J. Mar. Biotechnol. Immunol
Vol. 3 No. 1 (2025)

ISSN 3026-1457 (Online)
ISSN 3026-5274 (Print)

Journal of Marine Biotechnology and Immunology

Journal homepage : https://ejournal.immunolmarbiotech.com

Antioxidant Activity and Bioactive Coumpounds Extract of *Xylocarpus granatum* Fruit from Pengudang Village, Riau Island

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OPEN ACCESS

Article Info
Received: Oktober 11, 2024
Accepted: January 02, 2025
Published: January 31, 2025
Available online: January 31, 2025

Keywords:
Black Band Disease
Coral Disease
Montipora
Pari Islands
White Syndrome

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Abstract

Xylocarpus granatum is a mangrove species found in coastal waters that performs photosynthesis with the aid of chlorophyll pigments. These pigments are considered bioactive compounds and are thought to have antioxidant properties. Natural antioxidants are essential for combating free radicals and reducing the carcinogenic effects of synthetic compounds over prolonged use. This study aimed to evaluate the antioxidant activity and bioactive compounds of *Xylocarpus granatum* fruit extract from Pengudang Village. Antioxidant activity was assessed using the DPPH method, while bioactive compounds were analyzed through steroid and triterpenoid, tannin, and saponin tests. The total flavonoid and phenol content were determined using quercetin and gallic acid solutions, and chlorophyll and carotenoid pigments were analyzed using acetone as the solvent. The results showed that the antioxidant activity of the *Xylocarpus granatum* fruit extract from Pengudang Village was in the very weak category, with an activity value of 236.46 ppm. The total chlorophyll a and carotenoid content were found to be 70.9 mg QE/g of the sample, while flavonoid content was 3.21 mg GAE/g, and phenolic content was 0.35 mg/g. Tannin and saponin contents were 0.69 mg/g and 0.5 µmol/g, respectively. These findings indicate that although the extract exhibits some antioxidant properties, it is relatively weak in comparison to other natural sources.

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1. Introduction

The Nyiri plant (*Xylocarpus granatum*) is a mangrove plant often found in Indonesia. This plant can grow in tidal areas and along rivers. Antioxidants are chemical compounds that can inhibit oxidation reactions by donating one or more electrons to free radicals to reduce these free radicals. The term antioxidant is now familiar because antioxidants are known to positively influence human health, especially their ability to neutralize the negative impact of free radicals.

Free radicals are molecules with unpaired electrons, which can cause the molecule to donate or accept electrons from another molecule; this makes free radicals unstable and very reactive (Nosa *et al.,* 2020). Free radicals originate from external environmental exposure and metabolism (Nurjanah *et al.*, 2021). The DPPH method is one of the methods used to determine the antioxidant activity and has a simple, fast, and easy for screening the antioxidant activity of food ingredients or plant extracts. Antioxidant activity is indicated by the DPPH solution's color change from dark purple to pale yellow and antioxidant activity is expressed in Inhibition Concentration 50 (IC_{50}). This exploration of the use of mangroves from Pengudang Village needs to be carried out as an initial stage of information in developing a natural product.

2. Material and methods

2.1 Study location

The sampling location was taken in the Pengudang Village area, Riau Islands Province. Samples are taken as much as possible from the *X. granatum* fruit available at the sampling point (Figure 1).

2.2 Research Methods and Procedures

2.2.1 Sample preparation

The mangrove fruit samples that have been collected are washed clean using running water and dried in a place that is not exposed to direct sunlight or dry wind for ± 5 days.

Figure 1. Research areas

Next, the samples that have been collected will be cut into smaller sizes ± 2 cm.

2.2.2 Sample extraction

Sample extraction used a single maceration method. 50 gram samples of *Xylocarpus granatum* mangrove fruit that had been mashed were then soaked in 500 mL methanol solvent using a glass beaker for 1 x 24 hours and then filtered with rotary evaporator. The filtrate that has been obtained will be placed in a beaker the residue from the first soaking will be macerated again with methanol solvent for 1 x 24 hours.

2.2.3 Determination of maximum absorbance of DPPH

Four mg of DPPH powder is then dissolved with 100 ml of ethanol in an Erlenmenyer to produce a DPPH 0.1 mM. 4 ml of the DPPH solution was put into a cuvette to measure at a wavelength of 400-800 nm (Hidayati *et al.*, 2020).

2.2.3.1 Determination of antioxidant activity

The 1000 ppm extract solution was diluted to 50, 100, 150, 200, and 250 ppm concentration into an Erlenmeyer. 3 ml of each extract concentration was mixed with 0.1 mM DPPH solution. Next, the solution was incubated for 30 minutes, and the absorbance was measured using the maximum DPPH wavelength. The antioxidant strength value is measured using the %inhibition formula, and then the results of the linear regression equation were calculated for the IC50 value.

2.2.4 Phytochemical analysis

Phytochemical testing was done on steroids & triterpenoids, saponins, tannins, and flavonoids.

2.2.4.1 Flavonoids

Flavonoid testing was carried out by making a solution of 1000 ppm *Xylocarpus granatum* extract then taking 5 ml and adding 0.05 mg of magnesium powder, and followed by 1 ml of concentrated HCl (Erlidawati and Zahrina, 2023). The solution was shaken vigorously until a color change occurred. A positive test indicates the formation of a red, yellow, or orange color (Nur & Rahmawati, 2019).

2.2.4.2 Steroids and triterpenoids

Testing for steroids and triterpenoids uses the Lierbermann-Bouchard method. Add acetic acid and sulfuric acid to the 1000 ppm extract solution and observe the color changes. According to Novitasari and Putri (2016), the Lierbermann-Bouchard method is a color test for the characteristics of steroids and triterpenoids. Positive results are green-blue for steroids and red-purple for triterpenoids (Habibi *et al.*, 2018).

2.2.4.3 Saponins

Saponin testing was carried out with a solution of extract. 1000 ppm was added with 5ml of chloroform solution, shaken until homogeneous, and left for 2 minutes. Then, two drops of 2N HCl were added. Extracts containing saponin produce stable foam for 10 minutes (Pontoh *et al.*, 2019).

2.2.4.4 Tannin

Tannin testing was carried out by taking a solution of extract. 1000 ppm was added with 1% gelatin then a few drops of 10% NaCl were added. A positive result contains tannin if white precipitate forms (Makatamba *et al.*, 2020).

2.2.4.5 Total phenolic and flavonoid testing

The total phenolic content was carried out with gallic acid as a standard with a concentration of 5,10,15,20,25 ppm in 2 ml, then 5 ml of distilled water and 0.5 ml of 5% Folin-Ciocalteu reagent and 1 ml of 5% Na_2CO_3 solution, then incubated for 1 hour. The absorbance was measured using a spectrophotometer at a wavelength of 725 nm (Lakoro *et al.*, 2020). Next, the total flavonoid content was carried out with a quercetin solution as a standard with a concentration of 5, 10, 15, 20, 25 ppm with 1 ml of $AlCl_3$ and 8 ml of 5% acetic acid and left for 16 minutes, then the absorbance was measured at a wavelength of 415 nm. The standard curve for gallic acid and quercetin was obtained from the regression values by entering the absorbance value obtained on the x-axis and the concentration on the y-axis. The same steps were carried out on the *Xylocarpus granatum* extract solution. The total phenol value calculation is expressed in mg Galic Acid Equivalent (GAE)/1000mg, and total flavonoids is expressed in mg Quercetin Equivalent (QE)/1000mg.

2.2.4.6 Carotenoid and chlorophyll testing

A 1000 ppm *Xylocarpus granatum* extract solution used acetone and the absorbance was measured at wavelengths of 645nm, 663nm for cjlorophyll and 480 nm for carotenoid (Hidayati *et al.*, 2017).

3. Results

3.1 *Xylocarpus granatum* fruit extract

The yield percentage of *Xylocarpus granatum* fruit extract with a single maceration process was 17.85% (Tabel 1).

3.2 Bioactive Compound Content

The phytochemical tests carried out are steroid & triterpenoid, saponin, tannin, and flavonoid tests. *Xylocarpus granatum* contains bioactive compounds like triterpenoids, tannins, and flavonoids. (Table 2).

Table 1. Extract of *Xylocarpus granatum* fruit

Solvent	Dried samples (gram)	Extract (gram)	Yield Percentage (%)	Extract colour	Extract Form
Methanol	11.5	5.6	17.85	Reddish brown	pasta

Table 2. Phytochemical test of extract *Xylocarpus granatum* fruit

Extract	Bioactive compounds	Result
Xylocarpus granatum fruit	Flavonoids	+
	Steroids	-
	Triterpenoids	+
	Saponins	-
	Tanins	+

3.3 Total Phenolic and Flavonoid Content

The standard curve of the gallic acid solution and the quercetin solution has the same regression value with a value of r = 0.9888 for the gallic acid solution and r = 0.9925 for the quercetin solution. If the r value is close to 1, it shows a relationship between the solvent concentration and the absorbance value of gallic acid and quercetin. Next, the equation from the linear regression was used to calculate the total content of phenolic and flavonoid compounds. The following is a table of absorbance values for standard gallic acid, quercetin, and extract absorbance solutions (Table 3).

Xylocarpus granatum fruit extract has a total phenolic compound content (32.1 mg GAE/g sample) and a total flavonoid content (70.9 mg QE/ g sample).

Table 3. Total phenolic and flavonoids extract *Xylocarpus granatum* fruit

Test	Concentration (ppm)	Standart Abs. (ppm)	Concentration (ppm)	Extract Abs. (ppm)	Regression	Total
Phenolic	5	0.160	1000	0.0710	y = 0.0334x + 0.0364	32.1 mg GAE/g sample
	10	0.283				
	15	0.424				
	20	0.643				
	25	0.816				
Flavonoid	5	0.049	1000	0.0866	y = 0.0235x + 0.0801	70.9 mg QE/ g sample
	10	0.156				
	15	0.244				
	20	0.393				
	25	0.517				

3.4 Pigments Content

The chlorophyll a, chlorophyll b and carotenoid content of *Xylocarpus granatum* extract showed in (Table 4).

Table 4. Pigments content of extract *Xylocarpus granatum* fruit

Extract	Solvent	Absorbance			Pigment	Result
		663	645	480		
Xylocarpus granatum fruit	Acetone	0.038	0.038	0.031	Chlorophyll a (mg/g)	0.357
					Chlorophyll b (mg/g)	0.692
					Carotenoid (µmol/g)	0.5

3.5 Antioxidant Activity

3.5.1 DPPH maximum wavelength

DPPH or free radicals with unpaired electrons have a maximum absorbance at a 510-520 nm wavelength. In this research, the maximum absorbance of DPPH was obtained at a wavelength of 515 nm (Figure 2).

3.5.2 Antioxidant activity of *Xylocarpus granatum* fruit extract

The antioxidant activity test used five serial sample concentration, namely 50, 100, 150, 200, and 250 ppm (Table 5). The linear regression graph of antioxidant activity, extract concentration, and the resulting percentage of inhibition value is directly proportional. If the concentration is higher, the percentage of inhibition will also be higher. The following table shows absorbance values, percentage inhibition, and IC50 values.

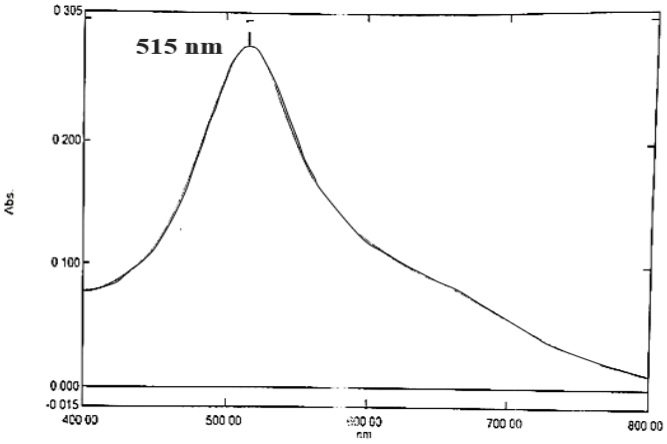

Figure 2. DPPH maximum wavelength

Table 5. Antioxidant activity of *Xylocarpus granatum* fruit extract

Solvent	Concentration (ppm)	DPPH Absorbance	Absorbance of extract + DPPH	Inhibition Percentage	IC_{50} (ppm)	Regression Equation
Methanol	50	0.192	0.168	12.50	236.46	y = 0.2176x + 1.7761
	100		0.154	19.79		
	150		0.121	36.97		
	200		0.107	44.27		
	250		0.957	50.15		

4. Discussion

Xylocarpus granatum extract has a yield value of 17.85% from 11.5 grams of dry sample. This result is greater than research (Ramadani *et al.*, 2020) of 10.05% using ethanol solvent. Methanol (polar) solvent has the property of being able to dissolve various compound components, which can influence the results of higher yield values (Saputri *et al.*, 2019). Differences in yield values in previous studies with the same sample species and different solvents influenced the yield values produced.

Triterpenoids are compound that function as antiseptic, antimicrobial, and antibiotic (Akasiah *et al*., 2021). Tannin compounds have a role in being antibacterial and antioxidant. Flavonoids are a class of phytochemical compounds with protective properties found in many plants. And flavonoids are bioflavonoids that function as antioxidants (sudirman *et al*.,2016). Another factor that influences phytochemical tests is the solvent used due to the role of the solvent in attracting compounds in the sample (Haryani *et al.*, 2022).

Compared with the other research, the Pedada fruit skin extract (*Sonneratia caseolaris*) using methanol as a solvent has a total phenolic compound content of 32.75 mg GAE/g. Flavonoid compounds from the phenol group have a role in reducing free radicals (Hudaifah and Utami, 2020). Flavonoid compounds are compounds derived from phenol which can act as antioxidants. One of the natural antioxidants is flavonoid compounds by inhibiting oxidation (Purwaningsih and Deskawati, 2020).

The other compounds that help *Xylocarpus granatum* extract reduce free radicals are chlorophyll a, b, and carotenoids. According to Sedjati *et al.* (2018), the effect of high and low antioxidant activity can be influenced by other compounds, namely chlorophyll, carotenoids, and flavonoids. *Xylocarpus granatum* has a higher chlorophyll b value compared to chlorophyll a. According to Febrianto *et al.* (2019), it is thought that the use of methanol (polar) will dissolve more chlorophyll b than chlorophyll a.

Inhibition percentage value that has been obtained will be analyzed to look for the Inhibition correction value of 50 with linear regression, namely looking for the x value at y=0.2176x+1.7761 for antioxidant activity. The value obtained for antioxidant activity was 236.46 ppm and was classified as very weak (Supriyana *et al*., 2019). Determination of the dampening power of antioxidants was carried out by calculating the decrease in the value of DPPH absorbance on the concentration of the *Xylocarpus granatum* extract solution. According to Hidayati *et al.* (2022), as the concentration of the extract increases, there is an increase in the inhibitory value and antioxidant capacity of the extract. The %inhibition graph obtained results from calculating the average absorbance value of each extract concentration and analyzing it using the inhibition presentation formula. Then, this value will be used as a linear regression equation with the form y= bx + a which will determine at what concentration of the sample can be an optimal antioxidant in the IC50 range. The higher the IC50 value, the smaller the strength of the antioxidant activity, whereas conversely, if the IC50 value is low, the greater the strength of the antioxidant activity (Andriani and Murtisiwi, 2020). IC50 value of *Xylocarpus granatum* extract amounting to 236.46 ppm, so that at this concentration, the sample used can inhibit or counteract 50% of the reactivity of free radicals. The resulting antioxidant activity values vary due to differences in sampling locations, sample handling after collection (Marraskuranto *et al.* 2021), and the type of solvent used during the sample extraction process (Purwaningsih and Deskawati, 2020).

5. Conclusions

The bioactive compounds of *Xylocarpus granatum* from Pengudang Village, Riau Islands, are positive for triterpenoids and tannins. The total flavonoid content, total phenolic content, chlorophyll a, chlorophyll b, and carotenoids had values of 70.9 mg QE/g sample, 32.1 mg GAE/g sample, 0.357 mg/g, 0.692 mg/g, 0.5 µmol/ ml, and has very weak antioxidant activity.

Ethics approval

No permits were required.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

JRH is doing conceptualization, data curation, funding acquisition, writing- review & editing and methodology. DP is doing formal analysis, project administration, and writing - original draft. MFBSA, RA and AP are doing investigation, resources and writing - original draft. MSR is doing methodology and writing - review & editing.

Funding

The author would like to thank the Raja Ali Haji Maritime University for providing research funds with contract number 646/UN53/PT/2023

Acknowledgments

The author would like to thank the Raja Ali Haji Maritime University

Declaration of competing Interest

None

Referrences

Akasia, A. I., Putra, I. D. N. N., & Putra, I. N. G. 2017. Skrining Fitokimia Ekstrak Daun Mangrove *Rhizhopora mucronata* dan *Rhizhopora apiculate* yang Dikoleksi dari Kawasan Mangrove Desa Tuban Bali. *Journal of Marine Research and Tehcnology*. 4(1): 16-22.

Andriani, D. & Murtisiwi, L. 2020. Uji Aktivitas Antioksidan Ekstrak Etanol 70% Bunga Telang (*Clitoria ternatea* L) dari Daerah Sleman dengan Metode DPPH. *Jurnal Farmasi Indonesia.* 17(1): 2685 5062. https://doi.org/10.23917/pharmacon.v17i1.9321

Erlidawati, E., & Zahrani, Z. 2023. Telaah Senyawa Metabolit Sekunder Dari Air Gebang dan Pelepah (*Corypha utan*). Jurnal Ilmiah Mahasiswa Pendidikan Kimia.8(1).

Febrianto, W., Djunaedi, A., Suryono, S., Santosa, G. W., & Sunaryo, S. 2019. Potensi Antioksidan Rumput Laut *Gracilaria verrucosa* dari Pantai Gunung Kidul Yogyakarta. *Jurnal Kelautan Tropis*. 22(1): 81-86. https://doi.org/10.14710/jkt.v22i1.4669

Habibi, A. I., Firmansyah, R. A., & Setyawati, S. M. 2018. Skrining Fitokimia Ekstrak *n*-Heksana Korteks Batang Salam (*Syzygium polyanthum*). *Indonesia Journal Of Chemical Science*. 7(1): 1-4.

Haryani, T. S., Hasanah, U., Komala, O., Lestari, I., & Agurtina, D. 2022. Potensi Ekstrak *Codium, Halimeda, Dictyota, Chondrus, Glacillaria* Sebagai Sumber Pigmen dan Antioksidan Alami. *Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup*. 22(1): 9 17.

Hidayati, J. R., Ridlo, A., & Pramesti, R. 2017. Aktivitas Antioksidan Ekstrak Rumput Laut *Padina sp.* dari Perairan Badengan Jepara dengan Metode Transfer Elektron. *Buletin Oseanografi Marina*. 6(1): 46-52. https://doi.org/10.14710/buloma.v6i1.15742

Hidayati, J. R., Karlina, I., Wijaya, A., Ningsih, D. P. N., & Bahry, M. S. 2022. Bioactive Coumpounds and Antioxidant Activity of Tropical Red Algae *Gracilaria sp.* from Bintan Island. *Maritime Continent Fulcrum International Conference*. http://dx.doi.org/10.1088/17551315/1148/1/012004

Hidayati, J. R., Yudiati, E., Pringgenies, D., Oktaviyanti, D. T., & Kusuma, A. P. 2020. Comparative Study on Antioxidant Activites Total Phenolic Coumpound and Pigment Contents of Tropical *Spirulina platensis Gracilaria arcuata* and *Ulva lactuca* Extracted In Different Solvent Polarity. *E3S Web of Conferences ISMFR*. http://dx.doi.org/10.1051/e3sconf/202014703012

Hudaifah, I., Mutamimah, D., & Utami, A. U., 2020. Komponen Bioaktif dari *Euchema cottonni, Ulva lactucata, Halimeda opuntia,* dan *Padina australis. Jurnal Ilmu Perikanan dan Kelautan.* 2(2): 63-70.

Lakoro, J. E., Runtuwene, M. R. J., & Yamlean, P. V. Y. 2020. Uji Aktivitas Antioksidan dan Penentuan Total Kandungan Fenolik Ekstrak Etanol Daun Nanamuha (*Bridelia monoica* Merr). *Pharmacon*. 9(2): 17-183. https://doi.org/10.35799/pha.9.2020.29268

Makatamba, V., Fatimawali., & Rudengan, G. 2020. Analisis Senyawa Tannin dan Aktivitas Antibakteri Fraksi Buah Sirih (*Piper betle* L) Terhadap *Streptococcus mutans. Jurnal MIPA*. 9(2): 75-80. https://doi.org/10.35799/jmuo.9.2.2020.28922

Marraskuranto, E., Nursid, M., Utami, S., Setyaningsih, I., & Tarman, K. 2021. Kandungan Fitokimia Potensi Antibakteri dan Antioksidan Hasil Ekstraksi *Caulerpa racemosa* dengan Pelarut Berbeda. *JPB Kelautan dan Perikanan.* 16(1): 1-10. http://dx.doi.org/10.15578/jpbkp.v16i1.696

Nosa, S. P., Karnila, R., & Diharmi, A. 2020. Potensi Kappa Karaginan Rumput Laut (*Euchema Cottonii*) Sebagai Antioksidan dan Inhibitor Enzim α Glukosidase. *Jurnal Berkala Perikanan Terubuk*. 48(2): 1-10. https://doi.org/10.31258/TERUBUK.48.2.434-449

Novitasari, A. E & Putri, D. Z. 2016. Isolasi dan Identifikasi Saponin dada Ekstrak Daun Mahkota Dewa dengan Ekstraksi Maserasi. *Jurnal Sains.* 6(12): 10-14.

Nur, R. M., & Rahmawati, R. 2019. Kombinasi Uji Aktivitas Antifoulinng (*Rhizhopora apiculata*) di Kabupaten Pulau Morotai. *Jurnal Ilmu-Ilmu Perikanan dan Budidaya Perairan.* 14(1). https://doi.org/10.31851/jipbp.v14i1.3365

Nurjanah., Ramli, R. L., Jacoeb, A. M., & Seulale, A. V. 2021. Karakteristik Fisikokimia dan Antioksidan Krim Lulur Kombinasi Bubur Rumput Laut Merah (*Eucheuma cottonii*) dan Cokelat (*Sargassum sp.*). *Jurnal Standardisasi*. 23(3): 227-240. http://dx.doi.org/10.31153/js.v23i3.895

Pontoh, F. W., Sanger, G., Kaseger, B. E., Wonggo, D., Montolalu, R. I., Damangilala, L. J., & Makapedua, D. 2019. Kandungan Fitokimia Kadar Total Fenol dan Aktivitas Antioksidan Ekstrak Rumput Laut *Halymenia durvillae*. *Jurnal Media Teknologi Hasil Perikanan*. 7(3): 62-67. https://doi.org/10.35800/mthp.7.3.2019.23615

Purwaningsih, S & Deskawati, E. 2020. Karakteristik dan Aktivitas Antioksidan Rumput Laut *Gracilaria sp.* Asal Banten. *JPHPI.* 23(3): 503-512. https://doi.org/10.17844/jphpi.v23i3.32808

Ramadhani, A., Saadah, S., Sogandi. 2020. Efek antibakteri ekstrak daun cengkeh (*Syzygium aromaticum*) terhadap *Escherichia coli* dan *Staphylococcus aureus*. *Jurnal Bioteknologi dan Biosains Indonesia*, *7*(2), 203-214.

Supriatna, D., Mulyani, Y., Rostini, I., & Agung, M. U. K. (2019). Aktivitas Antioksidan, Kadar Total Flavonoid dan Fenol Ekstrak Metanol Kulit Batang Mangrove Berdasarkan Stadia Pertumbuhannya. *Jurnal Perikanan Dan Kelautan*, 10(2), 35–42.

Sedjati, S., Supriyantini, E., Ridlo, A., Soenardjo, N., & Santi, V. Y. 2018. Kandungan Pigmen Total Fenolik dan Aktivitas Antioksidan *Sargassum sp. Jurnal Kelautan Tropis*. 21(2): 137-144. https://doi.org/10.14710/jkt.v21i2.3329

Sudirman, S. Nurjanah, A. M. J., Jacoeb, A. M. 2016. Identifikasi Struktur Senyawa Antioksidan Buah Lindur. *Jurnal Pengolahan Hasil Perikanan Indonesia.* 19(2): 94-99. https://doi.org/10.17844/jphpi.2016.19.2.94