Trop J Nat Prod Res, May 2021; 5(5):809-813

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Short Communication

Antioxidant Activity of *Diadema setosum* Gonads from Kelapa Island, District of Seribu Island, Indonesia

Joko Sulistiyo, Dian M. Ulfa*, Silvester M. Tulandi

Department of Pharmaceutical and Food Analyst, Health Polytechnic Jakarta II Ministry of Health Republic of Indonesia

ARTICLE INFO

Article history:
Received 27 January 2021
Revised 16 March 2021
Accepted 17 May 2021
Published online 03 June 2021

ABSTRACT

Oxidative damage caused by ROS (Reactive Oxygen Species) can trigger various chronic diseases. Marine antioxidant research has focused mainly on the antioxidant effects of crude extracts. This study aims to examine the antioxidant effect of polar and non-polar fractions of *Diadema setosum* gonads fraction. Sea urchin samples were obtained from Kelapa Island, Indonesia. The gonads of *D. setosum* were harvested, cut into small pieces, ground and macerated with methanol in a ratio of 1: 3 w/v to the mass of the sample. The methanol filtrate was concentrated with a rotary evaporator at 40^{o}C until a dry mass was obtained. The crude extract was fractionated in n-hexane, ethyl acetate and water, respectively. The crude methanol extract and fractions were tested for antioxidant activity using the DPPH free radical scavenging assay. The results showed that the n-hexane fraction had the highest antioxidant activity compared to the ethyl acetate and water fractions with IC_{50} value of 77.10 ppm.

Keywords: *Diadema setosum*, DPPH, Sea urchin gonad, Antioxidant activity.

Introduction

Oxidative damage caused by ROS (Reactive Oxygen Species) to macromolecules such as lipids, proteins, and nucleic acids can trigger various chronic diseases, such as coronary heart disease and atherosclerosis, cancer, liver injury, and ageing.[12] It has been reported that overexposure to ROS leads to inflammatory response, growth arrest and cell death by various mechanism.[3] Some of the most commonly used synthetic antioxidants, such as Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisole (BHA), have been known to induce hepatotoxic, carcinogenic, neurotoxic effects, and infertility in animal studies and which may also translate to humans.[4-8] Studies have shown that treatment with BHT in animal studies induces endoplasmic reticulum (ER) stress and DNA fragmentation, which results in simultaneous stimulation of intrinsic apoptosis signal transduction[9] and hyperactivity.[10] Other studies have also reported the environmental impact of BHT.[11]Therefore; it is essential to find safer and new sources of natural antioxidants.

In recent times, interest in the search for natural antioxidants, especially from plants and animal sources, has gained momentum in research groups around the world. It is believed these natural sources are reservoirs of antioxidants that could improve the health conditions of the human population by reducing oxidative damage caused by pro-oxidant components. Notwithstanding the bourgeoning search for antioxidants, the focus has been on terrestrial plants and microorganisms, with little or no attention given to marine invertebrates as a potential antioxidants source.[12]

Sea urchin (Echinoidea) is a type of marine organisms that East Asian residents widely consumed for its nutritional value and pharmacological properties.[13] The sea urchin is morphologically divided into two parts: the outer part in the form of hard shells dominated by thorns and the inside part (the gonads). The gonad organs have become a special delicacy for humans. Also, there is increasing interest in other body parts of sea urchin, such as the spines that have been a pigment source.[14] The sea urchin is distributed in almost all zones of marine waters. About 84 of the 950 types of sea urchins are scattered throughout Indonesian waters. [15] The economic value of sea urchin gonads is very high in several East Asian countries, especially in Japan. Sea urchin gonads also have antibacterial,[16] antioxidant effect in the brain[17] and anti-inflammatory properties as well as anticancer activities.[18]

The aquatic sources of the sea urchin have been shown to significantly influence the type of chemical constituents that is expressed. This has also influence the colours of the gonads, which is mainly related to the carotenoids content and carotenoids precursors taken from food that can be modified before being stored or directly stored without modification in the gonad tissue.[19] The leading roles of carotenoids in organisms include antioxidant, anti-inflammatory, pro-vitamin A activity, photo-protection, radical quenching, pigments, and immunological modulation.[20] *Diadema setosum* research in Indonesia is arousing a great deal of interest especially due its perceived pharmacological activities. Most of the Marine antioxidant research has primarily focused on the antioxidant effects of crude extracts and or whole organism.

This study aims to fractionate the crude extract of sea urchins gonad using polar, semi-polar, and non-polar solvents and subject these fractions to antioxidant screening.

Materials and Methods

The gonads were obtained from *Diadema setosum* (Echinoidea). Ethanol, methanol, ethyl acetate, n-hexane, Dimethyl sulfoxide (DMSO), and pre-coated silica Gel 60 TLC plates layer thickness 0.25 mm were purchased from Merck (Darmstadt, Germany). All solvents were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent and vitamin C (Ascorbic acid) standard were purchased from Sigma–Aldrich Chemical Co. (Singapore).

Collection of samples and authentification

The test samples used in this study were collected from Kelapa Island, District Seribu Island, Jakarta – Indonesia (Figure 1), on 26th July 2018.

*Corresponding author. E mail: ulfa.dian@gmail.com
Tel: 021-7805624

Citation: Sulistiyo J, Ulfa DM, Tulandi SM. Antioxidant Activity of *Diadema setosum* Gonads from Kelapa Island, District of Seribu Island, Indonesia. Trop J Nat Prod Res. 2021; 5(5):809-813. doi.org/10.26538/tjnpr/v5i5.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

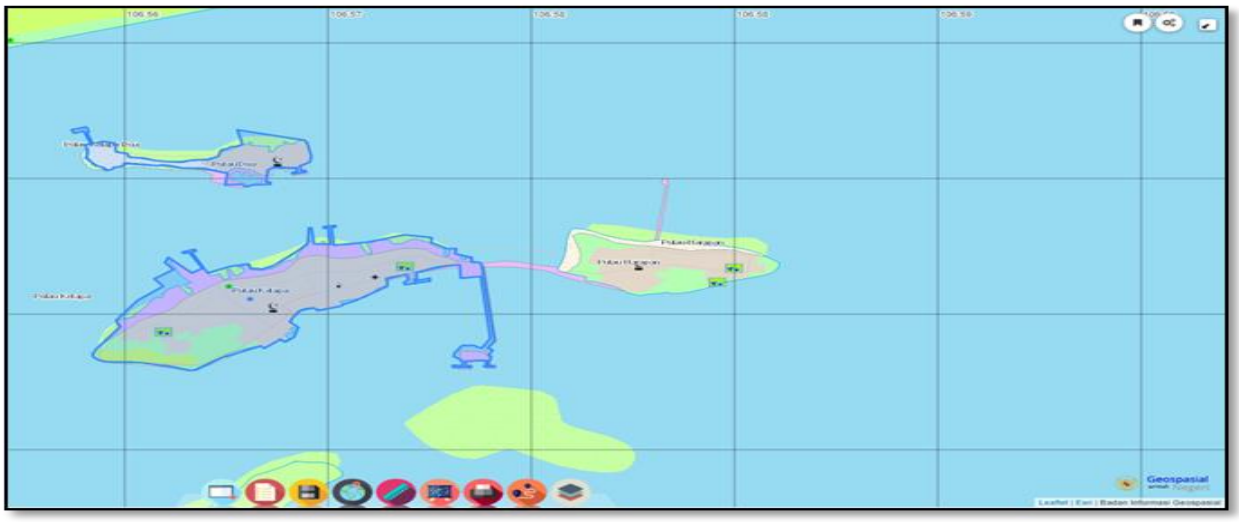

Figure 1: Map of Kelapa Island

The samples were identified macroscopically (the shape, colour, and specific characteristics of the sample were evaluated) by Indra Bayu Pramono, S.Si., M.App.Sc at the Research Centre for Oceanographic of LIPI (Indonesian Institute of Sciences). The voucher number for plant sample is B-3920/IPK.2/IF.07/XI/2018.

Extraction of Diadema setosum Gonad

Fresh *D. setosum* gonads (50 g) were washed thoroughly, air-dried, cut into small pieces, and milled. The samples were placed in a glass bottle and macerated with methanol for three days, with intermittent shaking on the first day. The macerated samples were filtered, and the filtrates were concentrated with a rotary evaporator at a maximum temperature of 40ºC. Nitrogen gas was passed through the concentrated extract until properly dried (4.2 g) and was stored at 4°C for future use.

Extract fractionation

The crude methanol extract of the sea urchin gonad was soaked with 400 mL of distilled water and homogenized in a round flask and then transferred to a separating funnel. Equal volume of n-hexane (400 mL) was added to the separating funnel. The content of the funnel was vortex and was left to stand to effect separation. The process was repeated several times until the n-hexane layer was clear. The combined filtrate (n-hexane fraction) was concentrated *in vacuo* in a rotary evaporator at a temperature of 40^{o}C and further oven-dried at 40 ^{o}C to obtain n-hexane fraction (2.8 g). The mother liquor was further extracted with ethyl acetate (400 mL). The filtrate obtained was concentrated with a rotary evaporator at a temperature of 40ºC to obtain a dried mass (2.2 g). The remaining water layer in the separating funnel was dried in a rotary evaporator at 40 °C (6.1 g). All the fractions were stored at 4 °C until further use.

TLC-DPPH Test

Preliminary tests for antioxidant activity were carried out on the crude extract of *D. setosum* gonads and other fractions, using thin-layer chromatography (TLC) techniques. About 0.2 mL of the crude extract and fractions were dissolved with a small amount of ethanol. Approximately 20 µL from the test samples were applied on a pre-coated TLC plate. The TLC plate was sprayed with a solution of DPPH (0.1 mg/mL). A positive antioxidant test is confirmed by decolourisation of the purple colour of the DPPH to yellow or colourless[21,22]

Potential Antioxidant Activity Screening

The antioxidant potentials of the test samples were determined by using DPPH assay. The samples (methanol, water, n-hexane, and ethyl acetate fraction) were dissolved in DMSO to obtain a concentration of 10,000 ppm. The samples were further diluted with methanol to a concentration of 100 ppm. The positive control drug (vitamin C) was also dissolved in methanol to obtain a concentration of 4 ppm. The DPPH solution (0.1 mM) was made by dissolving DPPH crystals in methanol. The solution was kept in the dark place throughout the experiment. An aliquot of the samples (100 µL) was pipette into microplates separately, followed with 100 µL DPPH solution. A blank solution was made by mixing 100 µL of DPPH solution and 100 µL of methanol in a microplate. The microplate was incubated in the dark at 37^{o}C for 30 minutes, and the absorbance was measured using a spectrophotometer at a wavelength of 517 nm. The antioxidant activity of each sample and vitamin C is expressed as percentage inhibition.[23]

Determination of IC_{50} value of the samples

The sample with highest antioxidant activity from the preliminary study was further subjected to antioxidant screening in order to determine its IC_{50} (the half maximal concentration that scavenge 50% of the DPPH radical). This fraction was diluted with methanol to obtain the following concentrations: 25, 50, 100, 200 and 400 ppm.[24] Again, vitamin C was used as the positive control drug at concentration range of 1.2; 2.4; 3.6; 4.8 and 6 ppm. The above procedure was repeated. The absorbance of the test samples were measured at a wavelength of 517 nm. IC_{50} values were obtained from the linear regression equation (y = a + bx) obtained by plotting sample and vitamin C concentration against percentage inhibition on the y-axis. The lower the IC_{50} values of a test sample, the more potent is its free radical scavenging capacity.

Ethical consideration

Ethical approval for this work was obtained from the Research Ethics committee of Health Polytechnic Jakarta 2. The study was done according to the ethical standards as laid down in the 1964 Helsinki Declaration and its later amendments or comparable ethical standards

Results and Discussion

Results of the preliminary study showed that the extract and fractions of the sea urchin gonad possessed antioxidant capacity based on the decolourisation of the DPPH reagent to yellow colour. The n-hexane and water fractions have the most concentrated colour zones, which implied that two fractions have the most potent antioxidant capacity than the methanol and ethyl acetate fractions in a qualitative test (Figure 2).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Spectrophotometer Potential Antioxidant Screening Activities

From the spectrophotometric measurement of the free radical scavenging activity of the extract and fractions against the stable DPPH free radical, the percentage inhibition was calculated to determine the antioxidant capacity of the test samples. The result of the screening is shown in Figure 3 and Table 1. The extract and fractions exhibited different antioxidant capacity based on percentage inhibition: n-hexane fraction (64.19%), water (55.67%), methanol (36.38%), and ethyl acetate (20.25%). While Vitamin C used as the positive control (standard antioxidant) had 85.51% inhibition of the DPPH free radical. Vitamin C is one of the more powerful and well-known antioxidants.[25]For the water fraction, the Lieberman Burchard reaction gave a negative response. However, the TLC and the quantitative antioxidant tests, the fraction exhibited relatively strong antioxidant activity (55.67%). The percentage of free radical inhibition of the water fraction was the closest to the n-hexane fraction (Figure 3). From the results, the antioxidant activities of the test samples may be due to the presence of compounds in the gonads with strong antioxidant activities such as terpenoids. Other water-soluble compounds with strong antioxidant activity include phenols, flavonoids, and alkaloids.[26,27] In this study, the IC_{50} value obtained in the n-hexane fraction for the sea urchin gonad extract was 77.1025 ppm (Table 2). According to Molyneux (2004), an ingredient is said to have a very strong antioxidant activity if it has an IC_{50} value <50 ppm, strong if the IC_{50} value is 51-100 ppm, moderate if the IC_{50} value is 101-150 ppm, and is weak if the IC_{50} value is 151 -200 ppm.[28] So it can be said that the antioxidant activity of the n-hexane fraction of the gonad extract of sea urchins is categorized as strong.

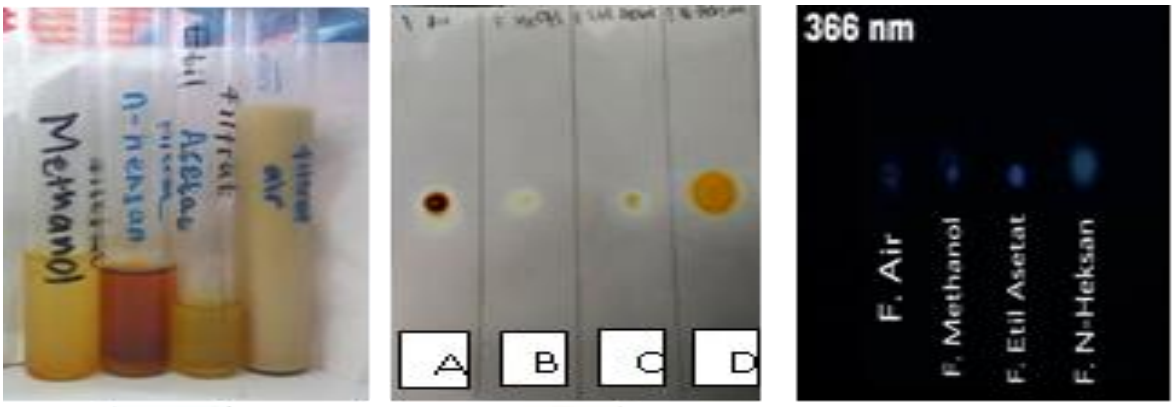

Figure 2 : TLC-DPPH Test. Water Fraction (A) Methanol Fraction (B) Ethyl Acetate Fraction (C) n-Hexane Fraction (D)

Table 1: Results of DPPH free radical scavenging activity of fractions and the standard Antioxidant agent (Vitamin C)

No	Sample	(λ)	Concentration (ppm)	Absorbance			Inhibition (%)
				OD I	OD II	OD III	
1	DPPH	517	100	0.688	-	-	
2	Methanol Fraction	517	100	0.447	0.431	0.435	36.38
3	n-Hexane Fraction	517	100	0.242	0.247	0.250	64.19
4	Ethyl acetate Fraction	517	100	0.542	0.553	0.551	20.25
5	Water Fraction	517	100	0.304	0.310	0.301	55.67
6	Vitamin C	517	4.00	0.102	0.098	0.099	85.51

Table 2: Comparison of the Antioxidant activity of the n-Hexane fraction and Vitamin C

C (ppm)	n-Hexane Fraction			C (ppm)	Vitamin C			Inhibition (%)	
	Abs I	Abs II	Abs III		Abs I	Abs II	Abs III	Fraction	Vit C
400	0.111	0.103	0.108	6.0	0.065	0.062	0.068	84.40	90.55
200	0.185	0.190	0.188	4.8	0.095	0.090	0.089	72.72	86.72
100	0.242	0.247	0.250	3.6	0.103	0.105	0.108	64.20	84.69
50	0.401	0.398	0.396	2.4	0.243	0.245	0.252	42.10	64.68
25	0.552	0.570	0.583	1.2	0.441	0.438	0.450	17.37	35.61

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

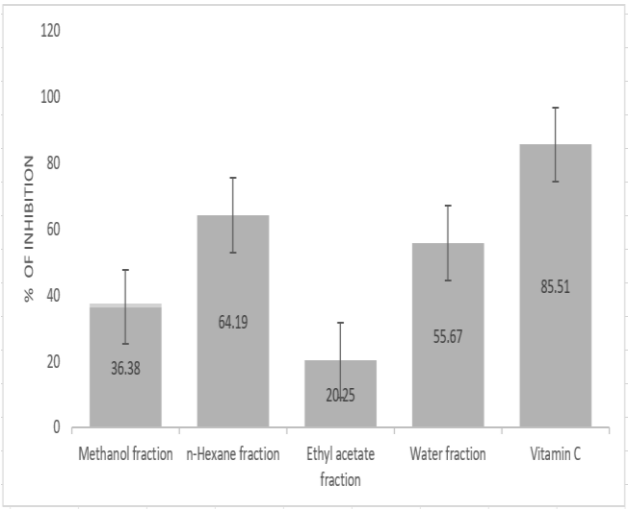

Figure 3: Antioxidant Activity of Sea Urchin Gonad Fraction

Conclusion

Based on the tests' results, the gonad extract of sea urchins has antioxidant activity. Therefore, it could be a source of natural antioxidants that could be used to prevent premature ageing (anti-ageing). The most promising fraction of the sea urchins gonad against free radicals is the n-hexane categorized as a potent antioxidant.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research was carried out with funding assistance from the Risbinakes program for prospective lecturers. Thank you to the Research and Development Section of the Jakarta Health Polytechnic II, who provided meaningful research improvement suggestions.

References

1. Luceri C, Bigagli E, Femia A Pietro, Caderni G, Giovannelli L, Lodovici M. Aging related changes in circulating reactive oxygen species (ROS) and protein carbonyls are indicative of liver oxidative injury. Toxicol Rep. 2018; 5:141-145.
2. Finkel T and Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000; 408(6809):239-247.
3. Sies H and Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2020; 21(7):363-383.
4. Ito N, Hirose M, Fukushima S, Tsuda H, Shirai T, Tatematsu M. Studies on antioxidants: Their carcinogenic and modifying effects on chemical carcinogenesis. Food Chem Toxicol. 1986; 24(10-11):1071-1082.
5. Safer AM. Hepatotoxicity induced by the anti-oxidant food additive, butylated hydroxytoluene (BHT), in rats: An electron microscopical study. Histol Histopathol. 1999; 14(2):391-406.
6. Ham J, Lim W, You S, Song G. Butylated hydroxyanisole induces testicular dysfunction in mouse testis cells by dysregulating calcium homeostasis and stimulating endoplasmic reticulum stress. Sci Total Environ. 2020; 702:134775.
7. Park S, Lee J, Lim W, You S, Song G. Butylated Hydroxyanisole Exerts Neurotoxic Effects by Promoting Cytosolic Calcium Accumulation and Endoplasmic Reticulum Stress in Astrocytes. J Agric Food Chem. 2019; 67(34):9618-9629.
8. Seifi-jamadi A, Kohram H, Zareh-shahne A. Effect of various concentrations of butylated hydroxyanisole and butylated hydroxytoluene on freezing capacity of Turkman stallion sperm. Anim Reprod Sci. 2016; (170):108-113.
9. Ham J, Lim W, Whang K, Song G. Butylated hydroxytoluene induces dysregulation of calcium homeostasis and endoplasmic reticulum stress resulting in mouse. Environ Pollut. 2019;256:113421.
10. Liang X, Zhao Y, Liu W, Li Z, Souders CL, Martyniuk CJ. Butylated hydroxytoluene induces hyperactivity and alters dopamine-related gene expression in larval zebrafish (Danio rerio). Environ Pollut. 2019; 113624.
11. Wang W and Kannan K. Inventory, loading and discharge of synthetic phenolic antioxidants (SPAs) and their metabolites in wastewater treatment plants,. Water Res. 2017; 129:413-418.
12. Shahidi F. Maximising the Value of Marine By-Products - Google Books. 1st ed. (Fereidoon Shahidi, ed.). CRC Press; 2007. Accessed January 28, 2019.
13. Lawrence MJ and Lawrence JM. Edible Sea Urchins : Biology and Ecology. Elsevier; 2001; 1. Accessed January 25, 2019.
14. Shikov AN, Makarov VG, Pozharitskaya ON, Krishtopina AS. Naphthoquinone pigments from sea urchins : chemistry and pharmacology. Phytochem Rev. 2018; 17(3):509-534.
15. Suwignyo S, Widigdo B, Wardiatno YKM. Avertebrata Air. Penebar Swadaya; 2005; 1.
16. Sidiqi FM, Pringgenies D, Setyati WA. Antibacterial Activity of Gonad Methanol Extract of the Sea Urchin Diadema Setosum Against Methicillin-Resistant Staphylococcus aureus and Escherichia coli. Earth Environ Sci. 2019; 246:012040.
17. Delianis P, Ana A, Sri S, Dwi H. The Potency of Sea Urchin (*Diadema setosum*) Gonad on Brain Cells of White Rats (Rattus norvegicus). Asian J Pharm. 2016; 10(2):100-107.
18. Mayer AM, Glaser KB, Cuevas C, Jacobs RS, Kem W, Little RD, McIntosh JM, Newman DJ, Potts BC, Shuster DE. The odyssey of marine pharmaceuticals: a current pipeline perspective. Trends Pharmacol Sci. 2010; 31(6):255-265.
19. Garama D, Bremer P, Carne A. Extraction and analysis of carotenoids from the new zealand sea urchin evechinus chloroticus gonads. Acta Biochim Pol. 2012; 59(1):83-85.
20. Lawrence EJM, Suckling CC, Kelly MS, Symonds RC. Carotenoids in Sea Urchins. Dev Aquacult Fisheries Sci. 2020; 43(4):209-214.
21. M. Christopher AMLS. Marine Natural Products as Novel Antioxidant Prototypes. *J* Nat Prod. 2003; 66(5):605-608.
22. Cieśla Ł, Kryszeń J, Stochmal A, Oleszek W, Waksmundzka-Hajnos M. Approach to develop a standardized TLC-DPPH test for assessing free radical scavenging properties of selected phenolic compounds. J Pharm Biomed Anal. 2012; 70:126-135.
23. Hatano T, Kagawa H, Yasahara HT OT. The effect of extracts on DPPH radical was estimated according to the method. Food Chem. 1988; 78(3):347-354.
24. Tamokou JD, Simo Mpetga DJ, Keilah Lunga P, Tene M, Tane P, Kuiate JR. Antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark of Albizia adianthifolia (Mimosoideae). BMC Compl Altern Med. 2012; 12(1):1-10.
25. Kirtawade R, Salve P, Kulkarni A, Dhabale P. Herbal antioxidant: Vitamin C. Res J Pharm Technol. 2010; 3(1):58-61.
26. Ghasemi K. Antioxidant Activity, Phenol, an Flavonoid Contents of Citrus. University of Agricultureal Science; 2008.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

27. Sayeed A. Introduction of Plant Constituents and Their Tests. New Delhi University; 2007.
28. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. J Sci Technol. 2004; 26(2):211-219.