International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.6, pp 343-347, 2016

Radical Scavenging Activity of Leaf Extract of Edible Hibiscus (*Abelmoschus manihot* (L.) Medik) Using 1,1- Diphenyl-2-Picryl Hydrazil (DPPH)

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Abstract : Edible Hibiscus (Abelmoschus manihot(L.) Medik) family Malvaceae have bioactive compound that flavonoid functioning as a free radical scavenging. Research has been done on free radical activity leaf extracts of edible Hibiscus (Abelmoschus manihot(L.) Medik), compared with the free radical activity of quercetin using graduate extraction method. Three hundred and fifty grams of leaf edible Hibiscus (Abelmoschus manihot(L.) Medik) was macerated with graduate extraction using different solvents, respectly n-hexane, ethyl acetate and ethanol. All of extract assayed with DPPH as free radical activity at 517 nm wavelength and IC_{50} values obtained for n-hexane extract of 35.83 µg/mL, the ethyl acetate extract of 19.50 µg/mL and ethanol extract 12.36 µg/mL. Leaf extract of edible Hibiscus (Abelmoschus manihot(L.) Medik) has high potency as radical scavenging (IC_{50}< 50 µg/mL).

Key word : Edible Hibiscus (Abelmoschus manihot(L.) Medik),IC_{50} , DPPH.

Introduction

Antioxidants are compounds that can inhibit reactive oxygen species, nitrogen species and other free radicals, for example H_2O_2so as to prevent degenerative diseases such as cardiovascular, cancer and aging. This compound have a molecular structure which can provide electrons to the free radical molecules without being disturbed at all functions and can break the chain reaction.[3]Basic method of radical scavenging assay, when a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present) as shown on Figure 1.[8]

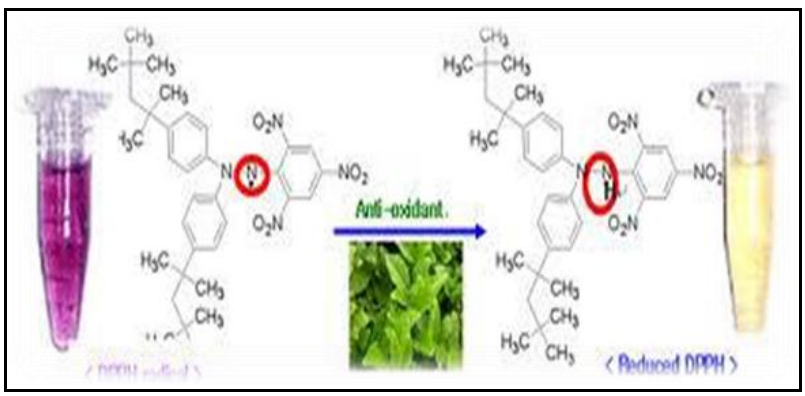

Figure 1 Basic reaction on DPPH assay

One plant that has the potential to be developed as a traditional medicine is edible Hibiscus (*Abelmoschus manihot*(L.) Medical) which some peopleth is plant are used as a vegetable, the other partcan be used as at raditional medicine, the root of which is used as a cure cancer anddiabetes.[10,13,15] Previous studies showed that flavonoids in *A. manihot* possess various biological activities, such as antiinflamatory, antibacterial and antioxidant.[4,5]

The species *Abelmoschus manihot* grows at low altitudes between 0 and 400 m, in the regions with a marked dry season of Indonesia, the Philippines, Papua New Guinea and New Ireland.[9]The flavonoid content in extract related with the effect of the samples. Flavonoid content such as quercetin, hyperoside, isoquercetin and myrcetin.[9]The different plant extracts will have different modes of action for curing diseases and in mixture form may exhibit enhanced activity than that of individual plants, which is known as 'synergistic action'. A particular principle in the pure form may have only a fraction of the pharmacological activity than it has in its plant matrix. This highlights the importance of using the plant as a whole or a mixture of plants for treating a disease.[11] The aim of research to measure antiradical scavenging on leaf extracts edible Hibiscus (*Abelmoschus manihot*(L.) Medik) by binding of free radical DPPH (1,1- Diphenyl-2-Picryl Hydrazil).

Material and Method

This reserach conducted on Laboratory of Pharmacognocy-Phytocemistry, Faculty of Pharmacy Universitas Muslim Indonesia, Makassar.

Collection of Plant Material

Edible Hibiscus (*Abelmoschus manihot*(L.) Medik) fresh plants were collected from Masohi Regency, Maluku. Determination of these plants were carried out at Department of Pharmacognosy-Phytocemstry, Faculty of Pharmacy Universitas Muslim Indonesia, Makassar.

Extraction plant materials& phytochemical screening

Dried sample (350 g) macerated using variation solvents, fisrt with n-hexane, ethyl acetate and ethanol respectively of 4000 mL in three days (this process was done in two times) then evaporated gave extract n-hexane 5.86 g, ethyl acetate 6.58 g and ethanol 15.82 g. Screening of phytochemical constituent on each extracts as the preliminary test.

Phytochemical analysis

Extracts were tested for the presence of active principles such as steroids / triterpenoids, saponins, flavonoids and phenols. Following standard procedures were used.[2,12]

Test Steroids and Triterpenoids:

Liebermann Buchard test - Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.[2,17]

Test for Saponins:

Foam Test – Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.[2,12]

Test for Flavonoids:

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.[2,12]

Test for Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.[2,12]

Radical Scavenging Assay

Radical scavenging activity of plant extracts against stable 2,2diphenyl 2 picryl hydrazyl hydrate (DPPH) was determined by the slightly modified method of Brand-Williams *et al*(1995). DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in colour (from deep violet to light yellow) was measured at 517 nm on a UV visible light spectrophotometer. The solution of DPPH in methanol 6×10^{-5} M was prepared fresh daily before UV measurements. Three ml of this solution was mixed with 100 microgram/ml concentration of individual plant extracts as well as herbal preparation. The samples were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula.

% Inhibition = [(A B –A A)/A B] × 100
Where: A B = absorption of blank sample (t= 0 min)
A A = absorption of test extract solution (t=15 mins) [14]

Result and Discussion

In this research, the extraction process used maceration method or immersion in a solvent without heating intended that the compounds contained not damaged. Maceration is done with n-heksan, ethyl acetate and ethanol. Ethanol capable of dissolving almost all substances, whether they are polar, semi-polarandnon-polar as well as its ability to precipitate proteins and inhibit the action of the enzyme so that it can avoid the process of hydrolysis and oxidation.[3]

From the results of maceration,the rendements of n-heksan, ethyl acetate, ethanolic extracts were obtained, they are 1.39 %, 1.88 % and 4,52 % respectively, of the weight of dry sample.Yield value indicate show large amount of content that can be extracted by solvents in percent(%).Antioxidant activity of Edible Hibiscus extract were tested with 2,2diphenyl 2 picrylhydrazyl hydrate (DPPH) method.

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as steroid, alkaloids, flavonoids, phenols, saponins, etc. The various solvent of Edible Hibiscus extracts have revealed the presence of steroids, saponins, phenols and flavonoids. Saponins and Steroids were present only in the whole Ethyl acetate and Ethanol extract while Phenols were found to be present only in Ethanol extracts and flavonoids were found to be present all extracts. From this analysis, ethanolic extract Edible Hibiscus have more constituents compared to n-heksan and ethyl acetat extracts. This difference may be caused by different growing conditions or also type of solvent. Flavonoids are polar compounds are generally soluble in polar solvents such as methanol, ethanol, butanol and water. The presence of sugar bound to the flavonoids tend to cause more soluble in water. Meanwhile reason loss of phenolic levels due to changes in the chemical structure or the possibility of the extraction method used.[6,7]The results of phytochemical analysis are shown in Table 1.

Table 1Result of Determination Chemical Constituens Extracts

Phytochemical Analysis	N-heksan	Ethyl acetat	Ethanol
Saponins	-	+	+
Flavonoids	+	+	+
Steroids	-	+	+
Phenols	-	-	+

Table 2 Inhibitory Activity of Edible Hibiscus extracts

	%Inhibition							
Sampel Ekstrak	10 ppm	20 ppm	40 Ppm	60 ppm	80 ppm	100 ppm	200 ppm	IC_{50} (ppm)
N-heksan	16.64	17.96	22.9	24.91	25.14	27.30	39.88	35.82
Etilasetat	15.68	16.52	22.8	28.14	34.73	47.18	55.08	19.50
Etanol	19.16	23.11	34.37	39.64	47.06	54.37	77.12	12.36

The parameters used to declare the antioxidant activity of DPPH is IC_{50} value that is the concentration of sample or standard that may lead to the inhibition of DPPH by 50 %.Based on the results of the study to the Table 2, the antioxidant activity of Edible Hibiscus Extract with DPPH hasIC_{50} value of extracts n-heksan 35.82 ppm, ethylAcetate 19.50 ppm and ethanol12.36 ppm. The antioxidant power levels according to Phongpaichit et al (2007)The antioxidant activity all extract are strongly active (IC_{50} between 10-50 ppm).[11]The inhibitory activity of edible hibiscus extracts are shown in Table 2.DPPH test is one of the most widely used test to estimating the efficiency of the performance of a substance that acts as a free antiradical.[8]Differences in antioxidant activity in an extract due to differences in the content of polyphenol compounds in which, the high content of polyphenols on an extract effect on the high antioxidant activity. Yanagida, (1997) reported that free radical scavenging activity also depends on the type of polyphenol.[16]The high content of antioxidant compounds in the extracts uses high free radical scavenging activity.[1]

Conclusion

Leaf extract of edible Hibiscus (*Abelmoschus manihot*(L.) Medik) has high potency as antiradical scavenging (IC_{50}< 50 µg/mL).

Acknowledgments

This reserach was supported by the Laboratory of Pharmacognocy-Phytocemystry, Pharmacy of Phaculty, Universitas Muslim Indonesia, Makassar.

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