Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

ANTIOXIDANT ACTIVITY OF FOREST MANGGOSTEEN (*Garcinia hombroniana* Pierre) FRACTION USING DPPH AND ABTS METHOD

Ismail[1], Mufidah[1], Sukamto S. Mamada[2], Amrianto[1], Yayu Mulsiani Evary[1]*

[1]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Hasanuddin, Tamalanrea, Makassar, Indonesia
[2]Department of Pharmacology, Faculty of Pharmacy, Universitas Hasanuddin, Tamalanrea, Makassar, Indonesia

Received – December 13, 2020; Revision – March 30, 2021; Accepted – May 11, 2021
Available Online – September 08, 2021

DOI: http://dx.doi.org/10.18006/2021.9(Spl-2-ICOPMES_2020).S280.S285

KEYWORDS

Garcinia hombroniana

DPPH

ABTS

Fraction Active Antioxidant

ABSTRACT

This study was carried out to determine the active antioxidant fraction of *Garcinia hombroniana* bark extract by the DPPH and ABTS method. Along with this, the study also attempts to identify the class of compounds present in the various extract of *G. hombroniana* by the active fraction. The bark extract of *G. hombroniana* was prepared by the multilevel maceration method by using three solvents including n-hexane, ethyl acetate, and 96% ethanol. Results of study suggested that n-hexane (HSE), ethyl acetate (EASE) and ethanol 96% extract (ESE) have antioxidant activity with IC_{50} value of 423 ± 16.72 µg/mL, 284.89 ± 2.7µg/mL, and 10.49 ± 0.19 µg/mL in DPPH assay, while these extracts showed IC_{50} value of 560.92 ± 48.86 µg/mL, 430.18 ± 16.65 µg/mL, and 13.92 ± 0.57 µg/mL respectively in ABTS assay. Further, fractionated using vacuum column chromatography revealed the presence of five fractions viz., A, B, C, D, and E. Among these, fractions D showed the highest antioxidant activity with an IC_{50} value of 4.83 ± 0.18 µg/mL and 6.82 ± 0.31 µg/mL in DPPH and ABTS assays. Results of the fractioned analysis and qualitative determination showed that the active fraction of *G. hombroniana* contained flavonoid, triterpenoid, alkaloid, and saponin compounds, and antioxidant activities of these extracts might be due to the presence of these active ingredients.

* Corresponding author
E-mail: yayuevary7@gmail.com, yayumulsiani@unhas.ac.id (Yayu Mulsiani Evary)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).
All rights reserved.

All the articles published by Journal of Experimental Biology and Agricultural Sciences are licensed under a Creative Commons Attribution-NonCommercial 4.0 International License Based on a work at www.jebas.org.

1 Introduction

Nowadays, people's lifestyles have been changed to practical and instant life, especially on their daily consumption. Recently, consumption of fast foods has been increased tremendously and excess consumption of instant or fast food is the way of high quantity free radical (FR) production (Poumorad et al., 2006; Lobo et al., 2010). Accumulation of these FR in the human body may cause cancer or cardiovascular disease like serious diseases which might be a leading cause of death in the current scenario. Further, oxidative stress may also contribute to the dysfunction and death of neurons which might be caused by neurodegenerative disease at higher oxidative stress (Bernd & Christian, 2002). Recent studies showed that phytochemicals being considerable for the prevention of atherosclerosis which also contributes to the pathophysiology of cardiovascular disease (Sen et al., 2010; Silvana & Christina, 2020). Therefore, recently, antioxidants that can neutralize the effect of various FR are strongly needed. Antioxidants are substances that react and neutralize FR by donating their electrons and can prevent oxidative damage of molecules in the human body. Natural antioxidants are in high demand, among these natural antioxidants, phenolic compounds are most common and are abundantly exist in plants, these antioxidants can donate their hydrogens to FR, and inhibit oxidation of low-density lipoproteins by chelating metal ions (Lobo et al., 2010).

Garcinia sp. has been well known for the higher concentrations of xanthone, benzophenone, and flavonoid compounds (Klaiklay et al., 2013; Nargis et al., 2014). Listyani et al. (2017) has been reported that the ethyl acetate extract of *G.hombroniana* stem bark has antioxidant activity and it can inhibit the lipoxygenase enzyme with EC_{50} and IC_{50} values of 15.34 µg/mL and 0.26 µg/mL, respectively. Further, Nargis et al. (2015) succeeded in isolating cycloartane, triterpene, and five other triterpenoid compounds from dichloromethane *G. hombroniana* extract. Antioxidant activities of the natural antioxidant can be determined by various methods but the two most common methods are 1,1-diphenyl-2 picrylhydrazyl (DPPH) and the 2,2‘azinobis (3-ethylbenzthiazoline-6-sufonic acid) method (ABTS). Therefore, in this study, both methods have been used to test the potency of forest mangosteen extract as a natural antioxidant. Further, this study was also conducted to obtain an active antioxidant fraction of *G.hombroniana* stem bark.

2 Materials and Methods

The materials required in this study are Chambers (CAMAG), exicators, UV lamps, micropipettes (Nesco®), simplicial ovens, and analytical scales (Sartorius®). All reagents used are pro analytic reagents. The bark of the forest mangosteen (*G. hombroniana*) was collected from the Rompegading Village. The collected bark samples were sorted wet, cleaned, and washed with running water, then dried in an oven at 40°C. The dried sample was used for the extraction. Identification of the collected sample was carried out at the Botany Laboratory of the Faculty of Mathematics and Natural Sciences, Hasanuddin University.

2.1 Extraction process

Dried forest mangosteen stem bark was macerated using the multilevel maceration method with 3 kinds of solvents, namely n-hexane, ethyl acetate, and 96% ethanol. The obtained 3 extracts viz., hexane soluble extract (HSE), ethyl acetate soluble extract (EASE), and 96% ethanol soluble extract (ESE) were evaporated using a rotary evaporator (Buchii®).

2.2 Determination of Chromatogram Profiles Using Thin Layer Chromatography (TLC)

About 100 mg of HSE, EASE and ESE extract were dissolved with suitable solvents, then spotted on the GF254 silica plate (Merck®), and eluted using 3 eluent mixtures which were hexane-ethyl acetate (1: 1), chloroform-ethyl acetate-formic acid (5: 2: 0.5), chloroform-ethyl acetate-formic acid (5: 4: 0.25), respectively in the different chambers. The spot was visualized under a UV 254 nm, 366 nm and observed directly after being sprayed with 10% H_2SO_4 reagent (Merck®) accompanied by heating and also sprayed with DPPH solution.

2.3 Fractionation of Ethanol Extract

Fractionation of ethanol extract was done using vacuum liquid chromatography (VLC). Silica gel 60 PF_{254} were used as stationary phase and 8 different mixtures of ethyl acetate and methanol viz., ethyl acetate 100%, different ratio of ethyl acetate-methanol (20:1; 10:1; 5:1; 3:1; 1:1; and 1:3), and methanol 100% were used as a mobile phase. After undergoing VLC of ESE, the fractions that resulted were qualitatively analyzed for their TLC profile. The same TLC profile was used to obtain 5 fractions which were fractions A, B, C, D, and E. The fractions were then tested for their antioxidant activity.

2.4 Determination of antioxidant activity

2.4.1 DPPH Method

Five different concentrations i.e. 2.5 ppm; 5 ppm; 7.5 ppm; 10 ppm and 12.5 ppm of HSE, EASE, and ESE were prepared and followed by the addition of 80 µL DPP @ 4 mM. While Trolox® was used as a positive control. Subsequently, this mixture was incubated at room temperature in the dark for 30 minutes, and absorption was measured using a microplate reader (Biorad®) at a wavelength of 515 nm (Nargis et al., 2014; Rajkumar et al., 2015).

2.4.2 ABTS Method

Like DPPH, here also five different concentrations i.e. 2.5 ppm, 5 ppm, 7.5 ppm, 10 ppm, and 12.5 ppm of HSE, EASE, and ESE were prepared and 120 µL of mixtures of ABTS and Potassium Persulfate solution was added with 200 µL MeOH. While Trolox® was used as a positive control. Subsequently, the mixtures were left for incubation at room temperature in the dark for 30 minutes. This was followed by the measurement of the absorption by using a microplate reader at a wavelength of 650 nm. The free radical scavenging activity is expressed as percent inhibition which can be calculated by the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

The sample concentration and the probit value of percent inhibition are plotted on the x and y axes, respectively, to obtain a linear regression equation. This equation is used to determine the IC_{50} value of each sample (Nargis et al., 2014).

3 Results and Discussion

The current study was carried out to obtained active antioxidant fractions from the natural source of *G.hombroniana*. In this research, the multilevel extraction process yielded a different amount of extract. Results presented in table 1 revealed that ESE is the most yield-giving extract. Further, the thin layer chromatography (TLC) profile of HSE, EASE and ESE can be seen in figure 1. It revealed that all extracts undergo a good separation process using the appropriate solvent. The ethanol extract was the most abundant and active extract which fractionated the compounds based on their polarity. The fractions with the same TLC profiles were combined and resulted in 5 fractions (Fraction A, B, C, D, and E), these can be seen in the chromatogram given in figure 3. The chromatogram showed a variety of compounds contained in each fraction which showed the different colors of spots under UV observation at 254 and 366 nm wavelength.

Table 1 Percent Rendement and Organoleptic properties of Forest Mangosteen Bark Extract

Extracts	Percent Rendement (%) w / w	Organoleptic
Hexane Soluble Extract (HSE)	0.12	Odorless, tasteless, light brown
Ethyl Acetate Soluble Extract (EASE)	0.34	Odorless, tasteless, light brown
Ethanol96% (ESE)	7.03	Odorless, tasteless, dark brown

3.1 Antioxidant Activity

Qualitative and quantitative analyses of the antioxidant activity of *G.hombroniana* extracts and fractions were carried out by ABTS and DPPH assay. Quantitative antioxidant activity analysis of the extracts showed that the ESE has the highest antioxidant activity to reduce the ABTS and DPPH radicals with IC_{50} of 13.92 and 10.49 µg/mL respectively. As shown in figure 2, the ESE has a potential activity as a natural antioxidant as it has a relatively similar activity to Trolox in scavenging the ABTS and DPPH radicals (IC_{50} 10.9 µg/mL against ABTS and 5.58 µg/mL against DPPH). While the lowest antioxidant activity was reported in the HSE, and EASE extracts, these extracts have an IC_{50} value of 423 µg/mL (HSE) and 284.89 µg/mL (EASE) in DPPH and 560.92 µg/mL (HSE) and 430.18 µg/mL (EASE), in ABST radical scavenging assay (Figure 1). In a previous study, Rajkumar et al. (2015) evaluated the antioxidant potential of *Garcinia imberti* stem bark methanol extract against DPPH and reported the IC_{50} value of 274.24 µg/mL, and this value was reported lower than the current study *G. hombroniana* bark extract.

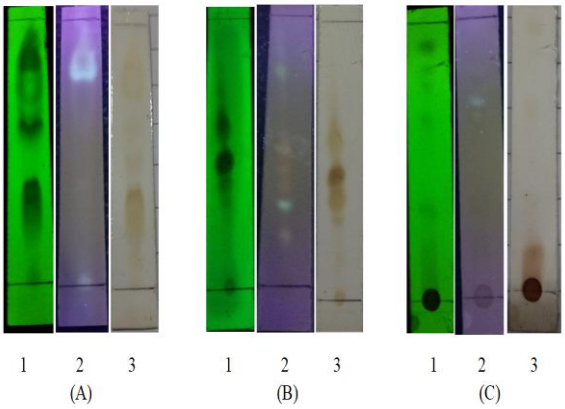

Figure 1 (A) Chromatogram profile of n-hexane extract with an eluent mixture of hexane: ethyl acetate (1: 1), (B) Chromatogram profile of ethyl acetate extract with eluent mixture of chloroform: ethyl acetate: formic acid (5: 2: 0.5), (C) Chromatogram profile of 96% ethanol extract with eluent mixture Chloroform: ethyl acetate: formic acid (5: 4: 0.25); here 1 is visualized under 254 nm UV Lamp; 2 under 366 nm UV Lamp; and 3 Visualized after spraying with 10% H2SO4 + heating

Furthermore, qualitative analysis of antioxidant activity was also done to obtain the fraction from different extracts. The chromatogram was sprayed with 0.2% DPPH solution (Figure 3). The chromatogram has shown five fractions A, B, C, D, and E as yellow spots on purple background in the TLC stationary phase. These results are in line with the qualitative antioxidant activity shown by the hexane fraction of red dragon fruit that faded the purple color of DPPH on the TLC plate (Budilaksono et al., 2014). To prove the qualitative results, a quantitative analysis of fractions A, B, C, D, and E was also conducted (Figure 4). ABTS assay to the fifth fraction showed that fraction D has the highest activity with IC_{50} of 6.82 µg/mL. While, fraction B, C, E, and A showed antioxidant activity with IC_{50} value of 8.04 µg/mL, 8.39 µg/mL,

16.91 µg/mL, and 102.37 µg/mL, respectively (Figure 4). According to Phongpaichit et al. (2007) compounds that have IC50 values <10 µg/mL is in the very active category and based on these recommendations, the antioxidant activity of fraction B, C, and D are in the very active category while fraction A has weak antioxidant activity.

Antioxidant analysis using the DPPH method showed that fraction D has the highest antioxidant activity with IC_{50} of 4.83 µg/mL. While, fractions C, B, and E showed antioxidant activity with IC50of 6.87 µg/mL, 8.15 µg/mL, and 9.93 µg/mL, respectively. While among the tested fraction, a weak antioxidant activity was shown by fraction E (IC_{50} Value of 186.64 µg/mL).

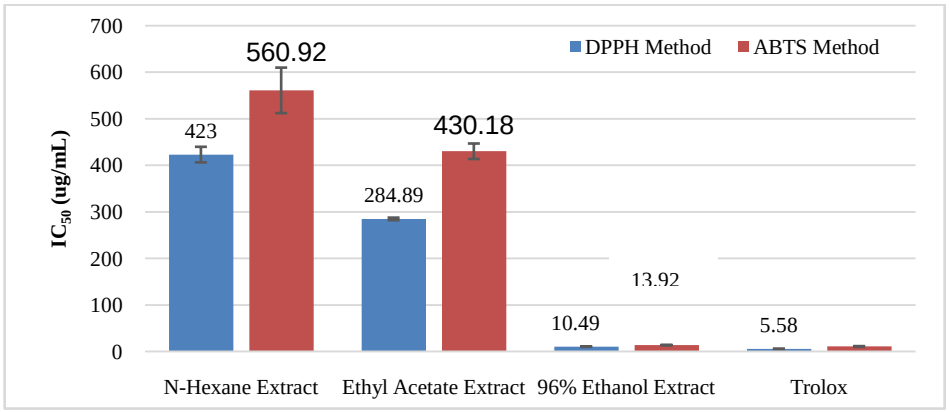

Figure 2 IC_{50} values of different extracts on free radical inhibitory activity using the ABTS and DPPH methods

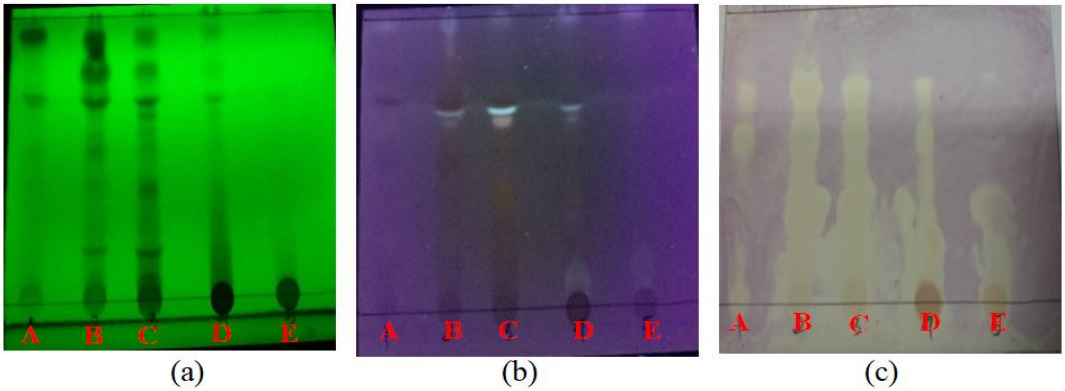

Figure 3 TLC chromatogram profile Fraction A, B, C, D, and E using Chloroform: Ethyl acetate: formic acid (2: 5: 0.5) as eluent (a) visualized at UV 254 nm, (b) visualized at UV 366 nm, (c) DPPH spraying

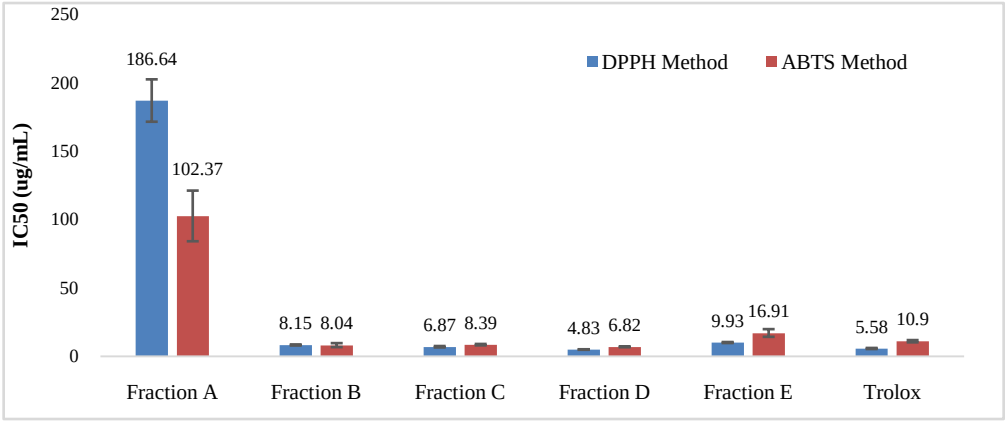

Figure 4 IC_{50} values of free radical inhibition activity for 96% ethanol fraction using the ABTS and DPPH methods

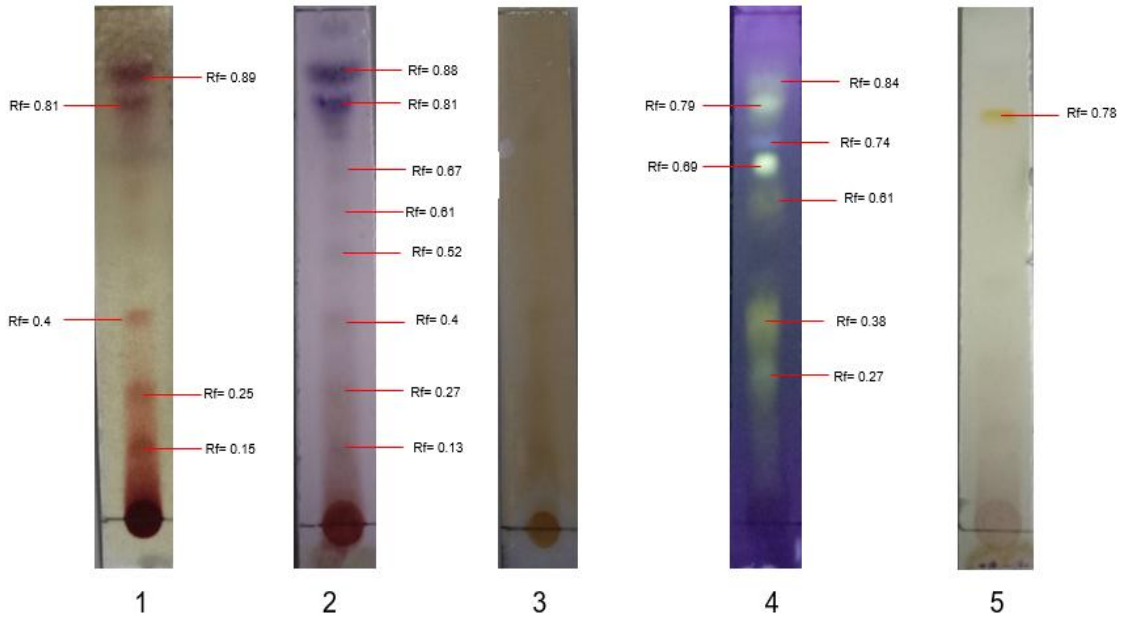

Figure 5 Chromatogram profile of active fraction using sprayed reagents after elution of fraction using mobile phase ethyl acetate-chloroform-formic acid (5: 1: 0.5). (1) Vanillin sulfate, (2) Anisaldehyde, (3) $FeCl_3$ (4) $AlCl_3$ at UV 366 nm, (5) Dragendroff

The identification of chemical compounds contained in the active fraction of forest mangosteen stem bark was done by spraying chemical reagents on the TLC plates spot. The results showed that the active fraction contains flavonoids, triterpenoids, alkaloids, and saponins (Figure 5). Identification of flavonoids using AlCl3 reagent, positive results are indicated by yellow, green, and blue stains that fluoresce at a wavelength of 366 nm after spraying the reagent (Wagner & Bladt, 1996; Galuh et al., 2018). The identification of alkaloid compounds using dragendroff reagent is marked with a brown or brownish-orange stain after spraying the reagent (Archana & Anubha, 2011). Identification of triterpenoid compounds was carried out using anisaldehyde solution marked with a blue-purplish stain, while the identification of saponin compounds was carried out using the vanillin-sulfuric acid reagent marked with a blue or red color change on the TLC stain (Sarker et al., 2006; Elzbieta et al., 2016). These results are in the line of previous research which found some terpenoid and saponin compounds in thymi oil on the TLC profile showed purple and blue spots (Elzbieta et al., 2016). However, qualitative identification of chemical compounds in the active fraction showed that compounds accumulation still occurred in this fraction. Further study needs to be done to isolate and characterized active antioxidants in this fraction.

Conclusion

Among the tested *G. hombroniana* bark extracts, 96% ethanol-soluble extract (ESE) has the highest antioxidant activities in both DPPH and ABTS assay. Further, among the tested five fractions, fraction D from ethanol extract is the active antioxidant fraction that has the highest free radical inhibition activity using both the DPPH and ABTS methods with IC_{50} of 4.83 ± 0.18 µg / mL and 6.82 ± 0.31 µg/mL respectively. Identification of positive chemical components indicates the presence of alkaloids, flavonoids, saponins, and terpenoids in fraction D.

References

Archana A, Anubha K (2011) An Overview in Thin Layer Chromatography. International Journal of Pharmaceutical Sciences Research 6: 256-267. DOI: 10.13040/IJPSR/0975-8232.2(2).256-67.

Budilaksono W, Wahdayaningsih S, Fahrurroji A (2014) Antioxidant N-Hexane Fraction from Red Dragon Fruit (*Hycloceres lemairei* Britton dan Rose) Using DPPH Method (*1,1-Diphenyl-2-Picryhydrazil).* Jurnal Mahasiswa Farmasi Fakultas Kedokteran *UNTAN* 1(1): 1-11.

Bernd M, Christian B (2002) Antioxidants as Treatment for Neurodegenerative Disorders. Expert Opinion on Investigational Drugs 11(10):1407. Doi: 10.1517/13543784.11.10.1407.

Elzbieta M, Anna W, Roman P, Robert R, Beata P, Helena (2016) The New TLC Method for Separation and Determination of Multicomponent Mixtures of Plant Extracts. Journal of Analytical Methods in Chemistry 1-6. Doi: 10.1155/2016/1813581.

Galuh W, Supiani, Yova T (2018) Antioxidant Activity and Toxicity of Puspa (*Schima wallicii*) Leaves Extract from Indonesia. The Journal of Tropical Life Sciences 8(2): 151-157.

Klaiklay S, Sukpondma Y, Rukachaisirikul V, Phongpaichit S (2013) Firedolanostanes and xanthones from the twigs of *Garcinia hombroniana.* Phytochemistry 85:161-166.

Listiyani A, Erla B, Puspitasari N(2017) Antioxidant Activity and Lipoxygenase Enzyme Inhibitory Assay with Total Flavonoids Content from *Garcinia hombroniana* Pierre Stem Bark Extract. Pharmacognosy Journal 9 (2):276-279.

Lobo V, Patil A, Phatak A, Chandra N(2010) Free Radicals Antioxidants and Functional Food: Impact on Human Health. Pharmacognosy Reviews 4(8): 118-126.

Nargis J, Melati K, Sadiq NK (2014) Phytochemicals from The Bark of *Garcinia homroniana* and Their Biological Activities. Records of Natural Product 8(3): 312-316.

Nargis J, Naeem K, Imran K, Amir AK, Sadiq NK (2015) A Bioactive Cycloartane Triterpen From *Garcinia hombroniana*. Natural Product Research: Formerly Natural Product Letters 30(12): 1388-1397 DOI : 10.1080/14786419.2015.1060594.

Phongpaichit S, Nikom J, Rungjindamai N, Sakayaroj J, Hutadilok-Towatana N, Rukachaisirikul V, Kirtikara K (2007) Biological Activities of Extract from Endophytic Fungi Isolated from Garcinia Plants. FEMS Immunology & Medical Microbiology 51(3): 517-525.

Poumorad F, Hosseinimehr SJ, Shahabimajd N (2006) Antioxidant activity phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology 11:1142 1145.

Rajkumar K, Shubarani R, Sivaram V (2015) Phytochemical Screening And Antioxidant Activity Of Leaves And Stem Bark Extracts Of *Garcinia imberti*– endangered Plant. International Journal of Pharmaceutical Sciences and Research 6(9):4016-4021. DOI: 10.13040/IJPSR.0975-8232.6(9).4016-21.

Sarker SD, Latif Z, Gray AI (2006) *Natural Products Isolation Second Edition*. United States: Human Press

Sen S, Chakraborty, Sridhar C, Reddy YSR, De B (2010) Free Radicals, Antioxidants, Diseases and Phytomedicines: Currents Status and Future Prospect. International Journal of Pharmaceutical Sciences Review and Research 3(1): 91-100.

Silvana H, Cristina A (2020) New Mechanism of Action of Natural Antioxidants in Health and Disease. Antioxidants 9:344. DOI: 10.3390/antiox9040344.

Wagner H, Bladt S (1996) *Plant Drug Analysis A Thin Layer Chromatography Second Edition*. Germany: Springer-Verlag Berlin Heidelberg.