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ISOLATION AND CHARACTERISATION OF (17*E*)-CYCLOART-17, 26-DIEN-3*β*-OL AND CYCLOART-3*β*, 25-DIOL FROM THE PEEL OF *ARTOCARPUS HETEROPHYLLUS* L.

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ABSTRACT

Two triterpenoids namely (17E)-cycloart-17,26-dien-3β-ol and cycloart-3β,25-diol, were isolated as a mixture from the column fraction by elution with n-hexane/30% ethyl acetate of methanol extract of ripe jackfruit (Artocarpus heterophylus) peel. This is the first report of their isolation from this plant. Their structures were elucidated by comparing the nuclear magnetic resonance (NMR) data with those published for these compounds. The antioxidant activity of these isolated compounds was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and showed a low scavenging attitude compared with standard phenolic compounds (2-tert-butyl-4-methoxyphenol (BHA), Trolox, L(+)-ascorbic acid and gallic acid). Extracts and isolated compounds did not exhibit any antibacterial activity against two Gram-positive and two Gram-negative bacteria.

Keywords: (17E)-Cycloart-17, 26-dien-3*β*-ol, Cycloart-3*β*, 25-diol, Antioxidant activity, *Artocarpus heterophyllus* L.

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INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* L.) trees belong to the Moraceae family. They grow abundantly in Bangladesh, India and many parts of Southeast Asia (Rahman *et al*. 1999). It is a medium-size tree typically reaching 28 ft–80 ft in height that is easily accessible for its fruit. The fruit is born on side branches and the main branches of the tree. The average weight of a fruit is 3.5 kg–10 kg and sometimes a fruit may reach up to 25 kg. *A. heterophyllus* is an important source of compounds like morin, dihydromorin, cynomacurin, artocarpin, isoiartocarpin, cycloartocarpin, artocarpesin, oxydihydroartocarpesin, artocarpetin, betulinic acid, artocarpanone and heterophylol (Prakash *et al*. 2009), cycloartenone, cycloartenol and a diastereomeric mixture of 2,3-butanediols 3a and 3b in a 3:1 ratio (Ragasa, Jorvina and Rideout 2004). Its leave contains compounds such as *n*-octadec-9-enoyl *α*-L-rhamnopyranoside, *n*-octadec-9,12-dienoyl *α*-L-rhamnopyranoside, *n*-octadec-9, 12-dienoyl *β*-D-glucopyranoside, *n*-octadec-9-enoyl-*β*-D-glucopyranoside, *n*-octadec-9-enoyl-*β*-D-arabinopyranoside, *n*-octadec-9-enoyl-*α*-D-xylopyranoside (Shahin, Kazmi and Ali 2012).

Mass spectrometry analysis of jackfruit powder of Tamil Nadu, India identified 27 compounds (Srinivasan and Kumaravel 2016). A structural study of low molecular weight rubbers from this plant has been documented (Mekkriengkrai *et al*. 2004). The methanolic extract of *A. heterophyllus* and its tegmen exhibited significant anticancer potential with no toxicity on normal cell line (Patel and Patel 2011) and antitumor agent (Rajendran and Ramakrishnan 2010), respectively.

The present study was aimed to isolate as many compounds as possible and elucidate the structure of them from the column fraction by elution with *n*-hexane/30% ethyl acetate of methanol extract of ripe peel of *A. heterophylus* using spectroscopic techniques, and the evaluation of their bioactivity (antioxidant and antibacterial activity).

METHODS

General Experimental Procedures

The nuclear magnetic resonance (NMR) spectra were recorded using a Bruker DPX-400 MHz instrument (Bruker BioSpin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany), with chemical shift (*δ*) data reported in ppm relative to the residual solvent peak. The spectra were taken by using $CDCl_3$ as a standard reference. Mass spectra were received using the Applied Biosystems MDS/SCIEX 3200 Q-TRAP ® LC/MS/MS, USA system. The UV absorbance was performed with a PerkinElmer Shelton, CT 06484 USA, Lambda 25 UV/Vis spectrophotometer. A vacuum rotary evaporator (BUCHI, Rotavapor R-210 Switzerland) was used for evaporating solvents. All solvents were of analytical grade and obtained from commercial sources (Sigma-Aldrich, St. Louis, MO, USA).

Plant Material

Fruit of *A. heterophylus* was collected from a garden in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh.

Extraction and Isolation

The powdered peel weighted 500 g (fresh sample was 1,670 g) was soaked in 3.0 L of methanol for five days. The whole mixture was then filtered through filter paper and the extracted powder was again soaked in 3.0 L methanol for three days and the filtrate thus obtained was concentrated at 50°C with a rotary vacuum evaporator to provide crude extract (110 g). The concentrated extract (100 g) was dispersed in water:methanol (1:1) in 200 mL. The aqueous methanolic solution was fractionated successively in chloroform (300 mL × 3) and ethyl acetate (300 mL × 3). Again, these fractionated chloroform and ethyl acetate was evaporated by rotary vacuum evaporator. A portion of a chloroform extract (5 g) was subjected to column chromatography (CC) over silica gel, using gradients of *n*-hexane followed by *n*-hexane and ethyl acetate mixtures of increasing polarity to afford a total of 28 fractions (each 100 mL). After elution with *n*-hexane/30% ethyl acetate, a mixture (JP-5) yielded a white powder compound (2.0 mg). After NMR and MS analysis, JP-5 was identified as a mixture of Compounds 1 and 2.

(17*E*)-Cycloart-17, 26-Dien-3*β*-Ol (Compound 1)

White amorphous powder; FTIR (ATR) υ_{max}: 3364, 2946, 2837, 1656, 1450, 1410, 1113, 1019, 697 cm^{-1}; ^{1}H NMR (400 $MH_{Z,}$ $CDCl_3$): δ 5.49 & δ 5.53 (2H, brs, CH_2-26), 0.54 (each 1H, d, *J* = 3.6 Hz, 19-CH_2), 0.31 (each 1H, d, *J* = 3.6 Hz, 19-CH_2), 3.28 (m, 1H, 3-CHOH), 1.33 (m, 3H, 21-CH_3), 0.79 (s, 3H, 28-CH_3), 0.95 (s, 3H, 29-CH_3), 0.95 (s, 3H, 30-CH_3), 1.24 (s, 3H, 27-CH_3), 0.95 (m, 3H, 18-CH_3), 1.30 (m, 2H, 23-CH_2), 1.99 (2H, m, 24-CH_2), 2.01 (m, 2H, 22-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 39.9 (C-4), 19.3 (C-9), 26.1 (C-10), 45.4 (C-13), 48.8 (C-14), 134.5 (C-17), 130.8 (C-20), 143.9 (C-25), 78.8 (3-CHOH), 47.1 (5-CH), 47.9 (8-CH), 114.6 (26-CH_2), 32.8 (1-CH_2), 32.0 (2-CH_2), 21.1 (6-CH_2), 26.1 (7-CH_2), 26.4 (11-CH_2), 35.6 (12-CH_2), 36.3 (15-CH_2), 28.1 (16-CH_2), 52.1 (22-CH_2), 18.3 (23-CH_2), 40.5 (24-CH_2), 18.1 (18-CH_3), 29.9 (19-CH_2), 30.4 (21-CH_3), 29.9 (27-CH_3), 19.9 (28-CH_3), 25.5 (29-CH_3), 14 (30-CH_3). ESI-MS: (*m/z* 424.1 [M^+], *m/z* (%); **424.1 (30),** 406.6 (28), 383.4 (28), 366.6 (28), 341.3 (28), 326.4 (30), 295.5 (28), 270.3 (30), 265.5 (28), 228.3 (28), 217.3 (31), 201.03 (31), 187.3 (32), 179.2 (30), 163.3 (31), 161.2 (34), 149.2 (32), 147.1 (34), 135.2 (34.5), 131.3 (34), 119.2 (39), 115.2 (31), 117.1 (32.5), 109.2 (80), 105.3 (67), 99.1 (28), 95.2 (100), 91.1 (67), 85.0 (28), 79.1 (38), 67.1 (74), 65.2 (30), 57.2 (28), 55.3 (38).

Cycloart-3*β*, 25-Diol (Compound 2)

White amorphous powder; FTIR (ATR) υ_{max}: 3364, 2946, 2837, 1656, 1450, 1410, 1113, 1019, 697 cm^{-1}; ^{1}H NMR (400 $MH_{Z,}$ $CDCl_3$): δ 0.54 (2H, d, *J* = 3.6 Hz, 19-CH_2), 0.31 (2H, d, *J* = 3.6 Hz, 19-CH_2), 3.28 (m, 1H, 3-CHOH), 0.85 (3H, d, *J* = 6.8Hz, 21-CH_3), 1.33 (s, 3H, 27-CH_3), 1.33 (s, 3H, 26-CH_3), 0.79 (s, 3H, 28-CH_3), 0.95 (s, 3H, 29-CH_3), 0.95 (s, 3H, 30-CH_3), 0.95 (m, 3H, 18-CH_3), 1.99 (m, 2H, 24-CH_2), 2.01 (m, 2H, 22-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 (30-CH_3), 18.1 (21-CH_3), 18.3 (28-CH_3), 19.3 (C-9), 19.9 (18-CH_3), 21.1 (6-CH_2), 24.4 (26-CH_3), 24.4 (27-CH_3), 25.5 (29-CH_3), 26.0 (16-CH_2), 26.1 (C-10), 26.4 (11-CH_2), 28.1 (7-CH_2), 29.6 (23-CH_2), 29.9 (2-CH_2), 30.9 (19-CH_2), 32.0 (1-CH_2), 32.8 (15-CH_2), 35.5 (12-CH_2), 35.6 (22-CH_2), 36.5 (20-CH), 39.9 (24-CH_2), 40.5 (C-4), 45.3 (C-13), 47.1 (5-CH), 47.9 (8-CH), 48.8 (C-14), 52.0 (17-CH), 78.8 (3-CHOH), 82.3 (C-25).

ESI-MS: (*m/z* 444.4 [M^+], **444.4 (6.25)**, 443.4 (15.6), 442.4 (40.6), 441.4 (100), 440.4 (5.6), 439.4 (10.6), 438.4 (4.68).

Initial Screening of the Fractions by a Thin Layer Chromatography

Preliminary qualitative chemical tests for the identification of secondary metabolites were carried out for the plant extract by the method described by Harbrone (1973). This screening of the extract was performed by no visual detection by UV light (in short and long wavelength). But the purple colour was obtained after spraying with a vanillin-H_2SO_4 reagent. A thin layer chromatography (TLC) was carried out using pre-coated silica gel aluminum sheets. Compounds were detected on TLC plates (silica gel 60 F_{254}; Merck KGaA, Darmstadt, Germany) under UV light (VUVGL-58 handheld UV lamp, 254/365 nm UV, made in USA) at 254 nm or 365 nm and by spraying with vanillin-sulfuric acid (1% vanillin in concentrated H_2SO_4) spray reagent.

Total Phenolic Compound Analysis

The amount of total phenolic content in crude sample extract was determined with the Folin-Ciocalteu reagent (FCR) using the method of Spanos and Wrolstad (1990), as modified by Lister and Wilson (2001). To 50 µL of crude extract (three replicates), 2.5 mL 1/10 dilution of FCR (concentration of FCR = 1.24 g/mL) and 2 mL of Na_2CO_3 (7.5%, w/v) were added and incubated at 45°C for 15 min. The absorbance of all samples was measured at 765 nm using a PerkinElmer Shelton, CT 06484 USA, Lambda 25 UV/VIS spectrometer. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

Antioxidant Activity Estimation by DPPH Method

The free radical scavenging activity of the plant extracts and pure compound were assayed spectrophotometrically by DPPH method of Brand-Williams, Cuvelier and Berset (1995) which was slightly modified of Blois (1958). The radical scavenging activity of DPPH (1,1-diphenyl-2-picryl-hydrazyl) (deep violet colour due to its unpaired electron) can be followed spectrophotometrically by a loss of absorbance at 525 nm. Sample stock solutions were diluted to final different concentrations (200, 400, 600, 800, 1,000 µg/mL) in methanol.

Two millilitre of methanol solution of the extract at different concentrations was mixed with 2.0 mL of a methanolic DPPH solution (20 µg/mL), shaken well by vortex and allowed to react at room temperature. The absorbance values were measured after 10 min at 517 nm by UV/Vis spectrophotometer. The free radical scavenging activity of samples was calculated according to the formula:

$$\text{DPPH radical scavenging activity (\%)} = [1\ (Abs_{sample} - Abs_{blank})/Abs_{control}] \times 100.$$

Where Abs_{sample} is the absorbance of the experimental sample; Abs_{blank} is the absorbance of the blank; $Abs_{control}$ is the absorbance of the control.

As a blank, methanol (2.0 mL) and the sample solution (2.0 mL) were used. DPPH solution (2.0 mL, 20 µg/mL) and methanol (2.0 mL) were used as a negative control. Tert-butyl-2-methoxy-4-phenol, Trolox (a water-soluble form of Vit-E) and L(+)-ascorbic acid were used as a positive control in this assay to compare the free radical scavenging activity of the test samples. Each treatment was replicated thrice.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Antibacterial Screening

The antibacterial screening was carried out by the disc diffusion method (Bauer 1966). The bacterial strains used for the experiment were collected as pure cultures from the Institute of Food Science & Technology (IFST) and Pharmaceutical Sciences Research Division (PSRD), BCSIR. Both Gram-positive (*Bacillus megaterium* and *Staphylococcus aureus)* and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli)* organisms were taken for the test. Nutrient agar medium was used for making plates on which antibacterial sensitivity tests were carried out. This medium was also used to prepare fresh cultures. Standard discs act as a positive control to ensure the activity of standard antibiotics against the test organisms and to compare the activity of that produced by the test samples. In this investigation, kanamycin (30 µg/disc) standard disc was used as the reference. Blank discs were used as a negative control to ensure that the residual solvents (leftover discs even after air-drying) and the filter paper were not active themselves. The amount of sample per disc was 100 mg, 250 mg and 500 mg. Test samples present were peel of jackfruit (methanol extract, chloroform extract, and fraction JP-5 which was elution with *n*-hexane/30% ethyl acetate). The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4°C for about 24 hours to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours. After incubation, the antibacterial activities of the test materials were determined by measuring the diameter of the zones of inhibitions in millimeters with a transparent scale.

Statistical Analysis

Three replicates of each sample were used for statistical analysis. Correlation analyses of antioxidant activity (*Y*) versus the total phenolic content (*X*) were carried out using the correlation and regression program in MINITAB 13.2 (Minitab 2002 Software Inc., Northampton, MA). Data were subjected to analysis of variance, and means were compared by the least significant difference (LSD). Differences at $P < 0.05$ were considered to be significant.

Result and Discussion

Compounds 1 and 2 (mixture) were obtained as a white powder. The ^{1}H-NMR and ^{13}C-NMR data together with the DEPT 135 spectrum proved the presence of 30 C-atom signals corresponding to 6 sp^3 Me, 12 sp^3 CH_2, 3 sp^3 CH, 1 sp^2 CH_2, 5 sp^3 and 3 sp^2 quaternary C-atoms for Compound 1. The molecular formula of Compound 1 was confirmed by the mass spectrum with $[M+H]^+$ at *m/z* 424.1, suggesting the molecular formula $C_{30}H_{48}O$ in agreement with the NMR spectra. The ^{1}H-NMR and ^{13}C-NMR data of Compound 1 were found identical to those reported for this compound isolated from *Aphanamixis grandifolia* (Meliaceae) (Quan *et al*. 2010). This is the first report of (17*E*)-cycloart-17, 26-dien-3*β*-ol, found in *A. heterophylus* L.

The NMR spectra of Compound 2 were similar to those of 1, except for the additional 1 sp^3 Me at δ 1.33 (s, 3H, 26-CH_3), 2 sp^3 CH at δ 1.28 (m, 1H, 17-CH) and 1.90 (m, 1H, 20-CH), and one sp^3 quaternary C-atoms at δ_c 82.3 (C-25) and lack of one sp^2 CH_2 at δ 5.53 (1H, brs, 26-CH_2) and 5.49, (1H, brs, 26-CH_2) and 3 sp^2 quaternary C-atoms at δ_c 143.9 (C-25), 134.5 (C-17) and 130.8 (C-20). The 1 sp^3 CH signal of Compound 1 was bound

to the hydroxyl group at δ_c 78.8 (3-CHOH) whereas Compound 2 contained 2 hydroxyl groups which were bound at δ_c 78.8 (3-CHOH) and a quaternary C-atom at δ_c 82.3 (C-25). The molecular formula of Compound 2 was determined by the ^{1}H-NMR and ^{13}C-NMR data, Compound 1, and reported data which were found identical to those for this compound. This is the first report of cycloart-3*β*, 25-diol which was found in *A. heterophylus* L.

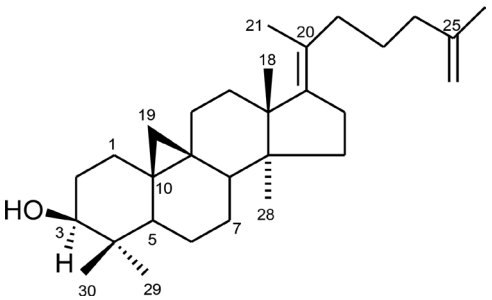

Figure 1: (17*E*)-cycloart-17,26-dien-3*β*-ol.

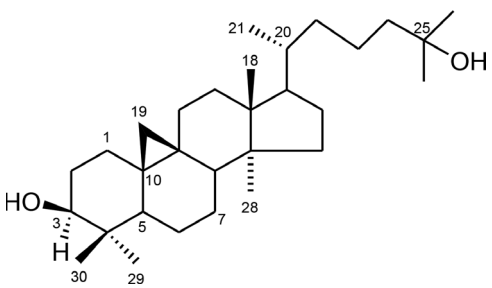

Figure 2: Cycloart-3*β*,25-diol.

The isolated compounds were assayed with the investigation of their antioxidant activity. Different water-soluble phenols such as BHA, Trolox, L(+)-ascorbic acid, and gallic acid were compared with it. At least three different concentrations were chosen for each tested compound in both methods (DPPH and ABTS·+) to avoid speculation of how to sample concentration influences the antioxidant capacity. Table 1 shows the antioxidant activity of phenols expressed as the ability to scavenge 50% of free radicals. Phenols with a high number of hydroxyl groups showed higher antioxidant capacity which is expressed as the lowest IC_{50} value, 2.64 µM, 3.44 µM, 3.747 µM and 5.98 µM for BHA, L(+)-ascorbic acid, gallic acid and Trolox, respectively. In contrast, the highest IC_{50} value (1014.93 µM for DPPH) was obtained for isolated compound JP-5 (Table 1). So, it is confirmed that the isolated compound does not contain a higher number of hydroxyls and it has very low antioxidant activity. FCR is used to measure the total amounts of phenolic content. By this investigation, total phenolic compounds content per gram sample in chloroform extract and methanol extract were 62.585 and 38.815 mg GAE/g dw (a milligram of gallic acid equivalent to per gram of dry weight), respectively.

Table 1: Antioxidant activity (DPPH free radical scavenging activity) of three different standards, two different solvent extracts and sample JP-5.

Standard						Solvent Extract				Sample	
BHA		α-tocopherol		L (+)-ascorbic acid		Methanol extract		Chloroform extract		Sample JP-5	
Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition
50	93.42	25	95.50	12.5	95.98	200	88.37	200	65.96	200	11.82
25	93.06	12.5	94.95	6.25	94.95	100	70.94	100	46.49	100	10.11
12.5	92.71	6.25	93.35	3.125	79.00	50	51.69	50	28.79	50	9.47
6.25	85.73	3.125	45.86	1.562	28.09	25	40.17	25	15.90	25	8.62
3.125	61.39	1.562	13.41	0.781	1.02	12.5	28.28	12.5	8.95	12.5	5.72
1.562	40.80	0.781	3.01	--	--	6.25	8.21	6.25	3.83	6.25	3.75
0.781	13.01	--	--	--	--	3.125	4.01	3.125	3.38	3.125	2.75
IC_{50} = 2.64 µM		IC_{50} = 5.98 µM		IC_{50} = 3.44 µM		IC_{50} = 83.06 µM		IC_{50} = 134.58 µM		IC_{50} = 1014.93 µM	

The crude methanol and chloroform extract and the isolated compounds from ripe jackfruit peel were tested for antibacterial activity against two Gram-positive and two Gram-negative bacteria. A standard disc of Kanamycin (30 µg/disc) was used for comparison purposes. All the extracts were tested at 100 µg/disc, 250 µg /disc, 500 µg/disc concentration and they exhibited no activity against the tested bacteria (Table 2). But the crude methanol extracts of the stem, root, barks, root heart-wood, leaves, fruits and seeds of this plant, and their subsequent partitioning with petrol, dichloromethane, ethyl acetate and butanol gave fractions that exhibited a broad spectrum of antibacterial activity (Prakash *et al*. 2009). The butanol fractions of the root bark and fruits were found to be the most active (Khan, Omoloso and Kihara 2003).

Table 2: Antimicrobial activity of methanol and chloroform extract and isolated compounds (JP-5) from jackfruit peel.

Bacterial strain	The diameter of the zone of inhibition (mm)			
	MeOH extracts (100–500 µg/disc)	**$CHCl_3$ extracts (100–500 µg/disc)**	**JP-5 (100–500 µg/disc)**	**Kanamycin (30 µg/disc)**
Gram-positive				
Bacillus megaterium	–	–	–	45
Staphylococcus aureus	–	–	–	31
Gram-negative				
Pseudomonas aeruginus	–	–	–	42
Escherichia coli	–	–	–	36

Note: – Indicates no activity.

CONCLUSION

It is concluded from spectroscopic evidence that a mixture of two cycloartane-type triterpinoids was isolated from the column fraction by elution with *n*-hexane/30% ethyl acetate of methanol extract of ripe jackfruit (*A. heterophylus* L.) peel. (17*E*)-cycloart-17, 26-dien-3*β*-ol and cycloart-3*β,* 25-diol were isolated for the first time from this plant. The antioxidant activity of this compound is not satisfactory compared with some standard phenolic compounds. Moreover, it has no antibacterial activity against two Gram-positive and two Gram-negative bacteria. Thus, subsequent research towards the discovery should continue as plants are the sources of medicinal agents.

REFERENCES

BAUER, A. W., KIRBY, W. M., SHERRIS, J. C. & TURCK, M. (1966) Antibiotic susceptibility testing by a standardized single disk method, *American Journal of Clinical Pathology*, 45(4): 493–496.

BRAND-WILLIAMS, W., CUVELIER, M. E. & BERSET, C. L. W. T. (1995) Use of a free radical method to evaluate antioxidant activity, *LWT-Food Science and Technology*, 28(1): 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

BLOIS, M. S. (1958) Antioxidant determinations by the use of a stable free radical, *Nature*, 181: 1199–1200. https://doi.org/10.1038/1811199a0

HARBORNE, J. B. (1973) Phenolic compounds. IN: Phytochemical methods, pp. 33–88 (Dordrecht: Springer). https://doi.org/10.1007/978-94-009-5921-7_2

KHAN, M. R., OMOLOSO, A. D. & KIHARA, M. (2003) Antibacterial activity of *Artocarpus heterophyllus*, *Fitoterapia*, 74(5): 501–505. https://doi.org/10.1016/S0367-326X(03)00120-5

LISTER, E. & WILSON, P. (2001) Measurement of total phenolics Ans ABTS assay for antioxidant activity (personal communication). (Lincoln, New Zealand.: Crop Research Institute).

MEKKRIENGKRAI, D., UTE, K., SWIEZEWSKA, E., CHOJNACKI, T., TANAKA, Y. & SAKDAPIPANICH, J. T. (2004) Structural characterization of rubber from jackfruit and euphorbia as a model of natural rubber, *Biomacromolecules*, 5(5): 2013–2019. https://doi.org/10.1021/bm040045i

PATEL, R. M. & PATEL, S. K. (2011) Cytotoxic activity of methanolic extract of *Artocarpus heterophyllus* against A549, Hela and MCF-7 cell lines, *Journal of Applied Pharmaceutical Science*, 1(7): 167–171.

PRAKASH, O., KUMAR, R., MISHRA, A. & GUPTA, R. (2009) *Artocarpus heterophyllus* (jackfruit): an overview, *Pharmacognosy Reviews*, 3(6): 353.

QUAN, L., CHEN, C. J., SHI, X., ZHANG, L., CHEN, H. J. & GAO, K. (2010) Chemical constituents from *Aphanamixis grandifolia*, *Chemical and Pharmaceutical Bulletin*, 58(11): 1431–1435. https://doi.org/10.1248/cpb.58.1431

RAGASA, C. Y., JORVINA, K. & RIDEOUT, J. A. (2004) Antimicrobial compounds from *Artocarpus heterophyllus*, *Philippine Journal of Science*, 133(2): 97.

RAHMAN, M. A., NAHAR, N., MIAN, A. J. & MOSIHUZZAMAN, M. (1999) Variation of carbohydrate composition of two forms of fruit from jack tree (*Artocarpus heterophyllus* L.) with maturity and climatic conditions, *Food Chemistry*, 65(1): 91–97. https://doi.org/10.1016/S0308-8146(98)00175-7

RAJENDRAN, N. K. & RAMAKRISHNAN, J. (2010) Polyphenol analysis and anti-tumor activity of crude extracts from tegmen of *Artocarpus heterophyllus*. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, 2(1): 63–66. https://doi.org/10.5958/j.0975-4261.2.1.009

SHAHIN, N., KAZMI, I. & ALI, M. (2012) Glycosides from the leaves of *Artocarpus heterophyllus* Lam, *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(3): 449–453.

SPANOS, G. A. & WROLSTAD, R. E. (1990) Influence of processing and storage on the phenolic composition of Thompson seedless grape juice, *Journal of Agricultural and Food Chemistry*, 38: 1565–1571. https://doi.org/10.1021/jf00097a030

SRINIVASAN, K. & KUMARAVEL, S. (2016) Mass spectrometry analysis of volatile constituents of jackfruit powder, *Indo American Journal of Pharmaceutical Sciences*, 3(4): 331–339.