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Phytochemical Screening and Antimicrobial Activity from *Sonneratia caseolaris* Fruit Extract

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Abstract. A study out to investigate the phytochemical screening and antimicrobial activity of *Sonneratia caseolaris* fruit, a mangrove plant from Maros Regency, South Sulawesi, Indonesia. The *Sonneratia caseolaris* extract was prepared by two different solvents: ethyl acetate and ethanol 70%. We tested the extract of the *Sonneratia caseolaris* for antioxidant activity by using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The phytochemicals were extracted separately with ethyl acetate and ethanol 70% by maceration. The evaluation of antibacterial activity for the different solvent with a different concentration of *Sonneratia caseolaris* fruit was measurements of inhibitory power are measured by measuring the diameter of the clear zone formed. In a phytochemical test *Sonneratia caseolaris* fruits extracted by ethyl acetate solvent confirmed the presence of flavonoids, saponin, tannin and phenolic and extracted by using ethanol 70% solvent confirmed the presence of alkaloid, saponin and phenolic. The antioxidant activity as indicated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of the *Sonneratia caseolaris* fruits extract from the species was found to be quite appreciable. The antioxidant scavenging activity of *Sonneratia caseolaris* fruit extract by ethyl acetate solvent was 0.38% and by ethanol solvent was 0.08%. Antimicrobial activity of *Sonneratia caseolaris* fruits ethyl acetate extract obtained significant ($P<0.05$) to *Escherechia coli* and *Candida albicans* but not significant to *Staphylococcus aureus*. The *Sonneratia caseolaris* extracted by ethanol 70% solvents was highly promising among *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* with level of significance was from $P<0.05$. The study suggests *Sonneratia caseolaris* fruits as a potential source of bioactive compounds with stable antioxidative and antimicrobial properties and can be used as natural antimicrobial/antioxidative agents in clinical, pharmaceutical and food processing industries.

Introduction

Infectious diseases are one of the main causes of death in developing countries. Microbes with multi drugs resistance characters are a source of concern that is now developing. This is due to the use of antibiotics carelessly to control a disease [1]. However, the use of synthetic antioxidants is increasingly limited due to their toxicity and health risks [2]. Therefore, the discovery of new antioxidant and antimicrobial agents from natural origin is an urgent need for clocks and plants can be a good source for this purpose. The tradition of using plants as a means of curing diseases is in many parts of the world since time immemorial. Moreover, nearly 30% of modern medicine used today comes from plants and extracts. These medicinal plants have a major role in homeopathic or ayurvedic medicines [3]. A number of plants reported to be rich in phytochemicals that have strong activity against human pathogens that have been found to be important both clinically and pharmacologically and various parts of these mangroves have been widely used in traditional medicine by traditional healers and local communities for a long time [4]. Various parts of this mangrove plant have been widely used in traditional medicine by traditional healers and local

communities for a long time and are known to produce various types such as steroids, triterpenoids, saponins, flavonoids, tannins, alkaloids etc.; These secondary metabolites have been shown to be basically responsible for biological activities such as antioxidant, antibacterial, antilarval, antiviral, antifungal, anti-insect, etc. along with other beneficial activities found associated with this plant [1].

Sonneratia caseolaris L. is one of the plants of the Sonneratiaceae family, which is known as a Mangrove with the local name "Pidada Merah." In Indonesia, this plant can easily be found in coastal and estuary areas where other plants are difficult to grow [5]. Several studies have reported that almost all parts of the plant have pharmacological properties, such as astringent, antiseptic [6], 2 analgesic, anti-inflammatory [7], antimicrobial [8,9,10], antidiabetic [11], antioxidants [12,13]. It is also used as traditional cosmetics by local people on the island of Borneo, Indonesia (especially the Dayak Tribe) is called "cold powder" [14]. These plants contain secondary metabolites such as flavonoids [15], phenolics, terpenoids, steroids, and alkaloids [7,16,17]. [18] In addition, research have been conducted on the content of pedada fruit were twenty-four components consisting of eight steroids, nine triterpenes, three flavonoids which function as antioxidants that can neutralize free radicals and can prevent diseases such as cancer, diarrhea, effectively repels viruses, effectively avoids thrombus, can prevent atherosclerosis, as an allergic repellent, and four carboxyl benzene derivatives.

Methods

Preparation of Plant materials

Sonneratia caseolaris plant samples were collected from the Maros Regency, South Sulawesi Province. Pedada fruits of this species was used for preparation of the solvent extracts.

Extraction Procedure

The mashed samples in powder form were weighed 500 grams and then macerated using ethanol 70% and ethyl acetate solvents as much as 5000 mL for 24 hours (combined from 3x extraction). Next, the filtrate was evaporated using a rotary evaporator. The results of extracts that have been evaporated are put in an oven at 40ºC until a thick extract is obtained. The extract obtained is calculated using formula 1:

$$\text{The yield} = \frac{(weight\ of\ extract)}{(dry\ weight\ of\ simplicia)}\ \text{X}\ 100\% \quad (1)$$

Phytochemical Screening

Analysis of the content of active compounds was carried out based on Harborner 1987 method. Analysis of the content of these active compounds was carried out in several ways, namely alkaloid test, flavonoid test, tannin test, saponin test, phenolic test.

Antibacterial Activity

Antibacterial activity is carried out by referring to the method of Murray *et al*., (1995) with little modification. The bacteria used are *Staphylococcus aureus* and *Escherichia coli*. To test the antibacterial activity of *Sonneratia caseolaris* fruits extract, the following were taken 100 µL of bacterial spectromes (108 CFU / mL bacteria) spread on nutrient agar (NA) medium. Then the paper disc was placed (9 mm in diameter), then it was pressed with 20 µL of extract with a concentration of 50 mg / mL of *Sonneratia caseolaris* fruits extract. As a positive control 10 µg Streptomycin was used on a paper disc. Aquadest is used as a pectrom control according to the extract solvent. The treatment was repeated three times and then incubated for 24 hours at 37ºC. After that, inhibitory power was measured by calculating the diameter of the clear zone formed [19].

Antifungal Activity

The antifungal activity of pedada fruit extract was carried out by the agar diffusion method by looking at the diameter of the inhibitory zone found around the perimeter of the paper disc. Tests were carried out on *Candida albicans*. The extract concentration tested was 50 mg / mL. The culture of each test mushroom is taken from the slant using an aseptic and rejuvenated needle in a liquid medium. In each media there is a spore density of 105 CFU / mL. Then Sabouraud was prepared in a

petri dish and each culture was scraped on top of the agar, then put a paper disc and continued by putting 20 µL of extract on the paper disc. Then it was incubated for 24 hours and measured the obstacle zone by using a ruler.

Antioxidant Activity

Antioxidant activity was carried out using DPPH method based on the method used by Millauskan *et al.* [20] with some modifications. A total of 0.025 grams of extract were dissolved in 10 mL of ethyl acetate and ethanol to get a concentration of 2.5 mg / mL repectively. Then 77 µL of extract solution was mixed with 3 mL of DPPH 6 x 10-5 M methanol solution. The solution was then stored in a dark place for 30 minutes at room temperature, and measured using spectrophotometer at wavelength 517 nm. DPPH radical concentration is calculated using the following formula:

$$\text{DPPH radical concentration (\%)} = \frac{((\text{A control} - \text{A sample}))}{((\text{A control}))} \text{ X } 100\% \quad (2)$$

Where, the control is the value of the absorbance of the control, and A sampel is the absorbance value of the extract tested in the sample.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA), and differences between means were determined by Duncan's multiple range test using the Statistical Analysis System (SPSS Statistics 17.0, SPSS Inc. Chicago, Illinois, USA) where applicable. $P \leq 0.05$ were regarded as significant.

Result and Discussion

Phytochemicals are non-nutritive plant chemicals possessing varying degrees of disease-preventive properties. They are invaluable sources of raw materials for both traditional and orthodox medicine [21] In this study, the phytochemical composition of the *Sonneratia caseolaris* fruits concentrates revealed the presence of alkaloids, flavonoids, saponin, tanin and phenolic (Table 1).

Table 1. Phytochemical Screening of *Sonneratia caseolaris* fruits from different Solvent Extract

Phytohemical Test	Extract	
	Ethanol 70%	Ethyl acetate
Alcaloid	-	+
Flavonoid	+	-
Saponin	+	+
Tannin	+	-
Phenolic	+	+

Notes: "+" means present; "–" means absent

The antioxidant activity as indicated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of the *Sonneratia caseolaris* fruits extract from the data was found to be quite appreciable. The antioxidant scavenging activity of *Sonneratia caseolaris* fruit extract by ethyl acetate solvent was 0.38% and by ethanol solvent was 0.08%.

The concentration of fruit extracts mangrove was utilized in this study include *E. coli; S. aureus; Candida albicans* and chloramphenicol a positive control. Whereas *E. coli; S. aureus; Candida albicans* was used at 2.5%, 5%, 7.5%, 10%, 12.5%, and 15% respectively.

The activity of *Sonneratia caseolaris* fruit extract using ethyl acetate solvent against the bacteria of *E. coli*, as can be seen in Figure 1a, showing the antimicrobial which was characterized by the partial inhibition zone around the paper disc. In Figure 1a, the highest and optimum antimicrobial activity partial inhibition zone was at a concentration of 15%. In Figure 1b demonstrated the antimicrobial activity of *S. caseolaris* fruit extracts against *S. aureus* which was characterized by the

partial inhibition zone at a concentration from 2.5% to 15% and the optimum inhibition zone at concentration 10% and 15%.

The antimicrobial activity of *S.caseolaris* fruit extract to *C. albicans* presented in Figure 1c and marked by the total inhibition zone around the paper disc. The highest and optimum of antimicrobial activity was demonstrated at consentration 15%.

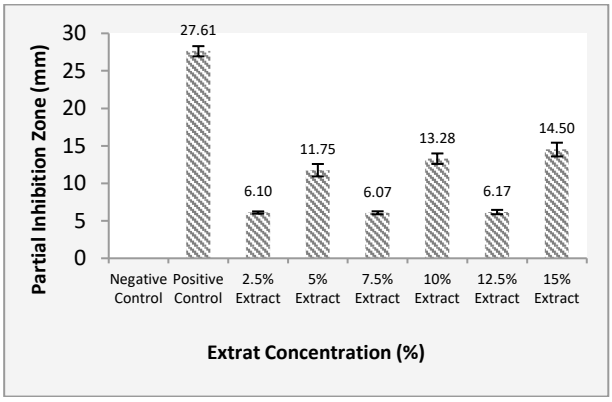

Figure 1a. Activity of *Sonneratia caseolaris* fruit extract using ethyl acetate solvent against *E. coli*

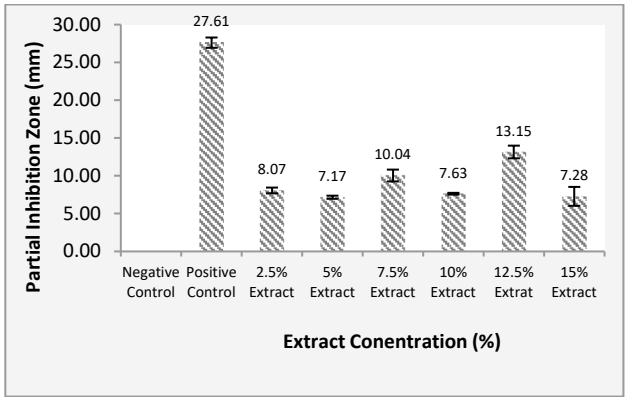

Figure 2a. Activity of *Sonneratia caseolaris* fruit extract using ethanol 70% solvent against *E. coli.*

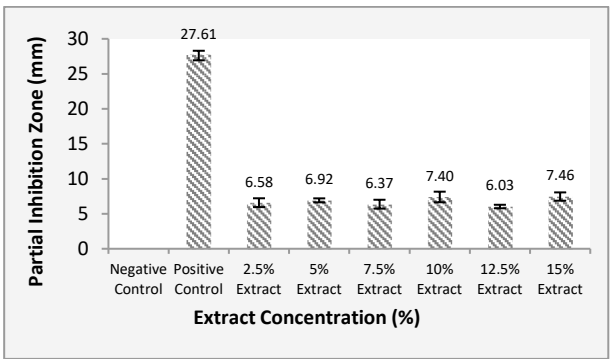

Figure 1b. Activity of *Sonneratia caseolaris* fruit extract using ethyl acetate solvent against *S. aureus*

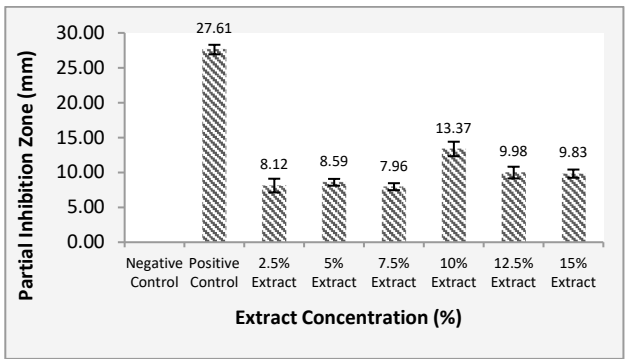

Figure 2b. Activity of *Sonneratia caseolaris* fruit extract using ethanol 70% solvent against *S. aureus.*

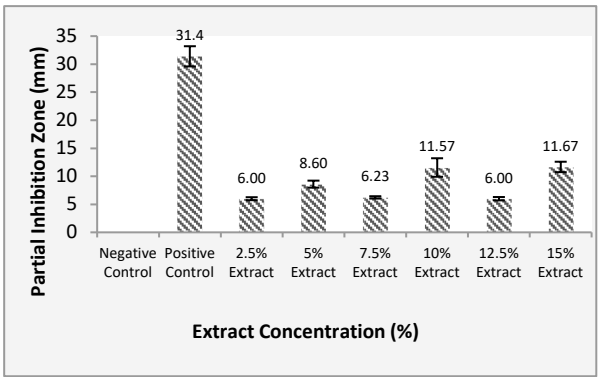

Figure 1c. Activity of *Sonneratia caseolaris* fruit extract using ethyl acetate solvent against *C. albicans*

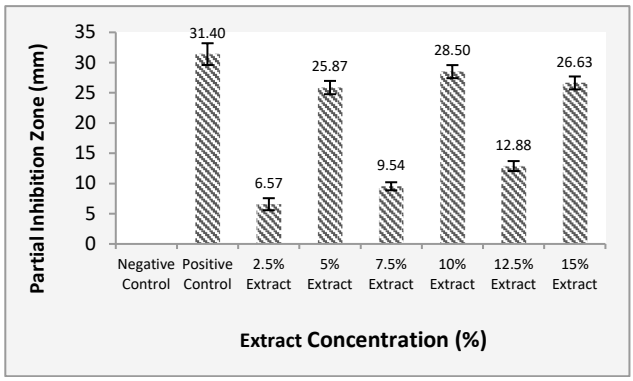

Figure 2c. Activity of *Sonneratia caseolaris* fruit extract using ethanol 70% solvent against *C. albicans*

The antimicrobial activity of *S. caseolaris* fruit extract using ethanol solvent 70% to *E. coli* presented in Figure 2a, which was characterized by the partial inhibition zone at a concentration from 2.5% to 15% and the optimum the partial inhibition zone at concentration 12.5%. As can be seen in Figure 2b, showed the antimicrobial activity *of S. caseolaris* fruit extract against the bacteria of *S. aureus* which was characterized by the partial inhibition zone around the paper disc. The highest partial inhibition zone was at a concentration of 10%, while at 12.5% has decreased. In Figure 2c, showed the highest and optimum of antimicrobial activity was demonstrated at 10% (28.50 mm) of *S. caseolaris* fruit extract to *C. albicans.* It can be concluded that the extraction of *Sonneratia*

caseolaeis fruit using 70% ethanol solvent with a concentration of 10% showed the most optimal inhibitory power compared to other concentrations and also with *Sonneratia caseolaris* extraction with ethyl acetate solvent at all concentrations.

The ANOVA analysis results showed P <0.05, this shows that ethyl acetate solvent in *Sonneratia caseolaris* fruits extraction significantly affected the growth inhibition of *E.coli* and *C. albicans*. However, it did not affect the growth capacity of *S. aureus* where P>0.05. *Sonneratia caseolaris* fruits extraction using ethanol solvent 70% significantly affected the inhibitory power of *E. coli, S. aureus*, and *C. albicans* based on ANOVA analysis results P<0.05.

The results of this study indicate that the extract of *Sonneratia caseolaris* contains phytochemical compounds and acts as an antibacterial. According to Qaiyum *et al.* [22], plants are the potential source of antioxidants, such as alkaloid, flavonoid, saponin, tannin, saponin, and phenol, which having the capability to scavenge the free radicals. It has been proven that these mechanisms may be important in the pathogenesis of certain diseases and aging. There are many reports that support the use of antioxidants supplementation in the reducing level of the oxidative stress and in slowing or preventing the development of complication associated with disease. It is also explained [23] that many synthetic antioxidants have shown toxic or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plants constituents have proved to show free radical scavenging/antioxidant activity.

Conclusion

The results obtained demonstrated that ethyl acetate and ethanol were the most effective solvent to extract phytochemical compounds compared to identified included alkaloid, flavonoid, saponin, tannin, phenolic. The antioxidant activity as indicated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of the *Sonneratia caseolaris* fruits extract by ethyl acetate solvent obtained had the highest was 0.38% than ethanol solvent. The *Sonneratia caseolaris* fruits extraction using ethanol solvent 70% significantly affected the inhibitory power of *E. coli, S. aureus*, and *C. albicans* and the highest partial inhibition zone was at a concentration of 10%, showed the highest and optimum of antimicrobial activity.

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