

## Effectivity Test Of Saga Tree Leaf Extract (*Adenantha pavonia*) Toward The Growth Of *Escherichia coli* Bacteria.

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### ABSTRAK

Indonesia memiliki kekayaan sumber daya alam berupa tanaman obat terbesar di dunia, dimana obat tradisional lebih berpeluang untuk dikembangkan. Salah satu tumbuhan tradisional yang sering digunakan sebagai obat tradisional adalah daun saga (*Adenantha pavonina*). Dari beberapa literatur diketahui bahwa daun saga mengandung senyawa kimia seperti minyak atsiri, alkaloid, dan flavonoid sebagai antibakteri, juga mengandung abrin yang sangat beracun, padahal daun saga banyak digunakan secara tradisional untuk menyembuhkan berbagai penyakit. Penelitian ini bertujuan untuk mengetahui apakah ekstrak daun pohon saga (*Adenantha Pavonina*) memiliki efektivitas antibakteri terhadap pertumbuhan *Escherichia coli* dengan konsentrasi 3,123%, 6,25%, 12,5%, 25%, 50%, 100 %. Sampel daun pohon saga (*Adenantha Pavonina*) yang digunakan diperoleh dari sekitar jalan Karya, kecamatan Medan Johor, provinsi Sumatera Utara. Preparasi sampel dilakukan dengan membuat ekstrak kental dengan metode maserasi. Uji efektivitas antibakteri dilakukan dengan difusi cakram. Hasil penelitian menunjukkan ekstrak daun pohon saga (*Adenantha Pavonina*) mengandung alkaloid, flavonoid, saponin, steroid, tanin, dan abrin serta mempunyai daya efektifitas terhadap pertumbuhan bakteri *Escherichia coli* dengan rata-rata luas zona hambat : konsentrasi 3,123% =5,2 mm, konsentrasi 6,25%=5,5 mm, konsentrasi 12,5%=6,1 mm, konsentrasi 25%=7,3 mm, konsentrasi 50%=8,8 mm, konsentrasi 100%=10,8 mm. Ekstrak daun saga (*Adenantha Pavonina*) memiliki efektivitas terhadap pertumbuhan bakteri *Escherichia coli* dengan kategori kuat

**Kata Kunci :** Daun pohon saga, antibiotik, *Escherichia coli*.

### ABSTRACT

Indonesia has a wealth of natural resources in the form of the biggest medicinal plants in the world, in which traditional medicine is more likely to be developed. One of the traditional plant which is often used as traditional medicine is the saga leaves (*Adenantha pavonina*). From some literature it is known that saga leaves contain chemical compounds such as essential oils, alkaloids, and flavonoids as antibacterial, it also contain abrins which is highly toxic, even though saga leaves are widely used traditionally to cure various diseases. The aim of this research is to find out if the extract of saga tree leaves (*Adenantha Pavonina*) have the antibacterial effectiveness against the growth of *Escherichia coli* with the concentration of 3.123%, 6.25%, 12.5%, 25%, 50%, 100%. The used sample of saga tree leaves (*Adenantha Pavonina*) were obtained from around the Karya road, Medan Johor sub-district, North Sumatra province. Sample preparation was done by making thick extract with maceration method. Antibacterial effectiveness test was done with disc diffusion. Research result showed the extract of saga tree leaves (*Adenantha Pavonina*) contains alkaloids, flavonoids, saponins, steroids, tannins, and abrins and has the power of effectiveness against the growth of *Escherichia coli* bacteria with the average area of inhibition zone : concentration 3.123%=5.2 mm, concentration 6.25%=5.5 mm, concentration 12.5%=6.1 mm, concentration 25%=7.3 mm, concentration 50%=8.8 mm, concentration 100%=10.8 mm. The extract of saga tree leaves (*Adenantha Pavonina*) has effectiveness against the growth of *Escherichia coli* bacteria with strong category..

**Keywords :** Saga tree leaves, antibiotic, *Escherichia coli*.

## I. PENDAHULUAN

### 1. Latar Belakang

Indonesia has a wealth of natural resources in the form of the biggest medicinal plants in the world, in which traditional medicine is more likely to be developed. One of the traditional plant which is often used as traditional medicine is the saga leaves (*Adenanthera pavonina*). From some literature it is known that saga leaves contain chemical compounds such as essential oils, alkaloids, and flavonoids as antibacterial, it also contain abirins which is highly toxic, even though saga leaves are widely used traditionally to cure various diseases. Saga leaves are wild plants in the forest, bushes, or planted in the yard by being propagated on the fence as medicinal plant. The taste of saga leaf seeds are spicy, bitter, neutral, astringent, and highly poisonous (toxic). Effective in killing parasites, sore, expel phlegm, vomiting stimulant. The taste of root, stem, and leaf are sweet, neutral. The leaves can soothe the skin and mucous membranes (Yusrandah dkk, 2015). *Escherichia coli* is a helical shaped gram negative bacteria (classified as a curved rod, not a spirochete) which length around 3  $\mu\text{m}$  with diameter around 0.5  $\mu\text{m}$ . *Escherichia Coli* can be demonstrated in the system by gram staining, giemsa staining, hematoxylin-eosin staining, warthin-starry silver staining, acridine orange staining (Ginns,2000) *Escherichia coli* is one of the normal flora in the body, but these bacteria will become pathogens with different virulence mechanism if their number surpass body limit. Disease that is often caused by *Escherichia coli* bacteria is colibacillos. These bacteria can cause gastrointestinal problems, one of them is diarrhea (Putri, 2016). Saga trees start producing at the age of 5 years, producing 3 times a year until they are 30 years old (Becker et al., 2016). Production of BK (dry matter), PK (crude protein) and TDN (total digestible nutrients) of tree saga leaves reached optimal at the age of 12 years, namely reaching 119.39; 18.08 and 8.86 kg/tree/year, with a cutting period of 3 months (Subagiyo et al., 2014). Kamaliyah report (2019) the total biomass of saga trees at the age of 15 years reached 289.1 kg fresh (178.0 kg DM with a moisture content of 48.9%) and leaf production was 25.0 kg fresh (9.9 kg DM with a moisture content water 50.1%) per tree per year. Saga trees take 3.5-4.0 months for the flowering process to form old pods (Becker et al., 2016). The process from flower buds to small pods takes about 25 days, and small pods to contain solid seeds and bursts around 64 days (Aprelia, 2020). Each tree saga plant can produce saga seeds of around 100-150 kg/year (Situmeang, 2019). The weight of tree saga seeds is around 0.26 gram/grain (269.5 gram/1000 grains) or per 1 kg of saga seeds there are 3,711-3,750 grains (Suita. 2013). Report of Jaganathan et al. (2018) the weight of saga seeds is 30.6 grams/100 seeds with a DM of 92.2%. Each pod contains 10-12 seeds or each hectare can reach 2000-5000 kg of seeds (Kumoro, 2019). The productivity of saga tree plants is influenced by several factors including seed scarification during seeding, cutting age, defoliation, level of shade and availability of groundwater (Maulana, 2021). Defoliation is the cutting of plant parts that are above the ground (Subagiyo and Kusmartono, 2017). The cutting period will affect regrowth (regrowth), the level of productivity and quality (Hasan, 2012). Optimal defoliation of saga trees with a cutting period every 3 months by cutting at the branching points of the branches, produces BK, BO (organic matter) and PK respectively 2.31 kg BK/tree (23.10 tons BK/ha); 2.15 kg BO/tree (21.47 tons BO/ha) and 0.37 kg PK/tree (3.74 tons PK/ha) (Kamaliyah et al., 2019). Scarification (skin injury) is a way to provide impermeable seed conditions to become permeable through pre-treatment such as injury (throwing, sanding, cutting with nail clippers), soaking (hot water, strong acid) and burning (Hasan, 2012). Injuries with nail clippers on tree saga seeds can maximize seed growth, namely it takes 8.71 days to grow and the seed vigor index is 2.28 seeds/day (Prananda, 2018). Germination of scarified saga tree seeds with sanding was higher than without scarification with indicators of

increased germination rate of 75%, germination rate of 13%, germination simultaneity of 76%, and normal sprout dry weight of 0.32 grams (Juhanda et al., 2013). Scarification by immersing H<sub>2</sub>SO<sub>4</sub> at 80% concentration for 25 minutes is the most efficient combination to break dormancy of tree saga seeds in terms of germination time, root length and hypocotyl length (Aprelia, 2020). Yuniarti (2002) reported that breaking the dormancy of saga tree seeds by soaking them in H<sub>2</sub>SO<sub>4</sub> for 30 minutes resulted in a germination rate of 92% and by filing them then soaking them in cold water for 24 hours resulted in a germination rate of 77.33%. Breaking the dormancy of tree saga seeds can be done by soaking in hot water at 90 0C and optimum germination of tree saga seeds is planted at a depth of 3 cm (Jaganathan et al., 2018). Saga tree seed growth is influenced by the level of shade and soil moisture content. Saga tree seedlings aged 12 weeks can grow in stress with a soil water content level of 40% field capacity and a shade level of up to 80% and a temporary wilting point at a water content of 12.2% (Maulana, 2021). Saga trees can grow optimally when water is provided at 100% field capacity (Prasetya, 2018). The level of shade will affect the process of plant photosynthesis in the leaves. Saga tree seed growth under 40% shade can increase growth and biomass (Krishnan and Rajendraprasad, 2000). Saga tree seeds require 60 ml of water until the age of 8 weeks of planting and increase by 20% every week to get maximum growth in the number of leaves and number of branches (Putra, 2018).

## 2. Perumusan Masalah

The formulation of the problem in this study was to see the results of the effectiveness test of saga tree leaf extract on the growth of e coli bacteria

## 3. Tujuan Penelitian

The purpose of this study was to see the results of the effectiveness test of saga tree leaf extract on the growth of e coli bacteria.

## 4. Manfaat Penelitian

The benefit of this study is to see the results of the effectiveness test of saga tree leaf extract on the growth of e coli bacteria which can be used as a reference and for developments in the world of health for patients.

## II. METODE

### Tools

Tools used in this research are aluminum foil, autoclave, stirring rod, Bunsen, beaker glass, blender, petri dish, porcelain dish, scissors, *hotplate*, incubator, caliper, ose wire, disc diffusion paper, Erlenmeyer flask, micropipette, oven, dropper pipette, bath, test tube rack, *rotary evaporator*, spatula, test tube, analytical balance.

### Material

The materials used in this research are ammonia, sulfuric acid, anhydrous acetic acid, hydrochloric acid, distilled water, pure culture of *Escherichia coli* obtained from North Sumatra University laboratory, magnesium powder, raw saga tree leaves (undried) obtained from around the Karya road, Medan Johor sub-district, North Sumatra province, ethanol 96%, FeCl<sub>3</sub>, chloroform, *dimethylsulfoxide* negative control, positive control *amoxicillin* tablet 500 mg, BaCl<sub>2</sub>·2H<sub>2</sub>O 1.175%, DMSO solution, *nutrient* agar (NA), Dragendorff's reagent, Lieberman-Bouchardat reagent.

## **Research Procedure**

### **Sorting, drying saga tree leaves**

Taking the saga tree leaves in order to get leaves that are fresh, not too old, not too young, and with the same size. The saga tree leaves then washed with flowing water and dried by using incandescent lamps (Marjoni, 2016)

### **Making simplisia powder**

Making simplisia powder (saga tree leaves) is saga tree leaves which have been dried then mashed with blender. Then the powder is weighed as much as 1 kg and then put into a container for extraction purposes by the maceration method.

### **Making medium for the growth of test bacteria**

#### ***Tools and materials sterilization***

Tools used in this research were sterilized first. Non-glass tools were sterilized by autoclaving at 121°C in 15 minutes. While glass tools were sterilized by using the oven at 180°C in 2 hours (Ratu, dkk. 2010).

#### ***Inclined Agar Medium for Bacterial Inoculum***

4.5 g of *nutrient* agar (NA) was taken, dissolved into 180 ml of distilled water using an Erlenmeyer. Then homogenized with a stirrer on a water bath until it boils. 5 ml each was poured into 2 test tubes then sterilized and covered with gauze. Media was sterilized in autoclave at 121°C for 15 minutes, then left at room temperature for  $\pm$  30 minutes until the media solidifies at an incline of 30°C. After solidifying inoculate the microorganism into the test tube and incubate at 35°C for 18-48 hours (Ngajow dkk, 2013).

#### ***Basic Media and Growth Media***

Basic media was made with weighing 4.5 grams of nutrient agar, then dissolved in 180 ml of distilled water using an Erlenmeyer (Ratu dkk. 2010)

#### **Test Bacteria Suspension**

Test bacteria on an inclined agar medium were taken with a sterile ose wire then suspended into a tube containing 2 ml NaCl 0.9% solution until the turbidity is the same as Mc. Farland standard solution turbidity (Ngajow dkk, 2013)

#### **Making the Concentration of the Saga Tree Leaf Extract Test Solution**

Saga tree leaf extract was weighed as much as 2.5 grams, then dissolved into DMSO in a 5 ml volumetric flask reaching the mark line and obtaining the extract concentration is 500 mg/ml. then the solution was re-melted with DMSO until obtaining the extract concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.123%.

#### **Antibacterial Effectiveness Testing**

Testing the antibacterial effectiveness of saga tree leaves (*Adenathera Pavonina*) was done with the disc diffusion method. Disc paper used has a circle diameter of 6 mm. NA media which has been melted poured into the petri glass. Then 0.5 ml of the suspension was taken and put into the petri glass containing NA media. Then homogenize by shaking. After hardening this medium is used for the inhibition zone test. On the petri glass place the disc paper that has been treated, which is the negative control that is given DMSO solvent, disc paper given the antibiotic amoxicillin 500 mg tablet as the positive control, and disc paper treated with ethanol extract of saga tree leaves, by dipping the disc papers in each concentration of the test extract then left them for 30 minutes in order for the solvent to be absorbed into the disc, then put on the hardened media. Then incubated at 36-37°C for 24 hours. Then measurement of bacterial inhibition zone was carried out by measuring the clear area that forms around the disc paper (Refriana dkk, 2011)

### III. HASIL PENELITIAN

#### Plant Identification

Identification result done by “Herbarium Medanense” North Sumatra University, the sample studied was pandanus amaryllifolius Roxb.

**Table 1.** Results of organoleptic examination of saga tree leaves

Inspected Components	Fresh Leaves	Simplisia
Shape	Long Thin	Powder
Smell	Fragrant	Fragrant
Taste	Bitter	Bitter
Color	Green	Greenish brown
Size	Length 10-35 cm Width 3-8 cm	Smooth

#### Saga Tree Leaf Extraction Result

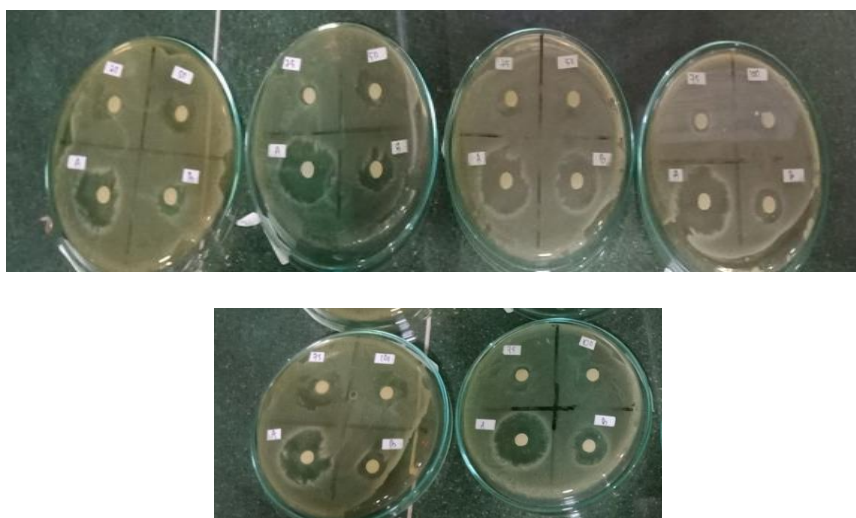
According to the extraction process done with maceration method using ethanol 96% solvent obtained a thick blackish brown extract with an extract yield of 4.988%.

#### Phytochemical Screening Results

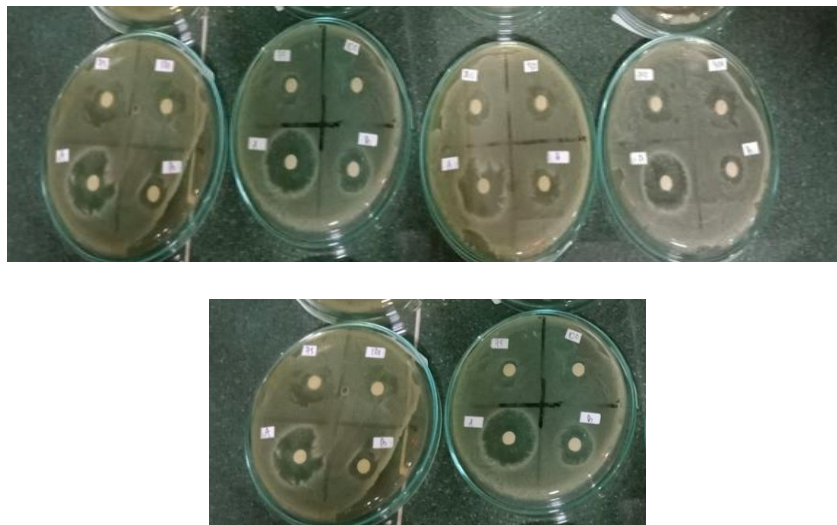
Phytochemical screening results towards this research show that the simplicia of saga tree leaves contain alkaloids, flavonoids, steroids, tannins.

#### 4. Discussion

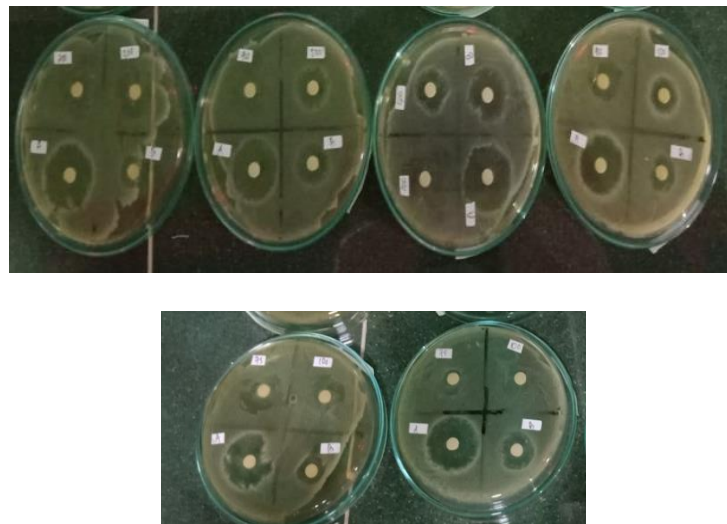
From the results on the effectiveness test of saga tree leaf extract (*Adenanthera Pavonina*) against the growth of *Escherichia coli* bacteria showed that all concentrations of saga tree leaf extract had anti-bacterial power against *Escherichia coli* bacteria. Observations were made after the media that had been mixed with the *Escherichia coli* bacteria suspension incubated at 37°C for 24 hours to see whether or not there is an inhibition zone around the disc papers that have been made on the media. Research results showed that there was a clear zone formed on each disc papers that had been dipped in each concentration of saga tree leaf extract namely 3.123%, 6.25%, 12.5%, 25%, 50%, 100%. Clear zone measurement were carried out using caliper.



**Picture 1.** Zone of Inhibition in *Escherichia coli* First Try



**Picture 2.** Zone of Inhibition in *Escherichia coli* Second Try



**Picture 3.** Zone of Inhibition in *Escherichia coli* Third Try

Note: A=Amoxicilin tablet (positive control); B=DMSO (negative control)

Based on the results of research using saga tree leaves simplicia (*Adenantha Pavonina*) with 8 treatment groups in 3 tries showed the diameter of the inhibition zone occurred in each group with different effectiveness. So, in order to see which group has the best effectivity or largest area of inhibition against each bacterium, the average is in the table below:

**Table 2.** The Average Area of the Inhibition Zone on *Escherichia coli* Bacteria

No.	Test Concentration (mg/mL)	Average Area of Inhibition Zone
1	Control -	0
2	3,123 %	5,5
3	6,25 %	6,1

4	12,5 %	7,3
5	25 %	8,8
6	50 %	10,8
7	100%	12,6
8	<i>Amoxicillin</i>	14,6

Results obtained from table 2 show that saga tree leaf extract with 100% concentration has the biggest average in zone of inhibition diameter which is 12.6. According to Susanto, Sudrajat and Ruga (2012), if the diameter of the inhibition zone is 5 mm or less, the inhibitory activity is categorized as weak, diameter of the inhibition zone is 6-10 mm it is categorized as medium, diameter of the inhibition zone is 11-20 mm it is categorized as strong, and if diameter of the inhibition zone is 21 mm or more then the inhibition activity is considered very strong. According to the research results show that the saga tree leaf extract produce inhibition zone in strong category for *Escherichia coli* bacteria.

#### IV. KESIMPULAN

Saga tree leaf extract (*Adenanthera Pavonina*) has anti-bacterial effectiveness against the growth of *Escherichia coli* bacteria marked with the zone of inhibition which formed around the disc papers. The most effective concentration of saga tree leaf extract in inhibiting the growth of *Escherichia coli* bacteria is at a concentration of 100%, with an average value of the largest inhibition zone in the diameter of 12.6 mm (strong category) against *Escherichia coli* bacteria.

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