Indian Journal of Traditional Knowledge
Vol 18(2), April 2019, pp 360-365

Identification of *Nephelium lappaceum* leaves phenolic and flavonoid component with radical scavenging, antidiabetic and antibacterial potential

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Received 12 November 2018; revised 04 February 2019

Nephelium lappaceum Linn. (Rambutan) is traditionally claimed, as a source of natural antioxidants and for its use in the treatment of diabetes and bacterial infections. The present study investigates the *in vitro* effect of ethanolic Rambutan leaves extract (NL) for its antioxidant effect, α-glucosidase, α-amylase enzyme inhibition, and antibacterial potentials. The total phenolic, total flavonoid content of NL was quantified and were expressed in terms of gallic acid (19.6±0.04 mg GAE/g) and rutin equivalents (16.7±0.01 mg RUE/g) respectively. The antioxidant assay revealed that NL exhibited significant inhibition of DPPH (IC_{50}±SEM: 1.52±0.03 µg/mL) and ABTS (IC_{50}±SEM: 1.295±0.05 µg/mL) radicals. NL also inhibited both α-amylase (IC_{50}±SEM: 2.624±0.07 µg/mL), α-glucosidase (IC_{50}±SEM: 2.416±0.06 µg/mL) enzyme activities, supported by its antioxidant potential and its phenolic and flavonoid content. The antibacterial activity was screened against seven human pathogenic ATCC strains for which the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were recorded. The selected MIC dose was tested, confirmed by Kirby-Bauer agar well diffusion method. NL exhibited MIC and MBC of 62.5 µg/mL and 125 µg/mL against *B.subtilis* and *E.coli* respectively. The results support the scientific claim of NL for its antioxidant, antidiabetic and antibacterial potential.

Keywords: Antibacterial, Free Radical Scavenging, *Nephelium lappaceum*, Rambutan, α-amylase, α-glucosidase.

IPC Code: Int. Cl.[19] : A61P 31/04, C07D 303/14, C12N 15/63, A01N 63/02, C12Q 1/40, C12P 19/16

The plant Rambutan (*Nephelium lappaceum*. Linn.) has been traditionally used in many Asian countries for several therapeutic purpose[1]. The vast majority of the developing nations have adopted a traditional therapeutic practice as a vital piece of their way of life. Historically, most therapeutic preparations were obtained from plants, and used either as a crude extract or as in the refined form. The journey for the new compound isolation from traditional plants has become an alluring research area. Plants with ethno-pharmaceutical significance were being considered on account of their medicinal values and additionally for their proficient medicinal properties[2].

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Rambutan falls under the Sapindaceae family and it is cultivated in the tropical regions such as Malaysia, Indonesia and Thailand[3]. Rambutan has many traditional uses and all its parts including its leaves, rind and seeds were reported to contain important phytochemical constituents[4]. Phenolic compounds were the most important phytochemical constituent investigated in Rambutan fruit peels and were reported to possess antioxidant[5-7], antibacterial[8], antidiabetic[9] and α-amylase inhibitory properties[10]. The Rambutan leaves are not fully explored, for their medicinal properties and hence the need arises as this tropical plant is abundant in many Asian countries, its medicinal properties can be explored further. In this context, we planned to analyse the antioxidant,

antidiabetic and antibacterial potential of ethanolic Rambutan leaves extract (NL).

Methodology

Chemicals

DPPH, α-amylase, α-glucosidase, Ascorbic Acid, Gentamycin and Gallic acid were purchased from Merck Millipore Corporation, USA. ABTS, Acarbose and Rutin were purchased from Sigma-Aldrich Corporation, USA. All the chemicals used were of analytical grade.

Collection of sample

The leaves of *N. lappaceum* were collected from Baling, 09100, Kedah, Malaysia. The matured and healthy plant was selectively chosen for sampling. The leaves were brought into the laboratory in sterile bags and processed within a few hours after sampling. The plant specimen was authenticated at the Department of Pharmacognosy, AIMST University, Malaysia (Ref. No.: AIMST/FOP/04).

Extract preparation

The leaves were cleaned under running tap water and air-dried for 2 days to avoid any chemical decomposition from taking place. 200 g of dried leaves were then finely grounded and macerated using 500 mL of 70% ethanol in a 1 L flask. The sample was macerated and kept on the shaker for 5 days at room temperature. The sample was filtered through a muslin cloth and the process was repeated until a clear colorless supernatant liquid obtained. Filtrates were collected and evaporated using a rotary evaporator[11].

Qualitative phytochemical screening

The ethanolic Rambutan leaves extract (NL) was used for preliminary screening of phytochemicals such as alkaloids, carbohydrates, phenols, amino acids, steroids, anthocyanins, proteins, flavonoids, saponins, mucilage, gums, glycosides and tannins using standard biochemical testing methods[12].

Quantification of total phenolic content

Total phenolic content of the NL was analyzed according to the protocol described with slight modifications[12]. 10 mg of NL was dissolved in 10 mL of 70% ethanol and different concentrations (0.1-1.0 mg/mL) were prepared by serial dilution. The reaction mixture was prepared by adding 100 µL of NL, 2 mL of 2% sodium carbonate solution and 100 µL of Folin-Ciocalteu's reagent and vortexed. The reaction mixture was incubated for 30 min at room temperature. The absorbance was measured at 750 nm. The procedure was repeated for gallic acid (GA) as a standard and the calibration curve was constructed. Based on the absorbance values, the phenolic concentration was expressed as mg/mL from the calibration curve. The total phenolic content of NL was expressed as gallic acid equivalents (GAE, mg/g).

Quantification of total flavonoid content

Total phenolic content of the NL was analyzed according to the protocol described[12]. Various concentrations of NL were prepared as mentioned in the total phenolic content. A reaction mixture containing 1 mL of NL, 10 mL of 30% ethanol and 0.7 mL of 5% sodium nitrite and 10% aluminium chloride was prepared and incubated at room temperature for 6 min. Followed by the addition of 10 mL of 1 M sodium hydroxide solution, the final volume was made up to 25 mL with 30% ethanol and kept for 10 min. The absorbance was measured at 450 nm. The procedure was repeated for rutin (RU) as a standard and the calibration curve was constructed. The flavonoid concentration was expressed as rutin equivalents (RUE, mg/g).

Antioxidant assay

DPPH radical assay

The ability of NL to scavenge DPPH radical was assessed using the procedures described earlier with minor modifications[13]. Different concentrations of NL were prepared using 70 % ethanol. A reaction mixture containing 0.5 mL of NL (10 to 1000 µg/mL), 0.5 mL of 2 µM DPPH were mixed and incubated for 20 min at room temperature. The absorbance was measured at 517 nm and the above procedure was repeated for the standard ascorbic acid. The percentage of scavenging was calculated using the equation (Eq 1).

$$\% \text{ Inhibition: } \frac{(\text{Absorbance Control} - \text{Absorbance Sample})}{\text{Absorbance Control}} \times 100 \quad \text{(Eq. 1)}$$

ABTS radical cation decolourization assay

The 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS*+) free radical cation scavenging ability of NL was assessed following the procedures described earlier[13].Various concentrations of NL were prepared as mentioned in DPPH assay. A reaction mixture containing 0.5 mL of NL, 0.5 mL of ABTS (7 mM) were mixed and incubated for 30 min

at room temperature and the absorbance was measured at 734 nm. The procedure was repeated for the standard ascorbic acid and the percentage of scavenging calculated using the equation (Eq. 1).

Antibacterial assay

Microbial inoculum standardization

The following bacterial strains used were *Bacillus subtilis* (ATCC 11774), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Streptococcus pyogenes* (ATCC 19615), *Neisseria gonorrhoeae* (ATCC 43069), *Pseudomonas aeruginosa* (ATCC 10145), and *Escherichia coli* (ATCC 10799). The strains were placed on Mueller Hinton agar plates at 36ºC and adjusted to 0.5 McFarland standard[14].

Determination of minimum inhibitory and bactericidal concentration

The minimum inhibitory concentration (MIC) was determined using the test tube dilution method. Nutrient broth (1 mL) was added into sterile tubes. Subsequently, 1 mL of 1 mg/mL extract was transferred in a tube and serially diluted to produce concentrations ranging from 31.25 µg/mL to 500 µg/mL. 0.1 mL of inoculum was inoculated into each test tube and incubated for 24 h at 37°C and the growth of microorganisms was monitored by the turbidity produced. 10% DMSO was used as solvent. MIC was determined with the lowest concentration of a sample that suppresses the bacterial growth[15].

The minimum bactericidal concentration (MBC) was determined by comparing the number of viable bacteria or inoculum with the initial number. Clear tubes from MIC study were diluted further and spread onto a nutrient agar plate and incubated for 24 h at 37°C.

Kirby-Bauer Agar well diffusion method

The selected bacterial inoculum was spread evenly onto a fresh Mueller Hinton agar plates with the help of a sterile cotton swab. 4 wells sized 12 mm in diameter were made into the agar plate and 50 µL of the extract was added to each well. These plates were incubated for 24 h at 37ºC, under aerobic condition. After the incubation period, the diameter of inhibition zones was measured. Gentamycin and 10% DMSO were used as positive and negative controls, respectively[15]

***In vitro* antidiabetic assay**

α-Amylase inhibitory assay

A reaction mixture containing 0.5 mL (100-1000 µg/mL) of NL and 0.5 mL of α-amylase solution (0.5 mg/mL) in 0.2 mM phosphate buffer (pH 6.9) was incubated for 10 min at 25°C. 0.5 mL starch solution (1% w/v) in 0.02 M sodium phosphate buffer (pH 6.9) was added. The samples were incubated at 25°C for 10 min and 1 mL of dinitrosalicylic acid (color reagent) was added. The reaction mixture was boiled for 5 min, cooled to room temperature followed by addition of 10 mL of distilled water. The absorbance was measured at 540 nm. Acarbose was used as the standard drug[16] and the percentage of inhibition was calculated using the equation (Eq. 1).

α-Glucosidase inhibitory assay

A volume of 1 mL α-glucosidase (1 U/mL) was pre-incubated with 1 mL of NL (100-1000 µg/mL) for 5 min at 37°C. Then, 1 mL of 2% sucrose in Tris buffer (pH 8) was added and incubated for further 20 min. The reaction was arrested by heating in boiling water for 2 min. Subsequently, the liberated glucose was measured by the GOD-POD method, where acarbose was used as the standard drug[17].

Statistical analysis

The inhibitory concentration 50% (IC_{50}) values were computed using the Graph Pad Prism Software (Version 5) by non-linear regression graph plotted between percentage of radical scavenging (x-axis) against concentration (y-axis) for antioxidant studies and percentage of enzyme inhibition (x-axis) versus concentration (y-axis) for antidiabetic studies.

Results and Discussion

Qualitative phytochemical screening

The phytochemical results showed evidences for the presence of various phytoconstituents such as reducing sugar, monosaccharides, carbohydrates, phenols, proteins, tannins, alkaloids, flavonoids, steroids, saponins and glycosides.

The flavonoid content present in NL was determined from the standard Rutin curve as portrayed in Fig. 1 (a). The linear regression equation of $y = 0.0003x + 0.025$ and a high degree of correlation ($R^2 = 0.987$) amongst concentration and the absorbance were depicted from the calibration curve as shown in Fig. 1 (a). Thus, the total flavonoid content of NL was found to be 16.7 ± 0.01 mg RUE/g of crude extract.

The phenolic content of the NL was determined from the standard GA curve as portrayed in Fig. 1 (b). A high correlation ($R^2 = 0.9665$) amongst concentration and as the absorbance was depicted from the standard calibration curve, which showed the

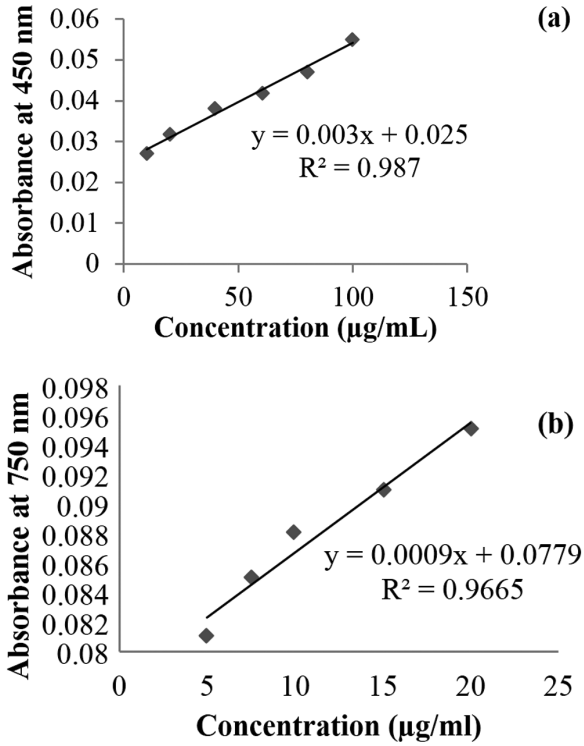

Fig. 1 (a) — The standard curve of Rutin to measure total flavonoid content; 1(b). The standard curve of Gallic acid to measure total phenolic content

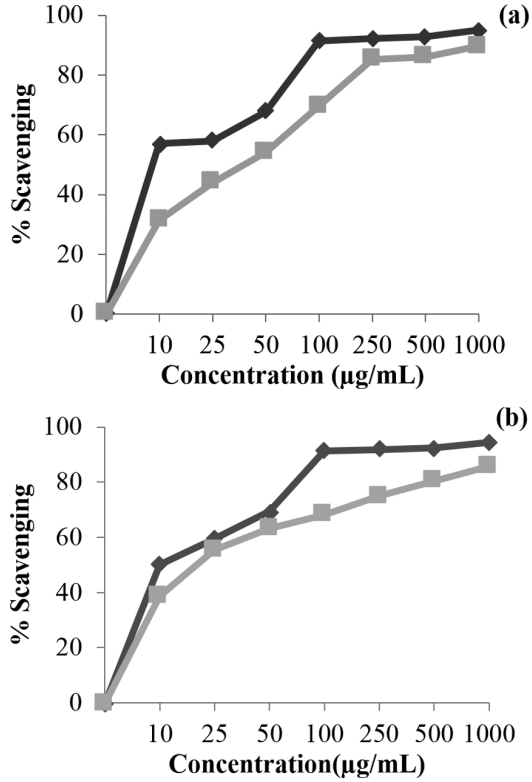

Fig. 2 (a) — DPPH radical scavenging assay of ascorbic acid (black color) and NL (grey colour); 2 (b). ABTS radical scavenging assay of ascorbic acid (black color) and NL (grey colour).

linear regression equation of y = 0.0009x+0.0779. Thus, total phenolic content of NL is found to be 19.6 ± 0.04 mg GAE/g of crude extract.

The aluminum chloride colorimetric technique was utilized to quantify the flavonoid content. The phenolic content of NL was measured by Folin–Ciocalteu technique. Under alkaline conditions, phenols able to dissociate the reagent into a proton and phenolate anion or by electron transfer. The phenolate anion decreases the Mo (VI) of Folin's reagent prompting the development of blue coloured complex with absorption taken at 750 nm. The outcomes uncover that the phenols in NL are profoundly polar in nature and subsequently ethanol could be the best dissolvable solvent to recover it. The earlier research findings also supports that polyphenols and flavonoids of Rambutan extract contributes for their antioxidant properties[6-7].

Antioxidant assay

Fig. 2 (a) and 2 (b) showed the DPPH and ABTS radical scavenging ability of NL and ascorbic acid. At the highest concentration (1000 µg/mL), NL exhibited 89.6% and 85.81% of inhibition of DPPH and ABTS free radicals respectively. The IC_{50} values of NL was found to be 1.52 ± 0.03 and 1.295 ± 0.05 µg/mL for DPPH and ABTS assays respectively. Similarly, the standard ascorbic acid showed an IC_{50} value of 0.93 ± 0.1 and 1.074 ± 0.09 µg/mL.

From the earlier research findings, it is clear that the hydroxyl moiety of Rambutan phenolic compound possesses radical scavenging properties and reduces lipid peroxidation capacity of cells[18]. Numerous polyphenolic constituents obtained from plants are more viable antioxidants and accordingly may contribute to protective impacts *in vivo*.

Antidiabetic assay

Table 1 showed α-amylase and α-glucosidase inhibitory effect of NL and standard acrabose. At the highest concentration (1000 µg/mL), NL exhibited 80 % and 86.8 % of inhibition of α-amylase and α-glucosidase respectively. The IC_{50} values of NL was found to be 2.62 ± 0.07 and 2.42 ± 0.06 µg/mL for α-amylase and α-glucosidase respectively. Similarly, the standard acarbose exhibited IC_{50} values 2.25 ± 0.15 2.22 ± 0.04 µg/mL.

The pancreatic α-amylase is one of the vital enzymes in the human digestive system, as it

Table 1 — α-Amylase and α-Glucosidase Enzyme Inhibition by NL and Acarbose.

Concentration (µg/mL)	% Inhibition α-amylase		α-glucosidase	
	Acarbose	NL	Acarbose	NL
100	39.39	15.15	40.00	33.28
200	57.58	38.18	52.00	40.16
400	54.55	46.36	68.80	53.84
800	69.70	56.06	80.80	75.92
1000	93.94	80.00	90.40	86.80

Table 2 — The MIC and MBC of NL

Microorganisms	NL	
	MIC (µg/mL)	MBC (µg/mL)
B.subtilis	62.5	125.0
E.faecalis	125.0	500.0
S. aureus	125.0	500.0
S.pyogenes	125.0	500.0
N.gonorrhoeae	250.0	1000.0
P.aeruginosa	250.0	1000.0
E.coli	62.5	125.0

breakdowns starch into oligosaccharides and disaccharides. The liberated glucose, which is later absorbed into the blood circulation. The inhibition of carbohydrate hydrolyzing enzymes would lessen the starch breakdown in the intestinal tract. Subsequently, the postprandial hyperglycaemia level may reduce[19]. According to previous studies[20], the phytoconstituents with strong antioxidant ability have been known to be good enzyme inhibitors, hyperglycemia regulators and ameliorate diabetic complications resulting from oxidative stress. The results showed that the NL had comparable potential inhibitors of α-amylase and α-glucosidase when compared with acarbose, this could be attributed due to its flavonoid, phenolic contents and antioxidant property.

Antibacterial assay

The MIC and MBC results of NL were depicted in Table 2. NL exhibited the highest MIC at 62.5 µg/mL and MBC at 125 µg/mL for both *B.subtilis* and *E.coli*. At MIC of 125 µg/mL, as shown in Table 3, NL portrayed significant zone of inhibition against *B.subtilis*, 13.7±0.58 mm and *E.coli*, 13.3±0.58 mm compared to gentamycin 14.0 ± 1.01 mm, 13.7±0.58 mm respectively (Fig. 3). NL showed moderate antibacterial activity (*E.faecalis* and *S.aureus*) and least antibacterial activity (*S.pyogenes* and *N.gonorrhoeae*) among the tested ATCC bacterial strains. Rambutan peel extracts were reported earlier

Table 3 — The Microbial Inhibition Zone of NL

Microorganisms	Zone of inhibition (mm, 125 µg/mL) ± SD	
	NL	Gentamycin
S.pyogenes	7.7±0.58	10.6±0.58
N.gonorrhoeae	6.0±1.00	14.3±1.15
S.aureus	12.0±1.15	14.0±1.00
E.faecalis	12.8±1.00	14.3±0.58
B.subtilis	13.7±0.58	14.0±1.01
P.aeruginosa	9.3±0.58	12.3±1.15
E.coli	13.3±0.58	13.7±0.58

Values are expressed as Mean ± Standard deviation (SD), (n=3).

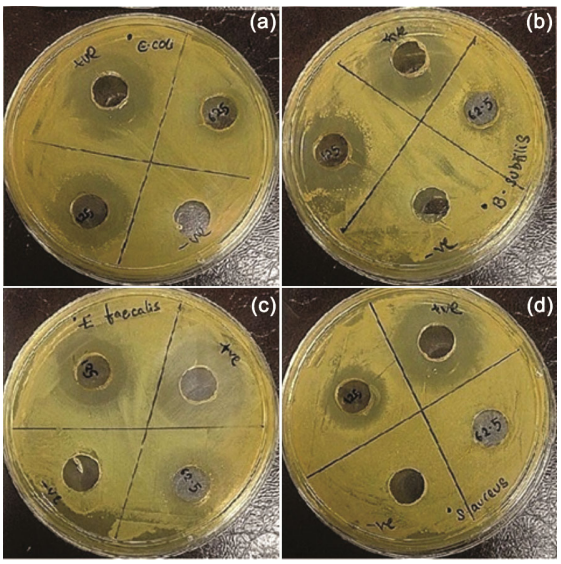

Fig. 3 — Zone of inhibition of NL against (a) *E. coli,* (b) *B.subtilis,* (c) *E. faecalis* and (d) *S.aureus*

for its antibacterial[8] potential and it's the first report to reveal the antibacterial effect of Rambutan leaves. Phytocompounds for instance sesquiterpenes, terpenoids, tannins, phenolic, flavonoids, polyphenols are known to act against a broad spectrum of microbes[11]. The study revealed that NL is a conceivable antibacterial agent comparable to standard gentamycin. The antibacterial activity of NL could be credited due to its high phenolic and flavonoid content. These phytochemical constituents have been accounted to hinder the nucleic acid synthesis and other metabolic activities[21]. The phenolic constituents with C3 side chain at lower oxidation level and the oxygen absences have also reported to be responsible for the antibacterial effect. The toxicity of polyphenol's mechanism against virulent microbes could be related to the hydrolytic enzyme inhibition particularly proteases or diverse

interactions that inactivate the microbial adhesion, cell envelope transport proteins and non- specific interactions with sugars[22].

Conclusion

The outcomes of the study uncover the significant free radical scavenging ability, antidiabetic and antibacterial potential present in the leaves of *Nephelium lappaceum* . Henceforth, these findings show that NL could be a rich natural source for bioactive metabolites as potential pharmacological agents. However, further studies are required to elucidate and comprehend these potentials using different biochemical and molecular biology tools to explore the complete mechanisms.

Acknowledgment

The authors are very much thankful to AIMST University, Malaysia for providing grants to carry out the research project and also thankful to Qassim University, Kingdom of Saudi Arabia for the encouragement for this collaborative work.

References

1 Quattrocchi U, CRC World Dictionary of Medicinal and Poisonous Plants: Common names, scientific names, eponyms, synonyms, and etymology, (Boca Raton: CRC Press) 2012, 2628.

2 Roy P, Abdulsalam FI, Pandey DK, Bhattacharjee A, Eruvaram NR, *et al*., Evaluation of antioxidant, antibacterial, and antidiabetic potential of two traditional medicinal plants of India: *Swertia cordata* and *Swertia chirayita, Pharmacogn Res,* 7 (Suppl 1) (2015) S57-62.

3 Rohman A, Physico-chemical properties and biological activities of Rambutan (*Nephelium lappaceum* L.) fruit, *Res J Phytochem,* 11 (2) (2017) 66–73.

4 Palanisamy U, Cheng HM, Masilamani T, Subramaniam T, Ling LT, *et al*., Rind of the Rambutan, *Nephelium lappaceum*, a potential source of natural antioxidants, *Food Chem,* 109 (1) (2008) 54–63.

5 Sun L, Zhang H, & Zhuang Y, Preparation of free, soluble conjugate, and insoluble-bound phenolic compounds from peels of Rambutan (*Nephelium lappaceum*) and evaluation of antioxidant activities *in vitro*, *J Food Sci*, 77 (2) (2012) C198-204.

6 Thitilertdecha N, Teerawutgulrag A, Kilburn JD, & Rakariyatham N, Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities, *Molecules,* 15 (3) (2010) 1453-65.

7 Hernández C, Ascacio-Valdés J, De la Garza H, Wong-Paz J, Aguilar CN, *et al*., Polyphenolic content, *in vitro* antioxidant activity and chemical composition of extract from *Nephelium lappaceum* L. (Mexican Rambutan) husk, *Asian Pac J Trop Med,* 10 (12) (2017) 1201-1205.

8 Yuvakkumar R, Suresh J, Nathanael AJ, Sundrarajan M, & Hong SI, Novel green synthetic strategy to prepare ZnO nanocrystals using Rambutan (*Nephelium lappaceum* L.) peel extract and its antibacterial applications, *Mater Sci Eng C Mater Biol Appl*, 41 (2014) 17-27.

9 Ma Q, Guo Y, Sun L, & Zhuang Y, Anti-Diabetic Effects of Phenolic Extract from Rambutan Peels (*Nephelium lappaceum*) in High-Fat Diet and Streptozotocin-Induced Diabetic Mice, *Nutrients,* 9 (8) (2017) E801.

10 Evaristus NA, Wan Abdullah WN, & Gan CY, Extraction and identification of α-amylase inhibitor peptides from *Nephelium lappacheum* and *Nephelium mutabile* seed protein using gastro-digestive enzymes, *Peptides,* 102 (2018) 61-67.

11 Chigurupati S, Eric WKY, Jahidul IM, Shantini V, Kesavanarayanan KS, Venkata RRM, & Sitansu SN, Screening antimicrobial potential for malaysian originated *Tamarindus indica* ethanolic leaves extract. *Asian J Pharm Clin Res*, 11 (3) (2018) 361.

12 Chigurupati S, Mohammad JI, Vijayabalan S, Vaipuri ND, Selvarajan KK, *et al*., Quantitative estimation and antimicrobial potential of ethanol extract of *Durio zibethinus* Murr. leaves, *Asian J Pharm Clin Res,* 10 (9) (2017) 1–4.

13 Chigurupati S, Shaikh SA, Mohammad JI, Selvarajan KK, Nemala AR, Khaw CH, Teoh CF, & Kee TH, *In vitro* antioxidant and *in vivo* antidepressant activity of green synthesized azomethine derivatives of cinnamaldehyde, *Indian J Pharmacol*, 49 (3) (2017) 229-235.

14 Ramezan Ali A, Ali Mehrabi T, Mohammad Javad HS, Khadijeh M, & Mahdi Ghorbananli Z, A method for antibiotic susceptibility testing: applicable and accurate, *Jundishapur J Microbiol,* 5 (1) (2012) 341–345.

15 Chigurupati S, Designing new vanillin schiff bases and their antibacterial studies, *J Med Bioeng,* 4 (5) (2015) 363–366.

16 Noreen T, Taha M, Imran S, Chigurupati S, Rahim F, *et al*., Synthesis of alpha amylase inhibitors based on privileged indole scaffold, *Bioorganic Chem,* 72 (2017) 248–255.

17 Kajaria D, Ranjana JT, Tripathi YB, & Tiwari S, *In-vitro* α amylase and glycosidase inhibitory effect of ethanolic extract of antiasthmatic drug—Shirishadi, *J Adv Pharm Technol Res,* 4 (4) (2013) 206–209.

18 Yuslianti ER, Bachtia BM, Suniart DF, & Sutjiat AB, Antioxidant activity of Rambutan honey: The free radical-scavenging activity *in vitro* and lipid peroxidation inhibition of oral mucosa wound tissue *in vivo, Res J Med Plant,* 9 (6) (2015) 284–292.

19 Tarling CA, Woods K, Zhang R, Brastianos HC, Brayer GD, *et al*., The search for novel human pancreatic α-amylase inhibitors: High-throughput screening of terrestrial and marine natural product extracts, *Chem Bio Chem,* 9 (3) (2008) 433–438.

20 Khorasani EA, Mat TR, Mohajer S, & Banisalam B, Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L.(red clover), *BioMed Res Int,* 2015 (2015) 11 pages.

21 Cushnie TT, & Lamb AJ, Antimicrobial activity of flavonoids, *Int J Antimicrob Agents,* 26 (5) (2005) 343-356.

22 Pyla R, Kim TJ, Silva JL, & Jung YS, Enhanced antimicrobial activity of starch-based film impregnated with thermally processed tannic acid, a strong antioxidant, *Int J Food Microbiol,* 137 (2-3) (2010) 154–160.