International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Comparative Study on the Phytochemical Screening and Antioxidant Activity of *Melastoma Malabathricum* and *Gynura Procumbens* Extracts

Nor Azila Maskam

Chemical and Food Technology Department, Polytechnic Tun Syed Nasir Syed Ismail, Pagoh, johor, Malaysia
Email: *norazilamaskam[at]gmail.com*

Abstract: ***Malaysia is blessed to have a variety of natural resources of plants that are given beneficial for human well-being. Plants are a rich source of natural phytochemical compounds such as secondary metabolites and antioxidant. Phenolic compounds are the richest secondary metabolites commonly found in both edible and non-edible plant. Antioxidants play an important role to protect damage caused by oxidation process. The present study was designed to determine the antioxidant activities of aqueous leaves extract of Melastomamalabathricum (senduduk) and Gynurap procumbens (sambungnyawa). M. malabathricum usually used to treat diarrhoea, dysentery, hemorrhoids, cuts and wounds, toothache, and stomachache. Traditionally, G. procumbens commonly used in several countries to treat a wide range of health conditions, including kidney discomfort, arthritis, diabetes, constipation, and hypertension. In this study, the sample was extracted using aqueous extraction. Next, the phytochemical screening test was done to determine the presence of tannin, terpenoids, steroid, phenolic, flavonoids and saponin content. The antioxidant activities in this study were determined by total antioxidant capacity, DPPH (1,1-diphenyl-2-picrylhydrazine) radical scavenging assay. From the phytochemical screening test of aqueous extraction of M. malabathricum showed the presence of phenolic, flavonoid, tannin, steroid but absence of saponin, tannin and steroid. Whereas, G. procumbens screening, it shows presence of all phytochemical but absent of saponin. The antioxidant activity expressed as IC50 ranged from 0.31mg/ml for G. procumbens and 0.42 mg/ml for M. malabathricum. The potency of radical scavenging effect of G. procumbens extract was greater than M. malabathricum. Further study is needed to identify and isolate the exact active compound underlying this high antioxidant activity.***

Keyword: Phenolic compounds, Antioxidant, DPPH, IC50

1. Introduction

Plants extracts give very are important information, due to their chemicals produced through primary or secondary metabolism in their natural form. Many Malaysian plants are usually used as side dish in daily mealinconspicuously contain phytochemical that give a lot benefit to the consumers (Vijendren et al., 2015). They also used as traditional medicinal either for remedy or even as a healthful purpose. (Embuscado et al., 2015) Very few research has been conducted for these selected herbal plants. Thus, two types of plants were selected to determine their phytochemicals and antioxidant activities which are *Melastoma Malabathricum* and *Gynura Procumbens*. The research of natural product like natural antioxidants from plant and herbal extract not only healthier and safer than the artificial but it also maybe more acceptable among nowadays consumers.

2. Literature Review

2.1 *Melastoma Malabathricum* (Senduduk)

According to (Zheng et al., 2021), Melastoma plants originate in the tropical and subtropics regions, with a total of more than 4000 species worldwide. In Malaysia at least contain 12 species, many of them are used by natives in folk medicine. In general, usually paste made from leaves and roots was apply to reduce scars from scurf or smallpox. A broth of the roots is utilized to soothe toothaches and to mellow the feet. A broth of the roots and leaves or roots alone is taken by ladies after childbirth. The mixing of roots and leaves are used to give strength to the uterus after childbirth, also it believed to reduce menstrual bleeding and cramps and relieve postmenstrual syndrome. Traditionally, combination of root and leaves in powder form could aid the healing process when apply at wounds or pox scars or used to relieve the discomfort of hemorrhoids. (Oladele et al., 2015). Also, the shoots can be ingested to treat puerperal infections, high blood pressure, and diabetes.

2.2 Gynura Procumbens (Sambung Nyawa)

Gynura procumbens, is a small plant 1–3 m in height. The stems are fleshy and leaf is commonly consumed and scientifically it has been shown to be safe for consumption. In Malaysia, the fresh leaves of *G.Procumbens* are commonly eaten raw and in Thailand, the leaves are also used for cooking (Kaewseejan et al., 2015). In Malay, *G.Procumbens* is called *Sambung Nyawa* which means “prolongation of life” whereas in Chinese, it is called Bai Bing Cao which means “100 ailments”. This is because it has been promoted in traditional medicine both systemically and topical application for treatment of different types of illnesses and diseases.

2.3 Antioxidant

Antioxidants play an essential role in the body as they quench free radicals. By damaging healthy cells, free radicals can leave the body vulnerable to advanced ageing, cardiovascular problems and degenerative diseases. The free radicals can be caused by many factors including sun exposure, pollution, stress, poor diet, alcohol and cigarette smoke (Nagarajappa KA et al,, 2015).

The use of natural antioxidants in food, cosmetic, and therapeutic industry would be good alternative for synthetic antioxidants because of their low cost, high compatibility with dietary intake and no harmful side effects (Skaperda et al, 2021). Many antioxidant compounds have been identified as free radical or active oxygen scavengers from plants. The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

3. Methodology

3.1 Plant Material and Sample Preparation

This study only used *M.malabathricum* and *G.procumben* leaves. The leaves must have rinsed with tap water followed by distilled water to remove the dirt on surface. Next, the leaves were tossed and dried. The leaves were weighed and dried inoven, at 40˚C for about ≥ 72 hours. After drying, the dried leaves were milled and store at 0-4˚C.

3.2 Aqueous Extraction

The aqueous extract of dried plant leaves was made in the distilled water. About 25 grams of each plant leaves powder (*M. malabathricum* and G.*procumben*) were taken and mixed in 250 ml of distilled water. The mixture was heated at water bath at 60˚C for 24 hours. The solution was filtered through muslin cloth, the filtered extracts are collected and placed at biomedical freezer in a -21℃ and dry at freeze dryer to at -50 until - 55℃ and pressure -0.26 until -0.37 for 48 hours.

3.3 Equipment and Apparatus

The apparatus and material used were test tube, spatula, glass rod, volumetric flask, aluminum foil, beaker, drying oven, biomedical freezer, freeze dryer UV-Visible Spectrometers.

3.4 Chemical and Reagents

The chemical and reagent used in this research were ethanol, aluminum chloride, ferric chloride, chloroform, Sulfuric Acid, distilled water, DPPH reagent (2,2-diphenyl-1-1-picrylhydrazyl), Butylated Hydroxytoluence (BHT).

3.5 Phytochemical Screening Test

5 mg of samples were weighed and each sample is diluted with 20ml of distilled water. The sample is stirred until fully dissolved in the solvents.

3.5.1 Tannin using Ferric Chloride Test

A few drops of the crude extract sample were placed in a test tube. About 2 ml of distilled water was added and the mixture was shaken and then placed in a water bath for 5 minutes at 80°C to 100°C. Next 2 or 3 drops of 0.1% iron (III) chloride was added and observed for brownish green or a blue-black coloration.

3.5.2 Terpenoids using Salkowski Test

A few drops of the crude extract sample were added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration indicates the presence of terpenoids.

3.5.3 Flavonoid Using Shinoda's test

A few drops of sample were dropped in the test tube. 5 ml of sodium hydroxide and 1ml of nitric acid were added. The test tube was shaken and yellow coloration disappear indicates the presence of flavonoids.

3.5.4 Steroid using Libermann-Burchard Test

100 mg of was shake with chloroform in test tube and a few drops of acetic anhydride and 2ml Sulfuric Acid (H_2 SO_4) was added. If the upper layer turns to green color it shows the presence of steroid.

3.5.5 Saponin using Foam Test

2ml of sample was mixed with 2 ml of distilled water. Shake 15 minutes, if a like foam is obtained, saponin are present.

3.6 Determination of Antioxidant

3.6.1 DPPH ASSAY

3.6.1.1 Preparation of Stock Sample

The stock sample of *M. Malabathricum* and leaves *G.Procumben*. The 0.025g of sample was weighed into a small beaker. Dissolved with ethanol in 50ml of volumetric flask.The volumetric flask was closed and shake until DPPH completely dissolve.

3.6.1.2 Preparation of DPPH Assay Stock

The stock 2-2-diphenyl-1-picrylhdrazyl (DPPH) was prepared. The 0.004g of 1-1-diphenyl-2-picrylhdrazyl (DPPH) was weight into a small beaker. Dissolved the DPPH with ethanol and top up until 100ml of volumetric flask. The volumetric flask was closedand shake until DPPH completely dissolve.

3.6.1.3 Preparation of Standard Solution of Butylated Hydroxytoluence (BHT).

The stock of the Butylated Hydroxytoluence (BHT) was prepared. The 0.025g BHT was weight into a small beaker. It is dissolved with ethanol and top up until 50ml of volumetric flask. The volumetric flask was closed and shake until BHT completely dissolve.

3.6.1.4 DPPH Scavenging Activity

The scavenging action of stable 2,2-diphenyl-1-picryl dyhydrazyl free radical was determined. 2 ml of samples/standards were loaded, and followed by 2 ml of DPPH reagent and 2 ml ethanol. The mixtures were then mixed vigorously and incubated at room temperature in the

dark for 30 minutes and the absorbance was measured spectrophotometrically at 517 nm. (Ndezo et al., 2022). The free radical activity (% inhibition) was calculated using the formula given below:

DPPH Scavenging (%) or Percentage inhibition

Eg 1: $\frac{A0 - Ae}{A0} \times 100$

A0= absorbance of blank (negative control)

Ae= Absorbance in presence of sample

4. Analysis and Result

Phytochemical Screening Test

Various phytochemicals screening test was used to find the phytochemical contents that were present in the samples. The results of the tests are shown in Table 4.1. From the phytochemical screening of the aqueous extraction of *M. malabathricum* showed the presence of phenolic, flavonoid, tannin, steroid but absence of saponin, tannin and steroid. Whereas, from *G. procumbens* screening test, it shows presence of all phytochemicals but absent of s*aponin.*

Table 4.1: Result of the phytochemical screening test of samples

Sample	Phenolic	Tannin	Steroid	Flavanoid	Saponin
G. Procumbens	+++	++	+	+	-
M. Malabathricum	++	-	-	+	-

+++: Highly Present ++: Moderately Present +: Low Present -: Absent

The Percentage of Inhibition

The DPPH test gave an info on the reactivity of the sample with a stable free radical. The degree of reduction in absorbance measurement is denotive of the radical scavenging (antioxidant) power of the sample. IC50 value was determined by plotted graph of scavenging activity against the different concentrations of samples. The scavenging activity was determined by the percentage of DPPH reduction after 30minutes of reaction. The measurements were triplicate and their scavenging effects were calculated based on the percentage of DPPH scavenged.

Table 4.2: The percentage of inhibition *Melastoma Malabathricum, Gynura Procumbens* leaves and standard sample Butylated Hydroxytoluence (BHT)

Concentration (mg/mL)	BHT	*G.procumbens*	*M.malabathricum*
0.05	41.23	23.82	24.69
0.10	42.71	24.19	25.80
0.15	44.69	24.44	26.91
0.20	49.50	24.93	27.53
0.25	53.33	25.30	29.01
0.30	79.62	29.87	50.08
0.40	82.71	44.44	63.75
0.50	91.85	63.82	66.04

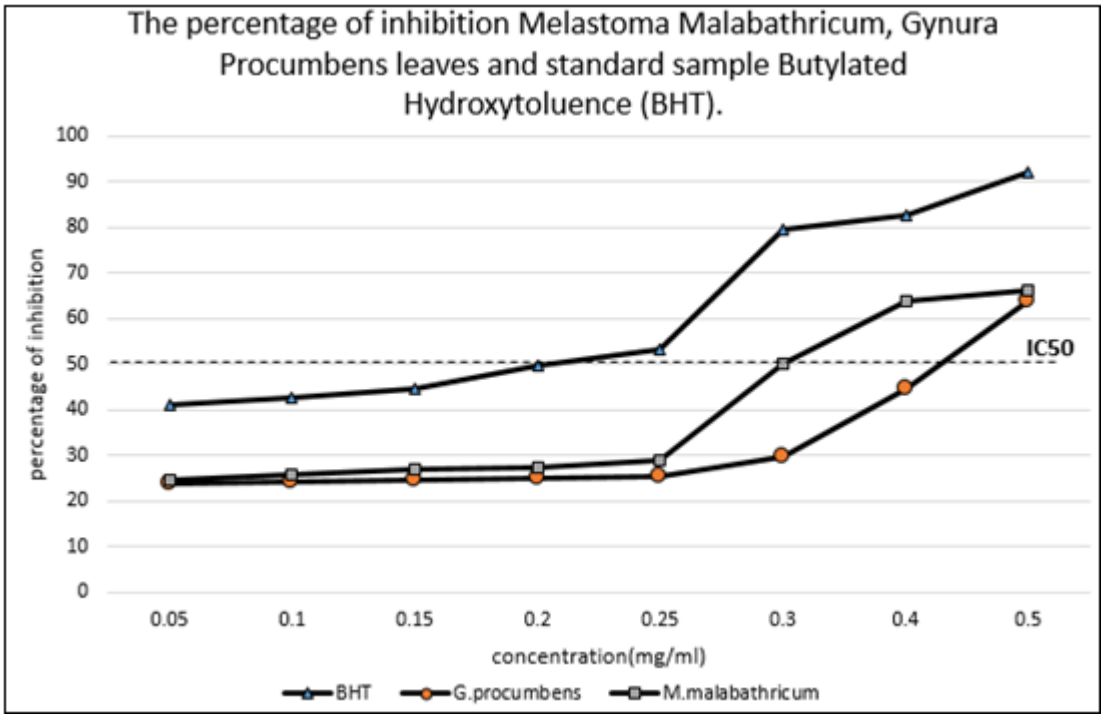

Figure 1: The percentage of inhibition of DPPH radical scavengingactivity at different concentration

The concentration of IC_{50}

The IC50 value was figured to determine the concentration of the sample needed to inhibit 50% of radical. The lower the IC50 value, the higher the ability of the sample act as antioxidant. In this research, the observed IC50 value showed that BHT exhibited highest antioxidant activity (0.20mg/ml) followed by *M. malabathricum* extract (0.31 mg/ml) and *G. procumbens* extract (0.42 mg/ml), respectively (Table 4.3). The less IC50 value, the more powerful of the antioxidant activities.

Table 4.3: IC50 value of DPPH radical scavenging activity of *M.malabathricum, G. procumbens* leaves and standard sample Butylated Hydroxytoluence (BHT)

Samples	IC_{50}Concentration (mg/ml)
Butylated Hydroxytoluence (BHT)	0.2
Gynura Procumbens	0.31
MelastomaMalabathricum	0.42

5. Conclusion

The results of this study have shown that the aqueous *M. malabathricum* and G.*procumben* leaf extracts have great potential as natural antioxidant to replace artificial antioxidant. Further study of the active components contains in these plant and the development of these new natural antioxidant used in oil and fat industry can be study in the future.

References

[1] Embuscado, Milda E. 2015. "Spices and Herbs: Natural Sources of Antioxidants – a Mini Review." Journal of Functional Foods 18: 811–19. https://www.sciencedirect.com/science/article/pii/S1756464615001127.

[2] J. O. Oladele and G. A. Ayoola, "Antioxidant activity among selected medicinal plants combinations (multi-component herbal preparation)," International Journal of Research in Health Science, vol. 3, no. 2, pp. 526–532, 2015.

[3] Nagarajappa KA, Divya P, Sreedevi, Ravi KS. (2015) Role of free radicals and common antioxidants in oral health, an update. British Journal of Medicine and Medical Research. 2015;9(4):1-12.

[4] Ndezo Bisso, B., Njikang Epie Nkwelle, R., TchuenguemTchuenteu, R., &Dzoyem, J. P. (2022). Phytochemical Screening, Antioxidant, and Antimicrobial Activities of Seven Underinvestigated Medicinal Plants against Microbial Pathogens. Advances in Pharmacological and Pharmaceutical Sciences, 2022, 1998808. https://doi.org/10.1155/2022/1998808

[5] Kaewseejan, N., Sutthikhum, V., Sirithon, S. (2015) Potential of *Gynura procumbens* leaves as source of flavonoid-enriched fractions with enhanced antioxidant capacity Journal of Functional Foods 12:120-128

[6] Skaperda, Z., Kyriazis D. I., Vardakas, P., Tekos, F., Antoniou, K., Giannakeas, N., & Kouretas, D. (2021). In vitro antioxidant properties of herb decoction extracts derived from Epirus, Greece. Int J FunctNutr, 2(5), 11. https://doi.org/10.3892/ijfn.2021.21

[7] Vijendren Krishnan, V., Syahida, A., &Maziah M.(2015)Antioxidant Potential in Different Parts and Callus of *Gynura procumbens* and Different Parts of *Gynura bicolor*. Volume 2015 |Article ID 147909

[8] Zheng, W. J., Ren, Y. S., Wu, M. L., Yang, Y. L., Fan, Y., Piao, X. H., Ge, Y. W., & Wang, S. M. (2021). A review of the traditional uses, phytochemistry and biological activities of the Melastoma genus. *Journal of Ethnopharmacology*, *264*(July 2020), 113322. https://doi.org/10.1016/j.jep.2020.113322