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Research Article

Antioxidant Activity of Senna (*Senna alexandrina* MILL.) Leaf Extracts

Aktsar Roskiana Ahmad[1,2]*, Abd. Malik[1,2], Selpida Handayani[1], Iskandar Zulkarnain[1], Amliati[1], Nur Lailatulqadri[1], Mardatilla Mamas[1]

ABSTRACT

Senna leaf plant (*Senna alexandrina* Mill.) is empirically effective in treating several diseases. Senna leaves contain saponins, alkaloids, glycosides, flavonoids, phenols, sesquiterpenes, tannins, and phytosterols. This study aims to assess Senna leaf extract's antioxidant activity, flavonoid, and phenolic content (*Senna alexandrina* Mill.) with various extraction methods and solvent variations. Senna (*Senna alexandrina* Mill.) leaves were extracted using Ethanol 96%, methanol, and ethyl acetate by maceration and ultrasonic methods. The extracts obtained were then tested for antioxidant activity and to determine the phenolic and flavonoid content using UV-Vis spectrophotometry. The highest total phenolic content obtained from maceration and ultrasonic extraction methods were ethanol extracts, with 12.96 and 14.08%. The highest total flavonoid content obtained from maceration and ultrasonic extraction methods was from ethyl acetate extract, with the same level of 7.4%. In conclusion, the highest phenolic and flavonoid content were obtained by ultrasonic extraction 14.08%. and 7.4%, respectively. Then, the reducing radical DPPH showed the potential antioxidant IC_{50} 119.9 μg/mL was collected by using UAE.

Keywords: Antioxidant, Senna leaf; Maceration; Phenolic, Flavonoid.

Aktsar Roskiana Ahmad[1,2]*, Abd. Malik[1,2], Selpida Handayani[1], Iskandar Zulkarnain[1], Amliati[1], Nur Lailatulqadri[1], Mardatilla Mamas[1]

[1]*Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, INDONESIA.*

[2]*Magister of Pharmacy, Universitas Muslim Indonesia, Makassar, INDONESIA.*

Correspondence

Aktsar Roskiana Ahmad

Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, INDONESIA.

E-mail: aktsar.roskiana@umi.ac.id

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INTRODUCTION

Senna plant (*Senna alexandrina* Mill.) part of the Fabaceae family, is widely distributed in Saudi Arabia. This species is also known as *Cassia senna* or *Cassia angustifolia* Vahl[1]. The plant is a shrub up to 3 meters tall. Senna's straight pale green stems have spreading branches consisting of four to five pairs of leaflets. The flowers are small and yellow in color, and the pods of the Senna plant (*Senna alexandrina* Mill.) are rectangular and wide and mostly contain about six seeds in each pod [2].

Senna leaves contain carbohydrates, saponins, alkaloids, glycosides, proteins, flavonoids, phenols, sesquiterpenes, tannins, and phytosterols[3]. *Senna alexandrina* Mill used as herbal extract in traditional medicine and found be rich in anthraquinone glycosides like sennoside A (SA) and sennoside B (SB), shows significant biological activity as a potential therapeutic agent[4].

Senna alexandrina Mill a versatile medicinal plant distinguished for its laxative effects, is distributed throughout the subtropical and tropical regions of the world. Studies on the antidiabetic and hypoglycemic effects of senna suggest that it might be a novel antihyperglycemic agent for the treatment of diabetes mellitus, especially type 2 diabetes. Extracts from different parts of senna showed considerable hypoglycemic effects in different animal models. Anthraquinone glycosides such as sennoside-A, sennoside-B, and saponin exhibit antihyperglycemic activity. Flavanols such as rutin are listed as showing anti-diabetic activity. These glycosides, saponin, and rutin, are found in high quantities in senna extract. *S. alexandrina* Mill. is also used in some areas of Iran to lower blood lipid levels. The aqueous extract of senna improves metabolic abnormalities and oxidative stress linked with diabetes, and reduces chronic hyperglycemia-related complications in rats. In vitro studies on the anti-diabetic effects of senna revealed that different solvent extracts of *S. alexandrina* Mill. can inhibit these enzymes. However, very few studies have been performed to understand the effect of *S. alexandrina* supplementation on HF diet-induced hepatic steatosis, hyperlipidemia and oxidative stress[5]

The treatment of obesity and its related complications is not easy. It is a time-consuming process, and sometimes patients express their unwillingness to participate in treatment approaches such as exercise. Very few drugs have been approved by the FDA to treat obesity or its related complications. The FDA recommends precautions against taking these drugs due to their undesirable side effects. Hence, alternative medicines, antioxidants, and polyphenolic-rich functional food might be good alternatives to treat obesity and its related complications. Considering the above discussion, this investigation was designed to assess the effect of *S. alexandrina* leaf powder supplementation on hepatic steatosis, hyperlipidemia and oxidative stress in HF diet fed rats[5-6]

The determination of solvent in the extraction process is determined by the type of compound to be extracted based on its polarity. Pharmaceutical industry regulations require that herbal extracts should only contain safe solvents such as water, methanol, ethanol, and ethyl acetate[7]. The type of solvent and the difference in solvent concentration affect the extraction rate, thus the solvent used during the extraction process must have the same level of solubility as the identified compound[8].

However, the extraction method has an effect on extracting the compounds. Maceration is one of the conventional methods that using a solvent with

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several shaking or stirring at room temperature. Other extraction method is Ultrasonic extraction or Ultrasonic Assisted Extraction (UAE). The use of ultrasonic waves in the extraction process of organic compounds contained in plants and grains using organic solvents can take place faster. The cell walls of the material are broken down by ultrasonic vibrations so that the content inside can escape easily. Ultrasonic waves are sound waves with frequencies above 20 KHz[9]. Therefore, this study aims to apply the maceration and UAE technique and assay Antioxidant of Senna leaves (*Senna Alexandrina* Mill.).

MATERIAL AND METHODS

Materials

Gallic acid, $AlCl_3$, CH_3COOK, DPPH, ethanol, ethyl acetate, folin-ciocalteu, quercetin, methanol, methanol p.a, Na_2CO_3.

Sample preparation

Senna leaves were obtained at an herbal shop in Makassar, South Sulawesi. The leaves were determined and deposited at Laboratory Pharmacognosy-Phytochemistry Faculty of Pharmacy Universitas Muslim Indonesia. Samples of Senna leaf (*Senna alexandrina* Mill.) were collected and powdered. Senna leaf powder (*Senna alexandrina* Mill.) was extracted by maceration method using Ethanol 96%, ethyl acetate, and methanol solvents for 3 x 24 hours. The extract obtained was evaporated using a rotary vacuum evaporator[9]. The ultrasonic method extraction was made by putting Senna leaf powder (*Senna alexandrina* Mill.) into an Erlenmeyer. Each solvent used was added, which consisted of methanol, ethanol, and ethyl acetate then extracted for 30 minutes using an ultrasonic bath. The filtrate obtained was later evaporated using a Rotavapor[9].

Determination of Phenolic Content

For each extract and standard, a concentration series was made then 0.4 mL of Folin-Ciocalteu reagent was added and shaken, and left for 4-8 minutes. About 4 mL of 7% Na_2CO_3 solution was added and shaken until homogeneous. Aquabidestilata was added up to 10 mL and allowed to stand for 2 hours at room temperature. The absorbance was measured at a maximum wavelength of 737 nm. Calibration curve was made for the correlation between gallic acid concentration and absorbance[10].

Determination of Flavonoid Content

For each test extract and standard, a concentration series was made. Each solution was taken 1 mL followed by an additional 3 mL of distilled water, added 0.2 mL $AlCl_3$ 10%, add 0.2 mL potassium acetate, and sufficed with aquadestilata up to 10 mL, stored for 30 minutes in a dark place with room temperature atmosphere. The absorbance was measured at UV-Vis spectrophotometry with a wavelength of 431 nm. The sample solution was made in three replications so that the flavonoid content was obtained as quercetin equivalent[12].

Antioxidant Assay

The test was performed by preparing 0.5 ml of quercetin solution and each extract of various concentrations that have been made. Then each was added 3.5 ml of DPPH. The solution was vortexed and incubated at 37°C in a dark room for 30 minutes. The absorbance was measured at a wavelength of 515 nm[11].

Data analysis

Data were analyzed with the Microsoft Excel application. Then the levels were calculated with the linear regression equation from the calibration curve $y = bx \pm a$ so that the sample concentration was known. Data analysis was also made using standard deviation (SD).

RESULTS AND DISCUSSION

The research related to comparison of maceration and ultrasonic methods on total phenolic content, total flavonoid content, and antioxidant activity of Senna (*Senna alexandrina* Mill.) leaf extracts with several solvents was observed. Extraction method in this study was performed by maceration and ultrasonic methods. The reason for selecting maceration and ultrasonic methods is for comparison because maceration method is a commonly used method for extraction by immersion. However, the maceration method takes a relatively long time and produces relatively fewer extracts. Some phenolic compounds are not heat-resistant or heat-damaged (thermolabile). Therefore, several non-thermal methods such as ultrasonic wave extraction are used to reduce the use of high temperatures during the extraction process and make the total extraction time shorter and the extract produced[13].

The solvents used for extraction were ethanol 96%, methanol, and ethyl acetate. The reason for choosing several solvents is that extraction with methanol, ethanol, and ethyl acetate solvents are able to separate important compounds in a material. In principle, a material will easily dissolve in a solvent of the same polarity[14].

Determination of phenolic content

Determination of phenolic content using UV-Vis spectrophotometry because the hydroxyl group in phenolic components with Folin-Ciocalteu reagent produces a blue color that can be detected by UV-Vis spectrophotometry[15]. In quantitative measurements using UV-Vis spectrophotometry, a blank solution is used. The blank solution used to determine total phenolic content is Folin- Ciocalteu reagent, 7% Na2CO3, and aquabidestillata.

Determination of total phenolic content for all extracts and gallic acid as a standard. Gallic acid is one of the stable phenols and is relatively cheap compared to others. The total phenolic content assay wich react with Folin-Ciocalteu method to determine the number of phenols contained in Ethanol, methanol, and ethyl acetate extracts of Senna leaves (*Senna alexandrina* Mill.)[16].

Based on the results obtained, the average content of total phenolics from maceration extraction obtained from Ethanol 96%, methanol, and ethyl acetate solvents were 129.691 mg EAG/g extract; 122.512 mg EAG/g extract; and 116.871 EAG/g extract, while the percent of total phenolic content from Ethanol 96%, methanol, and ethyl acetate solvents were 12.969%; 12.251%; and 11.687%, respectively. The average total phenolic content of ultrasonic extraction obtained from Ethanol 96%, methanol, and ethyl acetate solvents was 140.845 mg EAG/g extract, 132.576 mg EAG/g extract; and 120.076 EAG/g extract, respectively, while the percent total phenolic content of Ethanol 96%, methanol, and ethyl acetate solvents were 14.084%; 13.257%; and 12.007%, respectively. These results show that the highest percentage of total phenolic content is found in ultrasonic extraction with Ethanol 96% solvent. The data of this experiment showed on table 1.

According to the research that showed the phenolic content that were collected higher than maceration method. Its show the highest

Table 1: Phenolic content of Senna laves extracts.

Extraction methods	Solvents	Phenolic content (mg GAE/g)
Maceration	Methanol	12,250
	Ethanol	12,970
	Ethyl acetate	11,690
UAE	Methanol	13,260
	Ethanol	14,080
	Ethyl acetate	12,010

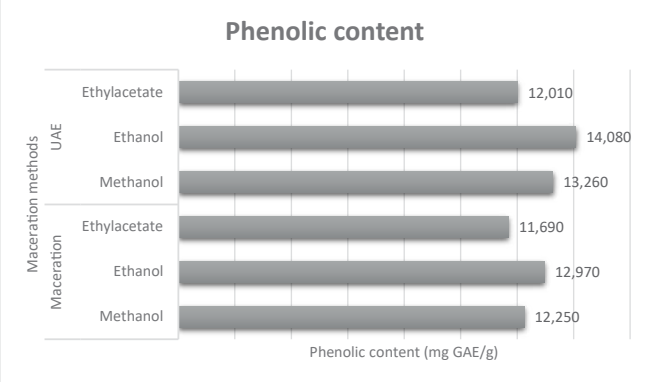

Figure 1: Graph of Phenolic content of Senna laves extracts.

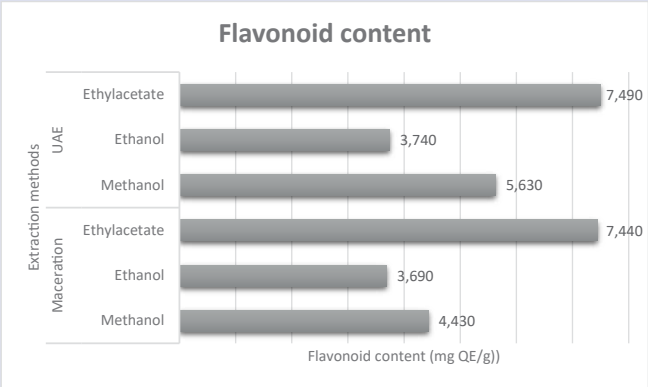

Figure 2. Graph of Flavonoid content of Senna laves extracts.

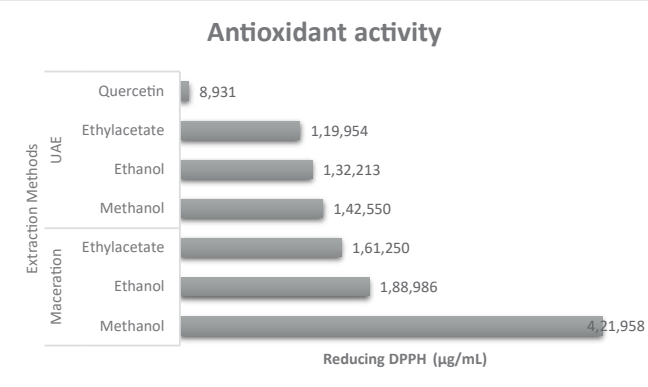

Figure 3. Graph of Antioxidant activity of Senna Leaves (*Senna alexandrina* Mill.).

phenolic content is extraction with ethanol solvent (Fig. 1). The higher total phenolic content of Senna (*Senna alexandrina* Mill.) leaves was obtained using Ethanol 96% solvent compared to methanol and ethyl acetate. The research of Riwanti *et al.* 2020 which determined the levels of phenolic compounds using ethanol and methanol solvents stated that ethanol and methanol solvents are polar extract phenolic compounds. Other research also determined the levels of phenolic compounds using ethanol, methanol, and ethyl acetate solvents stated that methanol and ethanol solvents have almost the same level of polarity so they are more effective in dissolving phenol compounds compared to ethyl acetate solvent whose level of polarity is below methanol and ethanol.

Determination of flavonoid content

In determining the flavonoid content of Ethanol 96%, methanol, and ethyl acetate extracts of senna leaves (*Senna alexandrina* Mill.), quercetin is used as a standard or comparator. Quercetin is a flavonoid of the flavonol group that has a keto group at the C-4 atom and also a hydroxy group at the adjacent C-3 and C-5 atoms. Determination of the maximum wavelength aims to determine the wavelength required to achieve maximum absorption. Measurement of analyte must use the maximum wavelength because at the maximum wavelength, the level of sensitivity or selectivity is high, so the change in absorbance for each unit of concentration is the greatest. The highest flavonoid content was obtained in the ultrasonic extraction method compared to the maceration extraction method. In addition, the highest flavonoid levels were successively produced by extraction with ethyl acetate, methanol, and Ethanol 96% solvents, as can be seen in the following figure.

The average content of total flavonoids from maceration extraction obtained from Ethanol 96%, methanol, and ethyl acetate solvents was 36.89 mgQE/g extract, 44.28 mgQE/g extract, and 74.40 mgQE/g extract, respectively. The results of the calculation of the total flavonoid content of Ethanol 96%, methanol, and ethyl acetate solvents were 3.689%, 4.428%, and 7.440%, respectively. While the results of the average content of total flavonoids from ultrasonic extraction obtained from Ethanol 96%, methanol, and ethyl acetate solvents were 37.41 mgQE/g extract, 56.29 mgQE/g extract, and 74.92 mgQE/g extract, respectively. And the calculation results of total flavonoid content from Ethanol 96%, methanol, and ethyl acetate solvents were 3.741%, 5.629%, and 7.492%, consecutively.

The effect of the choice of extraction method and solvent used on the flavonoid content of senna leaf extract (*Senna alexandrina* Mill.) is known. In Ethanol 96% and ethyl acetate, solvents did not have a significant effect on flavonoid results in both extraction methods. Whereas in methanol solvent, it can be seen that the highest flavonoid content is obtained from ultrasonic extraction, which is 56.29 mgQE/g extract or 5.629%, while in maceration extraction, it is 44.28 mgQE/g extract or 4.428%. But the highest flavonoid content results were obtained in ultrasonic extraction using ethyl acetate solvent, which is 74.92 mgQE/g extract or 7.492%.

The results showed that ethyl acetate solvent is able to extract flavonoid compounds better than Ethanol 96% and methanol. This shows that flavonoid components in senna leaves are more soluble in ethyl acetate organic solvent than other solvents. Ethyl acetate is a semipolar compound due to the structure of ethyl acetate containing polar groups (-COOR) and non-polar groups (-CH). Therefore, it can attract chemical compounds that are polar and non-polar. Ethyl acetate can dissolve semipolar compounds in cell walls, such as flavonoid aglycones. Flavonoid aglycones in plants are polyphenols that have chemical properties like phenol compounds[11].

Antioxidant Activity Test

Senna leaf extract was tested for free radical activity using DPPH. The DPPH method was chosen because it is simple, easy, fast, and sensitive and requires only a few samples. The solvent used to dissolve DPPH, and the sample was methanol. The solvent was used due to the reaction between the test sample as an antioxidant with DPPH as an anti-free radical. The calculation used in the determination of free radical capture activity is the IC_{50} (Inhibitor Concentration 50%) value, which describes the concentration of test compounds that can capture radicals by 50% [13].

Table 2: Flavonoid content of Senna laves extracts.

Extraction method	Solvents	Flavonoid content (m QE/g)
Maceration	Methanol	4,430
	Ethanol	3,690
	Ethyl acetate	7,440
UAE	Methanol	5,630
	Ethanol	3,740
	Ethyl acetate	7,490

Table 3: Antioxidant activity of Senna leaves (*Senna alexandrina* Mill.).

Extraction methode	Solvents	IC_{50} (µg/mL)
Maceration	Methanol	421,958
	Ethanol	188,986
	Ethyl acetate	161,250
UAE	Methanol	142,550
	Ethanol	132,213
	Ethyl acetate	119,954
	Quercetin	8,931

This study showed that antioxidant activity using a variety of solvents which had different results (Table 3). In line with extraction methods that including maceration and UAE extraction methods. The results show that the potential antioxidant activity is UAE with ethyl acetate solvent IC_{50} 119.954 µg/mL (Fig 3.).

CONCLUSION

The highest phenolic and flavonoid contents were obtained by ultrasonic extraction 14.08 mg GAE/g and 7.4 mg QE/g, respectively. However, the reducing radical DPPH showed the highest avtivity was collected by using UAE with ethyl acetate solvent IC_{50} 119,954 µg/mL.

CONFLICTS OF INTEREST

There's no conflict of interest in this article.

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