Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 11 [5]April 2022 : 23-26

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

DPPH scavenging activity of *Ficus septica* leaf ethanolic extract

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ABSTRACT

The study aims to determine the antioxidant activity of Ficus septica leaf ethanolic extract.TheDPPH scavenging activity test was conducted with four different level of concentrations at 100%, 75%, 50% and 25% respectively of Ficus septicaleaf ethanolic extract. The DPPH assay method was a modified version adapted from Clark et al. (2013), in which twenty microliters (20 L) of the extract was diluted appropriately in Dimethylsulfoxide (DMSO) with 180 L of DPPH in methanol (40 g/mL) in 96-well plate wells. The plate was left in the dark for 15 minutes before the absorbance of the solution was measured in a plate reader at 540 nm. The blank was DMSO, and the standard was Ascorbic Acid. The study showed that the leaf ethanolic extract of Ficus septica exhibited an antioxidant activity. The results also revealed that as the concentration of Ficus septica ethanolic extract increased, the percentage of DPPH inhibition decreased as compared to Ascorbic acid.

Keywords: *Antioxidant property, Ficus septica, DPPH scavenging activity, Ascorbic acid,*

Received: 15.02.2022 Revised: 02.03.2022 Accepted:21.03.2022

INTRODUCTION

Antioxidants can be found naturally in plants, animals, and microorganisms, or they can be synthesized chemically. Naturally occurring antioxidants can be isolated from their source material as pure compounds for possible use in nutraceutical/pharmaceutical applications. Higher plants and their constituents are high in natural antioxidants like tocopherols and polyphenols, which are abundant plant materials [6, 20]. By-products from the food and agricultural industries have been investigated for their potential use as antioxidants. Antioxidant activity has been discovered in the hulls, shells, and skins of nuts and cereals, citrus peels and seeds, canola meal, and fish viscera extracts [14, 4].

The DPPH radical scavenging assay is one of the most commonly used methods and is the first approach for assessing antioxidant activity. It is an ET-based method, with the HAT mechanism serving as a secondary reaction pathway in the assay [15]. The DPPH scavenging assay is based on electron donation of antioxidants to neutralize DPPH radical. The reaction is accompanied with color change of the DPPH measured at 517 nm, and the discoloration acts as an indicator of the antioxidant efficacy. The DPPH assay is a simple technique and requires only a UV spectrophotometer or an EPR (electron paramagnetic resonance) spectrometer. However, it has been argued that the scavenging of DPPH does not mimic the radical scavenging mechanism of antioxidants in real food or biological systems due to lack of oxygen radical in the assay [8]. Hence, this method is largely based on the assumption that antioxidant activity is equal to its electron donating capacity. Modifications to the assay have been proposed by different authors in an attempt to minimize the aforementioned problems and further simplify and automate the method [16].

*Ficus septica*is one of the oldest traditional medicine used by the Filipinos.The leaves were used as cure for skin disease, appendicitis, abscesses, poisonous snakebites and shortness of breath[19] while folks believed that it can cure skin diseases, gastrointestinal problems and others[18].

Many phytochemical studies of the *Ficus septica*has been conducted and showed to have anti-microbial properties and anti-oxidant properties. Presence of coumarins, flavonoids, saponins, steroids, alkaloids and tannins has been reported in the extracts of *Ficus septica*. In 2005, it was found out that itincludes (-)-tilosrebin (hauptalkaloid), tiloforin, septisin, and antofin, but it also contains flavonoids[3]. In 2002,it was reported the isolation of seven triterpene derivative, 13,27-cycloursan-3□-yl acetate, and two lignans from the non-alkaloidal fractions of the stem of *Ficus septica*[11].In the study conducted in 2017, phytochemical screening showed positive results with alkaloid, reducing sugar, saponin, terpene, phenol,

flavonoids, sterol, unsaturated lactone and deoxysugar[9]. Literature shows that antioxidant activity of plant extract can be explained by the fact that plants contain specific metabolites that are acknowledged to perform a range of purposeful activities [10]. Majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarines, lignans, catechins, and isocatechins[1]. The same concern reported that plants that are rich in phenolic and flavonoid compounds and induced antioxidant potentials[13].

MATERIAL AND METHODS

Plant collection and Extract Preparation

Fresh *Ficus septica*leaves were collected in Rizal, Cagayan. Leaves were thoroughly washed with distilled water for three times. The leaves were air dried under shade. The dried plant was pulverized using a disintegrator. The powdered samples were kept in airtight containers for extraction purposes. 150g of powdered *Ficus septica*leaves was mixed in 1,350ml of ethanol in erlenmeyer flask, covered with aluminum foil and stored for five days.

The mixture was filtered using analytical filter paper and the resulting filtrate placed in rotary evaporator. After the procedure, the extract was collected and stored in the refrigerator.

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

The method was adapted from Clark et al. (2013), twenty microliters (20 µL) of the extract was diluted appropriately in Dimethylsulfoxide (DMSO) with 180 µL of DPPH in methanol (40 µg/mL) in wells of a 96-well plate. The plate was kept in the dark for 15 minutes after which the absorbance of the solution was measured at 540 nm in a plate reader. DMSO served as a blank and Ascorbic Acid served as the standard.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Working procedure

Different volumes (2 - 20µl) of plant ethanolic extract treatments was made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 540 nm.

Antioxidant Assays

The % DPPH inhibition activity of the plant extracts was calculated using the formula,

$$\%DPPHInhibition = \frac{Abscontrol - Abssample}{Abscontrol} \text{ x } 100$$

Where, % Inhibition is the Radical Scavenging Activity; *Abs control* is the absorbance of DPPH radical + ethanol; *Abs sample* is the absorbance of DPPH radical + plant extract. The analysis was done in triplicates and the average readings were calculated[17].

RESULTS AND DISCUSSIONS

DPPH scavenging activity of Ficus septica leaf ethanolic extract

The quality of the DPPH scavenging activity of *Ficus septica* leaf ethanolic extract was determined by the percent inhibition values shown in Table 1.A decreasing percentage of DPPH inhibition was observed from the *Ficus septica*ethanolic extract with increasing concentration.

The same plant was studied for antioxidant activity of researchers in Indonesia. Ficus septicais locally know in Indonesia as “awar-awar”. Antioxidant screening of awar-awar leaves ethanol extract can reduce DPPH radicals or have antioxidant activity due to the flavonoid compounds present in the plant[7]. They described the antioxidant activity of *Ficus septica*as very strong compared to quercetin standard. Furthermore, in the study conducted in the University of San Carlos in Cebu, also proved the antioxidant activity of *Ficus septica*ethanolic extract, however of bark extract[12]. A similar observation can be inferred from the results of their study. Compared to the other treatments (25, 50, 100, 150, and 250 µg/mL), the greatest antioxidant activity (97.11%) was observed at 100 µg/mL among the test solutions and not at the highest concentration. They concluded that *Ficus septica* bark ethanolic extract showed antioxidant activity. In another study, it revealed that *Ficus septica* methanolic leaf extract has an antioxidant activity[2].

Table 1. Mean Percent DPPH Inhibition of *F.septica*leaf ethanolic extract at various concentrations.

Treatment	Concentration	Mean %DPPH Inhibition Activity
***Ficus septica*ethanolic extract**	100%	64.55
	75%	72.42
	50%	80.12
	25%	88.53
Positive Control	Ascorbic Acid	99.08

CONCLUSION

The study revealed that *Ficus septica* leaf ethanolic extract exhibited an antioxidant activity. A decreasing percentage of DPPH inhibition was observed from the *Ficus septica* ethanolic extract with increasing concentration. The presenceof secondary metabolites like alkaloid, flavonoid, saponine and terpeneshas a potential antioxidant activity.

ACKNOWLEDGMENTS

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REFERENCES

1. Aqil, F., Ahmad, I., & Mehmood, Z. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish journal of Biology*, *30*(3), 177-183.
2. A. Mu'nisa et al (2018). Bioactive compound and Antioxidant Activity Analysis of SomeMedicinal Plants of Province of Western Sulawesi J. Phys.: Conf. Ser. 1028 012023
3. Berg, C. C., Corner, E. J. H., &Noteboom, H. P. (2005). Moraceae (Ficus)(Vol. 17). National Herbarium Nederland.
4. C. Liyana-Pathirana, J. Dexter, F. Shahidi. Antioxidant properties of wheat as affected by pearling. Journal of Agricultural and Food Chemistry, 54 (2006), pp. 6177-6184
5. Clarke, G., Ting, K. N., Wiart, C., & Fry, J. (2013). High correlation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants*, *2*(1), 1-10.
6. F. Shahidi, R. Amarowicz. (1996). Antioxidant activity of protein hydrolysates from aquatic species. Journal of the American Oil Chemists' Society, 73,, pp. 1197-1199
7. Guntarti, A., Koto, D. S. P., Ahda, M., & Zainab, Z. Determination of Polifenol Content and Antioxidant Activity Test of Ethanol Extract 70% Awar-Awar (Ficus septicaBurm. F) Leaf using DPPH Method (1, 1-Difenil-2-Pikrilhidrazil). *Journal of Food and Pharmaceutical Sciences*, 3-3.
8. I.F.F. Benzie, J.J. Strain.(1999). Ferric reducing/antioxidant power assay: Direct measure of totalantioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentrationMethods in Enzymology, 299, pp. 15-27
9. Judah Israel, Joanna Ruiz. (2001). Antioxidant activity of Lagnob, *Ficus septica*(Burman, 1768) bark extract by 2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay. https://www.herdin.ph/ index.php/herdinpartners ?view=research& cid=68723#study
10. Khalil, M. M., Ismail, E. H., & El-Magdoub, F. (2012). Biosynthesis of Au nanoparticles using olive leaf extract: 1st nano updates. *Arabian Journal of Chemistry*, *5*(4), 431-437.
11. Kuo, Ping-Chung & Chm, Chao-Chen & Shi, Li-Shian& Li, Chia-Ying &Wu, Shwu-Jen &Damu, Amooru& Wu, Pei &Kuoh, Chang-Sheng & Wu, Tian-Shung. (2002). Non-Alkaloidal Constituents from the Stem of Ficus septica. Journal of the Chinese Chemical Society. 49. 10.1002/jccs.200200019.
12. Lescano J.I. & Ruiz J., (2017). Antioxidant activity of Lagnob, *Ficus septica*bark extract by 2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay. University of San Carlos
13. Moussa, A. M., Emam, A. M., Diab, Y. M., Mahmoud, M. E., & Mahmoud, A. S. (2011). Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of Punicagranatum leaf extract on rats. *International food research journal*, *18*(2).
14. N. Cumby, Zhong Y., M. Naczk, F. Shahidi. (2008). Antioxidant activity and water-holding capacity of canola protein hydrolysates Food Chemistry, 109, pp. 144-148
15. R.L. Prior, Wu X., K. Schaich. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry, 53, pp. 4290-4302
16. S.B. Kedare, R.P. Singh. (2011). Genesis and development of DPPH method of antioxidant assay Journal of Food Science and Technology, 48, pp. 412-422
17. Shekhar, T. C., & Anju, G. (2014). Antioxidant activity by DPPH radical scavenging method of Ageratum conyzoides Linn. leaves. *American journal of ethnomedicine*, *1*(4), 244-249.
18. Stuart, GU. (2011). Philippine Medicinal Plants. Retrieved from http://www.stuartxchange.org/index.html

19. Tsai, Z. I., Huang, P. S., & Tso, I. M. (2006). Habitat management byaboriginals promotes high spider diversity on an Asian tropical island.Ecography, 29(1), 8494.
20. Y. Athukorala, K.W. Lee, Song C., C.B. Ahn, T.S. Shin, Y.J. Cha, F. Shahidi, Y.J. Jeon (2003). Potential antioxidant activity of marine red alga *Grateloupiafilicina* extracts. Journal of Food Lipids, 10, pp. 251-263

CITATION OF THIS ARTICLE

Catherine Fugaban-Hizon. DPPH scavenging activity of *Ficus septica*leaf ethanolic extract. Bull. Env. Pharmacol. Life Sci., Vol 11[5] April 2022: 23-26