	3,9	4,7
	2000	4,7	4,2	5,1
	2500	5,4	4,9	5,8
Bacillus subtilis	**Kontrol -**	0	0	0
	1500	3,8	3,4	4
	2000	4,2	3,9	4,9
	2500	4,8	4,5	5,1

This test aims to determine the ability of *Pluchea indica* L. extract to inhibited the growth of *Escherichia coli* and *Bacillus subtilis* bacteria. Based on the interpretation of the test results (**Table 2**), it is known that the inhibition response of the *Escherichia coli* and *Bacillus subtilis* bacteria carried out by the *Pluchea indica* L.

extracts, both extracted using maceration, percolation and soxhletation methods, is in the moderate inhibition category (Pan *et al*, 2009). However, the mean results show that each has a different value. Beluntas leaves extract with a concentration of 2500 ppm with the Soxhletation method was known to be able to form the largest inhibition zone in *Escherichia coli* and *Bacillus subtilis*. This result caused by the extraction method, the solvent used, the well test method and the antibacterial compounds which affect the bacterial inhibition mechanism.

The maceration and percolation methods were chosen because they are cold extraction methods or without heating, so they can prevent damage to chemical components that are not resistant to heating (Syukur *et al*, 2012). The purpose of maceration is also to get the chemical components in the sample, where the solvent will penetrate the cell wall and enter the cell cavity containing the active compound. However, the mean results of the inhibition zone diameter of the extracts obtained by the percolation and maceration methods were not higher than the extracts obtained by the Soxhletation method. This can be because the extraction process carried out in the Soxhletation method is more effective so that the results obtained are better and the solvent used is relatively small. In addition, the extraction process carried out by the soxhletation method was able to produce high rendemen and high total phenolic (Puspitasari and Proyogo, 2017) including flavonoids, alkaloids, saponins and tannins which affected the diameter of the inhibition zone of each bacteria.

This study used methanol as a solvent because it was the polar compound, so it is able to dissolve polar compounds (tannins, alkaloids, flavonoids, saponins). This statement is in accordance with previous research (Thompson, 1985) which states that methanol can attract alkaloids, flavonoids and saponins from plants. Methanol was also chosen because the flavonoid content in *Pluchea indica* L. is a polar compound so it must be dissolved with a polar type solvent (Koreiwa *et al*, 2009). Methanol can affect the effectiveness of antibacterial substances so that it can inhibit the growth of the tested bacteria.

The test method used is the well method. This method was chosen because in the process, bacterial cells were not only present on the surface of the medium but also inside to the bottom of the agar medium (Endang and Maimunah, 2018). This is in accordance with the types of tested bacteria (*Escherichia coli* and *Bacillus subtilis*) which are facultative anaerobes whose life is scattered from the surface to the inside of the media. In the well method, the pit is filled with a concentration of *Pluchea indica* L. extract to determine its antibacterial properties. The well method can produce a clearer diameter of the inhibition zone than the disc disk method (Prayoga, 2013). This is because the well method occurs better osmolarity than the disk method, because each hole is filled with extract concentrations. The well method allows the osmolarity process to occur more thoroughly and more homogeneously and the resulting extract concentration is higher and stronger to inhibit bacterial growth.

Factors that influence the activity of antibacterial include the amount of antibacterial substances contained in *Pluchea indica* L., antibacterial compounds in *Pluchea indica* L. extracts such as flavonoids, alkaloids, saponins and tannins. The mechanism of antibacterial action is saponins which interfere with cell wall

permeability resulting in the release of important components, namely proteins, nucleic acids and others (Ganiswarna, 1995). Tannin compounds can shrink cell walls so that they can interfere with the permeability of the cell itself, flavonoids can cause damage to the permeability of bacterial cell walls (Kurniawati, 2001). Alkaloids have antibacterial activity by disrupting the components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact and causes cell death (Robinson, 1991). These results indicate that natural ingredients such as *Pluchea indica* L. have a fairly good ability to be applied as natural antibacterial agents (Paz *et al*, 2017).

CONCLUSION

Pluchea indica L. extract that obtained from maceration, percolation and soxhletation extraction contained flavonoids, alkaloids, saponins and tannins. In addition, the extracts from the three extraction methods have antibacterial ability to inhibit *Escherichia coli* and *Bacillus subtilis* which are included in the moderate inhibition category. The highest diameter was obtained from *Pluchea indica* L. leaves extract using the Soxhletation method.

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