Journal of Pharmaceutical Research International

33(46B): 186-192, 2021; Article no.JPRI.75276
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Antioxidant Activity of Red and Purple Rosella Flower Petals Extract (*Hibiscus sabdariffa* L.)

Mareetha Zahra Shafirany[1], Iin Indawati[2], Lela Sulastri[3], Asman Sadino[4], Anggun Hari Kusumawati[5] and Maulana Yusuf Alkandahri[5*]

[1]Department of Pharmaceutical Biology, School of Pharmacy Muhammadiyah Cirebon, Cirebon, West Java, Indonesia.
[2]Department of Pharmaceutical Analysis and Medicinal Chemistry, School of Pharmacy Muhammadiyah Cirebon, Cirebon, West Java, Indonesia.
[3]Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy Muhammadiyah Cirebon, Cirebon, West Java, Indonesia.
[4]Department of Pharmacy, Faculty of Mathematics and Natural Science, Garut University, Garut, West Java, Indonesia.
[5]Faculty of Pharmacy, Buana Perjuangan Karawang University, Karawang, West Java, Indonesia.

Authors' contributions

This work was carried out in collaboration among all authors. Authors MZS and MYA conceived the study, concept, and design and conducted most of the laboratory experiments, analyzed and interpreted experiment results. Authors II, LS, AS and AHK contributed to the supervision of the study, drafting, and critical revision of the manuscript of the article. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i46B32931
Editor(s):
(1) Dr. Debarshi Kar Mahapatra, Rashtrasant Tukadoji Maharaj Nagpur University, India.
Reviewers:
(1) Arpita Chatterjee, University of Nebraska Medical Center, USA.
(2) Nagham Mahmood Aljamali, Kufa University, Iraq.
Complete Peer review History: https://www.sdiarticle4.com/review-history/75276

Original Research Article

Received 02 August 2021
Accepted 06 October 2021
Published 21 October 2021

ABSTRACT

Aims: This study aims to test the antioxidant activity of red and purple *H. sabdariffa* flower petals extract and conduct qualitative phytochemical screening.
Study Design: Antioxidant potential of red and purple *H. sabdariffa* flower petal extract analyzed by spectrometric assays.
Place and Duration of Study: This study was carried out at School of Pharmacy Muhammadiyah Cirebon, Cirebon, West Java, Indonesia from the year of 2020 to 2021.

**Corresponding author: E-mail: alkandahri@gmail.com;*

Methodology: Red and purple *H. sabdariffa* petals extracted with 70% ethanol. The extract was then examined for its antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, after the qualitative phytochemical screening.
Results: In this study, the concentration of red and purple *H. sabdariffa* petals extract dependently demonstrated the ability to scavenge DPPH. In the DPPH radical scavenging activity test, the red and purple *H. sabdariffa* petasl extracts designating IC_{50} values of 63.77 and 37.19 μg/ml and fall into the category of strong and very strong antioxidant activity. Meanwhile, phytochemical screening tests showed the existence of flavonoids and polyphenols in the extract of red and purple *H. sabdariffa* petals.
Conclusion: This study shows that the red and purple *H. sabdariffa* petals extract has potential as a promising natural antioxidant agent for the treatment of oxidative stress.

Keywords: Hibiscus sabdariffa; antioxidant; DPPH (1,1-diphenyl-2-picrylhydrazyl); phytochemical screening; oxidative stress.

1. INTRODUCTION

The shift in people's lifestyles to become practical and instantaneous, especially in the fulfillment of needs such as food, has a negative impact on health. Instant or fast food processed by high heating or by burning can trigger the formation of free radical compounds [1]. Free radicals are defined as molecules whose electrons are lost causing the molecule becomes unstable and attempts to reclaim electrons from other molecules or cells [2]. In the body, free radicals are very reactive and will interact through destructive oxidation reactions with certain body parts and cells composed of proteins, fats, DNA, carbohydrates, and RNA, thereby prompting the development of chronic degenerative diseases such as cancer, aging, and coronary heart disease [1, 3]. Owing to the decreased performance of cellular antioxidant defense system, cell damage caused by these radicals can be more widespread. Antioxidant defense mechanisms exist in all biological systems to eliminate damaged molecules, however they can be inefficient. As a result, consuming antioxidant foods is critical for protecting cells from free radical damage [4]. Antioxidants are any substance that put off or prevent oxidative damage to a target molecule in a broad sense [5]. Antioxidants are best known for its capability to scavenges the free electrons directly or it enhances the expression and activity of free scavenging enzymes in the body. Antioxidant compounds including polyphenols, flavonoids, and phenolic acids can scavenge free radicals including hydroperoxides, peroxides, or lipid peroxyls, thereby inhibiting oxidative mechanisms that lead to degenerative diseases [6]. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are the most often utilized synthetic antioxidants in food (BHA). Both are powerful antioxidants, however their usage in food is discouraged due to their volatility and also because they are thought to act as promoters of carcinogenesis [7]. Therefore, research on antioxidants is needed that focuses on natural compounds from natural sources, one of which is herbal plants which have been considered the besr antioxidants since ancient times [8]. One of the herbal plants that efficacious as a source of natural antioxidants are rosella flower petals (*Hibiscus sabdariffa* L.). This plant is often used as food and drink. *H. sabdariffa* has many pharmacological activities including antibacterial, antifungal, antiparasitic, antipyretic, antinociceptic, antiinflammatory, nephroprotective, diuretic, anticancer, hepatoprotective, anticholesterol, antiobesity, antidiabetic, antihypertensive and antianemia [9]. Therefore, this study aimed to examine the antioxidant activity of red and purple *H. sabdariffa* flower petals extract using the DPPH scavenging assay.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma Chemical Co. (St. Louis, MO, USA) (Catalogue No. 300267-50MG), methanol absolute (CH_3OH) (Catalogue No. 1070182511) (CH_3OH), sulfuric acid (H_2SO_4) (Catalogue No. 4803641000), diethyl ether ($(C_2H_5)_2O$) (Catalogue No. 1009311000), Magnesium powder (Mg) (Catalogue No. 1058151000), Zinc powder (Zn) (Catalogue No. 1087890500), hydrogen chloride (HCl) (Catalogue No. 1090631000), ferric chloride ($FeCl_3$) (Catalogue No. 1039430250), ethanol (C_2H_5OH) (Catalogue No. 1009831000), chloroform ($CHCl_3$) (Catalogue No.

1070242500), amyl alcohol ($C_5H_{12}O$) (Catalogue No. 1009751000), ascorbic acid ($C_6H_8O_6$) (Catalogue No. 1004680100). All reagents are of analytical grade (Merck, Darmstadt, Germany).

2.2 Plant Materials

Each 5 kg of fresh red and purple *H. sabdariffa* flower petals were taken from Macanbang Village, Tulungagung Regency, East Java, Indonesia, and brought to the Pharmacognosy and Chemical Laboratory, School of Pharmacy Muhammadiyah Cirebon for cleaning, drying, grinding and extraction. The plant was identified as *H. sabdariffa* by Herbarium Jatinangor, Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran (No.677/HB/04/2018).

2.3 *H. sabdariffa* Extraction

Red and purple *H. sabdariffa* flower petal powder 100g each were macerated thoroughly in 70% ethanol for 72 hours. The liquid extract was attained and concentrated using a rotary evaporator (Eyela OSB-2100) at a temperature of 50°C produces a concentrate of about 30.00% for red *H. sabdariffa* flower petals and 41.99% for the purple *H. sabdariffa* flower petals (fixed weight of extract divided by weight of simplicia multiplied by 100%).

2.4 Phytochemical Screening

Red and purple *H. sabdariffa* flower petal extract each was screened qualitatively to identify the presence of secondary metabolites which include alkaloids, flavonoids, phenolic compounds, saponins, and triterpenoids/steroids [10].

2.5 DPPH Radical Scavenging Assay

Determination of antioxidant activity carried out using DPPH [11]. Stock solution of 100 µg/ml DPPH was set by dissolving 10 mg of DPPH in 100 ml of methanol. Solution sample obtained by dissolving each 10 mg red and purple *H. sabdariffa* flower petals extract with 100 ml of methanol, then solution diluted to 10, 20, 30, 40, and 50 µg/ml for red *H. sabdariffa* flower petals extract and 5, 10, 15, 20, and 25 µg/ml for purple *H. sabdariffa* flower petals extract. After that as much as 2 ml of each solution mixed with 2 ml of DPPH stock solution until homogeneous and incubated at temperature 30°C for 30 minutes. Antioxidant activity examined using spectrophotometry UV-Vis (Shimadzu UV Mini-1240) on long wave 515.50 nm and repeated three times. The blank sample was 1 ml of DPPH solution in 10 ml of methanol measured at the same time and wavelength (Ab). Ascorbic acid was used as a comparison with various concentrations of 2, 3, 4, 5 and 6 µg/ml. The following equation was used to calculate the percentage of DPPH radical scavenging activity:

$$Inhibition\ rate\ (\%) = \frac{Ab - As}{Ab}\ x\ 100$$

Where **Ab** is the absorbance of blank sample and **As** is the absorbance of sample. % inhibition plotted against concentration and calculated from the IC_{50} chart.

2.6 Statistical Analysis

Data were tabulated as mean±standard deviation (SD) of three replicates and statistical analysis was doneusing Graph Pad Prism software (Version 9).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Chemical components including alkaloids, flavonoids, polyphenols, saponins, and triterpenoids/steroids were found in phytochemical screening of red and purple *H. sabdariffa* flower petals extract. Summary of red and purple *H. sabdariffa* flower petals extract served on Table 1.

3.2 Antioxidant Activity

The DPPH method or known as IC_{50} was used to quantify antioxidant activity as a concentration needed to inhibit 50% of DPPH free radicals [11]. Antioxidant activity of red and purple *H. sabdariffa* flower petals extract carried out at different concentrations. We found that the higher the extract concentration, the higher the percentage of inhibition (Fig. 1).

Based on the results of the IC_{50} value in Table 2, the antioxidant power of red *H. sabdariffa* flower petals extract is in the strong antioxidant range, compared with purple *H. sabdariffa* flower petals extract and ascorbic acid is in the very strong antioxidant range. Classification of antioxidants based on IC_{50} value is presented in Table 3.

Table 1. Phytochemical screening of red and purple *H. sabdariffa* flower petals extract

Phytochemical screening	Reagents	Observation	Red *H. sabdariffa* flower petals extract	Purple *H. sabdariffa* flower petals extract
Alkaloids	Dragendorff Bouchardat Mayer	(+) Light brown (+) Dark brown (+) Muddy and white sediment	-	-
Flavonoids	Zn + HCl (p) Mg + HCl (p)	(+) Red	+	+
Polyphenols	1% $FeCl_3$	(+) Dark blue	+	+
Saponins	Hot water + HCl	(-) Bubble	-	-
Triterpenoids and Steroids	Liebermann-Burchard	(+) Purple	-	-

(+) = Contained, (-) = Not Contained

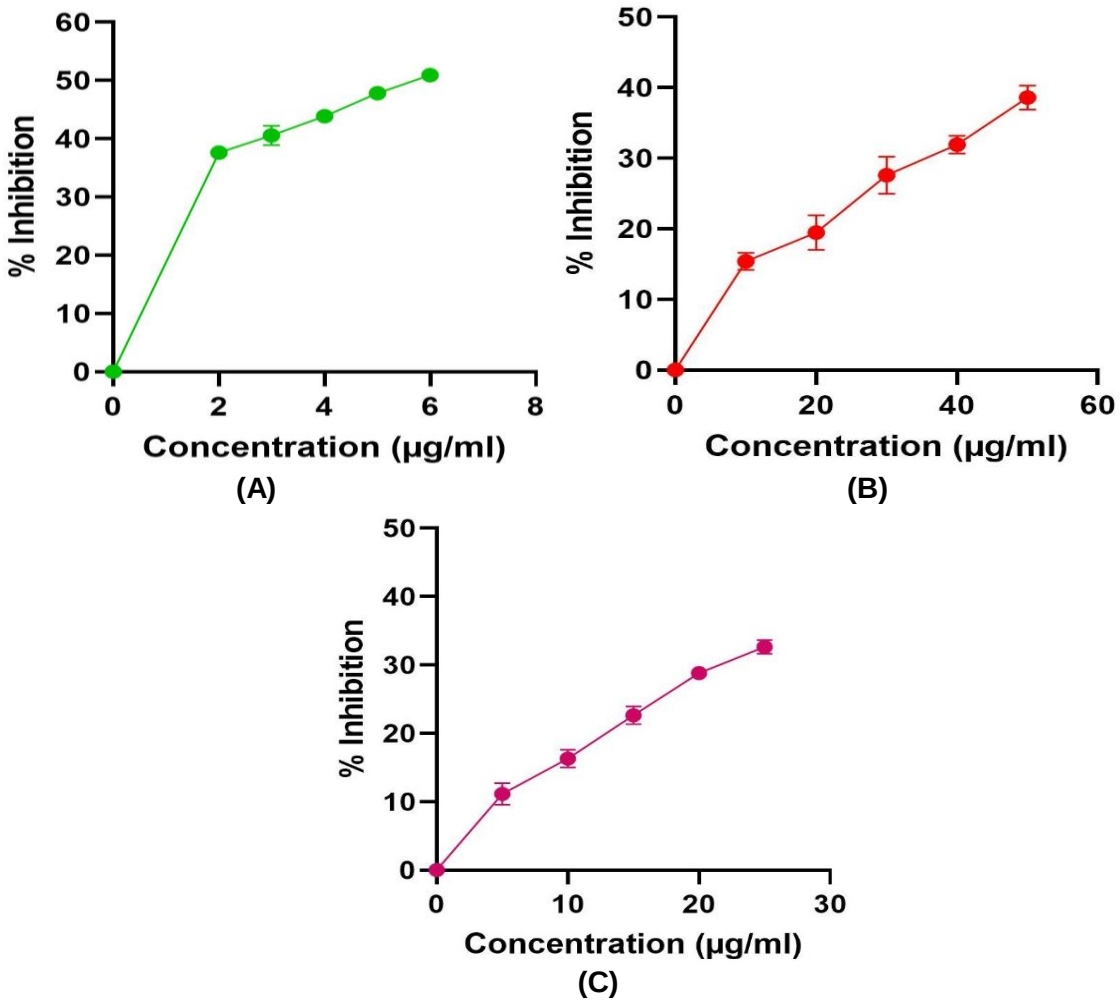

Fig. 1. Antioxidant activity of red and purple *H. sabdariffa* flower petals extract. The graph represents the % inhibition of (A) Ascorbic acid, (B) Red *H. sabdariffa* flower petals extract, and (C) Purple *H. sabdariffa* flower petals extract. X is sample concentration and Y is % inhibition. Data are presented as mean±SD of three replicates.

Table 2. IC_{50} value of red and purple *H. sabdariffa* flower petal extract compared with ascorbic acid

No	Sample	IC_{50} (µg/ml)	Antioxidant activity
1	Ascorbic acid	5.03	Very strong
2	Red *H. sabdariffa* flower petals extract	63.77	Strong
3	Purple *H. sabdariffa* flower petals extract	37.19	Very strong

Table 3. Classification of antioxidants based on IC_{50}value [12]

IC_{50} value (µg/ml)	Antioxidant activity
< 50	Very strong
50-100	Strong
101-250	Moderate
250-500	Weak
> 500	Not active

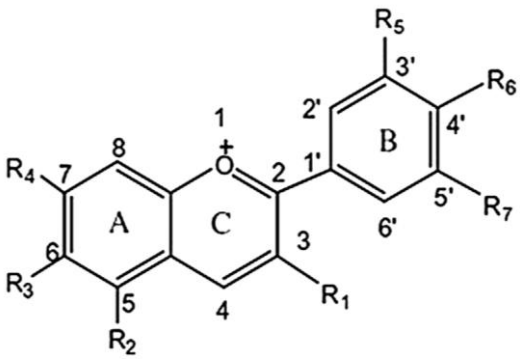

Fig. 2. Anthocyanidins chemical structure [22]

The significant scavenging effect of this plant extract on reactive oxygen and free radicals is responsible for its antioxidant activity [13, 14], that can protect cells from damage caused by lipid peroxidation [13, 15], inhibit xanthine oxidase activity and plays a role in protection against tert-butyl hydroperoxide-induced oxidative damage (t-BHP) [16], inhibit mediated Cu^{2+} LDL oxidation reactions and the formation of thiobarbituric acid reactive substances (TBARs) [17, 18], inhibit the formation of malondialdehyde content [13, 19], reduce glutathione deficiency and decrease the activity of superoxide dismutase and catalase in the blood [19], as well as increasing glutathione, catalase, and superoxide dismutase and lowering malondialdehyde in the liver [20]. Meanwhile, *H. sabdariffa* plant has been reported to contain active compounds anthocyanins [21]. Anthocyanins are compounds that inhibit oxidation reactions by scavenging free radicals and decreasing oxidative stress [2], this is due to differences in chemical structure, number, and position of hydroxyl groups (OH), conjugate groups, degrees of glycosylation and the presence of electron donors in their ring structure, so they are able to withstand the loss of electrons [22, 23, 24].

In anthocyanins, the amount of free OH around the pyron ring and the higher number of OH groups scattered throughout the molecular structure determine their potential antioxidant activity. Meanwhile, the presence of the number of OH at positions C3′ and C4′ on ring B and C3 of ring C on the basic structure of flavonoids appears to be the main structural requirement for anthocyanins to inhibit endothelial cell oxidative injury and intracellular free radical activity [22].

4. CONCLUSION

This study shows that the red and purple *H. sabdariffa* flower petals extract has potential as a promising natural antioxidant agent for the treatment of oxidative stress. However, further in vivo testing will be required as this is a pre-clinical requirement before it can be consumed or used for humans.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology. 2006;5(11):1142-1145.
2. Tena N, Martin J, Asuero AG. State of the art of anthocyanins: antioxidant activity, sources, bioavailability, and therapeutic effect in human health. Antioxidants. 2020;9(5):1-28.
3. Miguel MG. Anthocyanins: Antioxidant and/or anti-inflammatory activities. Journal of Applied Pharmaceutical Science. 2011;1(6):7-15.
4. Rahman MM, Islam MB, Biswas M, Alam AHMK. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC Research Notes. 2015;8(621):1-9.
5. Yamagishi S, Matsui T. Nitric oxide, a janus-faced therapeutic target for diabetic microangiopathy-friend or foe?. Pharmacological Research. 2011; 64(3):187-194.
6. Wu YY, Li W, Xu Y, Jin EH, Tu YY. Evaluation of the antioxidant effects of four main theaflavin derivatives through chemiluminescence and DNA damage analyses. Journal of Zhejiang University Science B. 2011;12(9):744–751.
7. Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A, Saija A. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chemistry. 2005;89(4):549-554.
8. Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pacific Journal of Tropical Biomedicine. 2012;*2*(12):960-965.
9. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. Hibiscus sabdariffa L. – A phytochemical and pharmacological review. Food Chemistry. 2014;165:424-443.
10. Alkandahri MY, Berbudi A, Utami NV, Subarnas A. Antimalarial activity of extract and fractions of *Castanopsis costata* (Blume) A.DC. Avicenna Journal of Phytomedicine. 2019;9(5):474-481.
11. Alkandahri MY, Nisriadi L, Salim E. Secondary metabolites and antioxidant activity of methanol extract of *Castanopsis costata* leaves. Pharmacology and Clinical Pharmacy Research. 2016;1(3):98-102.
12. Jun M, Fu HY, Hong J, Wan X, Yang CS, Ho CT. Comparison of antioxidant activities of Isoflavones from kadzu root (*Puerari lobata* ohwi). Journal of Food Science. 2003;68(6):2117-2122.
13. Farombi EO**,** Fakoya A. Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of *Hibiscus sabdariffa* L. Molecular Nutrition & Food Research. 2005;49(12):1120-1128.
14. Sayago-Ayerdi SG, Arranz S, Serrano J, Goni I. Dietary fiber content and associated antioxidant compounds in roselle flower (*Hibiscus sabdariffa* L.) beverage. J. Agric. Food Chem. 2007;55(19):7886-7890.
15. Olalye MT, Rocha JBT. Commonly used tropical medicinal plants exhibt distinct *in vitro* antioxidant activities against hepatotoxins in rat liver. Experimental and Toxicologic Pathology. 2007;58(6):433-438.
16. Tseng TH, Kao ES, Chu CY, Chou FP, Lin Wu HW, Wang CJ. Protective effects of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes. Food and Chemical Toxicology. 1997;35(12):1159-1164.
17. Hirunpanich V, Utaipat A, Morales NP, Bunyapraphatsara N, Sato H, Herunsalee A, Suthisisang C. Antioxidant effects of aqueous extracts from dried calyx of *Hibiscus sabdariffa* Linn. (Roselle) *in vitro* using rat low-density lipoprotein (LDL). Biological and Pharmaceutical Bulletin. 2005;28(3):481-484.
18. Ochani PC, D'Mello P. Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* Linn. leaves and calyces extracts in rats. Indian Journal of Experimental Biology. 2009;47(4):276-282.
19. Usoh IF, Akpan EJ, Etim EO, Farombi EO. Antioxidant actions of dried flower extracts of *Hibiscus sabdariffa* L. on sodium arsenite-induced oxidative stress in rats. Pakistan Journal of Nutrition. 2005;4(3):135-141.
20. Mossalam HH, Aty OAAE, Morgan EN, Youssaf SMS, Mackawy AMH.

Biochemical and ultra structure studies of the antioxidant effect of aqueous extract of *Hibiscus sabdariffa* on the nephrotoxicity induced by organophosphorous pesticide (malathion) on the adult albino rats. Journal of American Science. 2011;7(12):407-421.

21. Tsai PJ, McIntosh J, Pearce P, Camden B, Jordan BR. Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. Food Research International. 2002;35(4):351-356.
22. Reis JF, Monteiro VVS, Gomes RS, do Carmo MM, da Costa GV, Ribera PC, Monteiro MC. Action mechanism and cardiovascular effect of anthocyanins: A systematic review of animal and human studies. Journal of Translational Medicine. 2016;14(315):1-16.
23. Alkandahri MY, Patala R, Pratiwi MI, Agustina LS, Farhamzah, Kusumawati AH, Hidayah H, Amal S, Frianto D. Pharmacological studies of *Durio zibethinus*: A medicinal plant review. Annals of the Romanian Society for Cell Biology. 2021;25(4):640-646.
24. Alkandahri MY, Shafirany MZ, Rusdin A, Agustina LS, Pangaribuan F, Fitrianti F, Farhamzah, Kusumawati AH, Sugiharta S, Arfania M, Mardiana LA. *Amomum compactum*: A review of pharmacological studies. Plant Cell Biotechnology and Molecular Biology. 2021;22(33&34): 61-69.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/75276