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ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF THE AQUEOUS EXTRACT OF THE PEEL OF A SRI LANKAN VARIETY OF *NEPHELIUM LAPPACEUM* LINN.

Uduwelage Dona Harshani Kaushalya Uduwela[1], *Srianthie A. Deraniyagala[1] and Gobika Thiripuranathar[2]

[1]Dept. of Chemistry, University of Colombo, PO Box 1490, Kumaratunga Munidasa Mawatha, Colombo 03, Sri Lanka.

[2]Institute of Chemistry Ceylon, Adamantane House, 341/22, Kotte Road, Welikada, Rajagiriya, Sri Lanka.

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***Corresponding Author**
Srianthie A. Deraniyagala
Dept. of Chemistry, University of Colombo, PO Box 1490, Kumaratunga Munidasa Mawatha, Colombo 03, Sri Lanka.

ABSTRACT

Plant-based remedies play a significant role in healthcare due to low side effects compared to that of synthetic drugs. Excess free radicals generated in the human body are implicated in numerous diseases as well as in aging. This study involves the determination of antioxidant and anti-inflammatory activities of the aqueous extract of the peel of a Sri Lankan variety of rambutan (AEPR); Malwana special. The AEPR was prepared according to "kasaya" preparation method in Ayurvedic medicine. The total phenolic content and the total flavonoid content of AEPR were 463.5±5.2 mg (PGE)/g and 375.0±13.2 mg (QE)/g respectively. (PGE=Pyrogallol Equivalence, QE=Quercetin Equivalence). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of AEPR was 3.9-64.5% for concentrations of 10–500 µg/ml. Ascorbic acid gave values of 6.5-96.4%. The hydroxyl radical scavenging activity of AEPR ranged from 10.3-35.0% for concentrations of 1000–1500 ppm. Ascorbic acid showed values of 22.6-51.1%. The extract exhibited a good reducing power in the iron reducing power assay. Nitric oxide radical scavenging activity of AEPR was 4.1-31.7% for concentrations of 100–500 µg/ml. Ascorbic acid gave values of 30.1-43.3%. The protection reported for Human Red Blood Cell (HRBC) assay was 17.1-34.8% for concentrations from 35.5-250.0 ppm. Aspirin protection ranged from 32.3-54.5%. These results show that the AEPR prepared from peel

waste has a potential to be used as an antioxidant and also it possesses anti-inflammatory activity.

KEYWORDS: *Nephelium lappaceum*, antioxidant, anti-inflammatory, HRBC.

INTRODUCTION

Use of plants to combat diseases or symptoms of diseases can be dated back to ancient times.[1] Despite the advances in modern medicine, plant-based remedies play an important role in healthcare due to their low cost and less side effects.[1] As such there is a growing interest for natural sources of antioxidants and anti-inflammatory compounds. The peel of rambutan is considered a waste and recently it has been identified as a breeding site of dengue mosquitoes. Investigation of beneficial properties of this discarded plant material increase the economic value of rambutan and also it provides a fine solution for the prevention of a re-emerging mortal disease which has become a burning social issue in Sri Lanka at present time.

In a healthy individual there is a balance between the production of free radicals and the defensive mechanisms against them.[2] Collapse of this balance leads to oxidative stress.[2] It causes damage to important cellular components in the body.[2,3] Excess free radicals are implicated in numerous diseases and disorders such as Parkinsons' disease, Alzheimers' disease, cancer, atherosclerosis, diabetes and immunosuppression.[3,4,5] Furthermore, the role of free radicals in the process of aging is also well known.[3] Antioxidants play a vital role in preventing such complications by stabilizing or deactivating free radicals. Therefore, a special attention is drawn towards natural antioxidants which have the capability to scavenge free radicals and avoid the possible destructive processes. Inflammation is the method which human body responds to an infection, irritation or an injury of cells and tissues.[6] Acute and chronic inflammations play a significant role in many of the diseases including rheumatoid arthritis[6] and anti-inflammatory drugs are extensively used in treating them.

Sri Lanka with its' wide distribution of flora has many plants of medicinal value. A number of medicinal plants are used as antioxidants and to combat inflammation in traditional systems of medicine including Ayurvedha. *Nephelium lappaceum* Linn. commonly known as rambutan is reported to have many biological activities.[7,8] Rambutan belongs to the family of Sapindaceae and is distributed in South–East Asia including Malaysia, Indonesia and Thailand.[7,8,9] These fruits are native to humid tropical regions.[7,9] Rambutan fruits are ovoid

with a red pericarp covered with soft spines.[7] Inside the pericarp is a seed covered with a thick white gelatinous fleshy substance, which is a delicacy among Sri Lankan fruits. Previous studies in different countries have revealed that the peel of rambutan contains vitamin C, vitamin E, carotenes, xanthophylls, tannins and phenolics among many other natural products.[8] This study is aimed at determining the antioxidant capacity and anti-inflammatory activity of the aqueous extract of the peel of Malwana special variety of rambutan (AEPR).

MATERIALS AND METHODS

Collection of Plant Material

Fresh rambutan fruits were collected in December 2017 from Madegama, Monaragala district, Sri Lanka. Fruits were selected based on their uniformity and colour. Variety of collected fruits was botanically identified by a scientist at Fruit Research and Development Institute, Department of Agriculture, Kananwila, Horana, Sri Lanka.

Preparation of Water Extract

The peels were separated from fruits and washed thoroughly with portable water to remove contaminants. Then the peels were chopped into small pieces and dried under shade for 14 days. After that the dried peels were ground into a powder using a domestic grinder.

Water extract of powdered rambutan peels was prepared according to Ayurvedic traditional method which is used to prepare 'kasaya'.[10] A weight of 60 g of powder was simmerly boiled in 960 ml of distilled water to obtain the decoction of 240 ml. The water extract was filtered through a fine silk cloth and the filtrate was freeze dried. The freeze-dried compound was kept at - 4 ℃ in air tight containers until required. Total phenol content, total flavonoid content, antioxidant capacity and anti-inflammatory activity were evaluated according to previously published methods with slight modifications.

Preparation of Sample Concentrations

A stock solution of known concentration was prepared for each experiment separately by dissolving a known amount of freeze-dried compound in a known volume of solvent. A concentration series was prepared by diluting the stock solution.

Estimation of Total Phenolic Content

Folin Ciocalteu Assay

A volume of 4 ml of 2% Sodium bicarbonate was added to 200 µl of sample and incubated in darkness for 2 minutes. Folin Ciocalteu reagent 200 µl was added to the same mixer and incubated in darkness for 30 minutes. The absorbance was measured at 750 nm. The blank was prepared by replacing the Folin Ciocalteu reagent with 200 µl of distilled methanol. The same procedure was carried out for standard concentration series using 200 µl of pyrogallol instead of the sample.[4] The total phenolic content of the sample was calculated using the pyrogallol standard curve.

Estimation of Total Flavonoid Content

$AlCl_3$ Colourimetric Assay

A volume of 0.5 ml of sample was added to 2.0 ml of distilled water and 150 µl of 5% sodium nitrite solution. The mixture was incubated in the dark for 5 minutes. A volume of 150 µl of 10% aluminium chloride ($AlCl_3$) was added to the same mixture and it was kept in darkness for 6 minutes. Then 1.0 ml of 1 M sodium hydroxide solution and 1.0 ml of distilled water were added to the reaction mixture. The absorbance was measured at 510 nm. The blank was prepared without adding $AlCl_3$. The same procedure was carried out for standard concentration series using 0.5 ml of quercetin instead of the sample.[4] The total flavonoid content of the sample was calculated using the quercetin standard curve.

Estimation of Antioxidant Activity

DPPH Radical Scavenging Activity

A volume of 3 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) working standard was added to 100 µl of each concentration of the sample. The reaction mixtures were shaken well and incubated in the dark for 15 minutes at room temperature. The absorbance was measured at 517 nm. The control was prepared using DPPH working standard only. Methanol was used as the blank. The same procedure was carried out for standard concentration series using 100 µl of ascorbic acid instead of the sample. Percentage of scavenging activity (SA) was calculated using the following equation where A^o is the absorbance of the control and A^s is the absorbance of the sample.[11]

$$SA\ (\%) = \left(\frac{A^o - A^s}{A^o}\right) \times 100$$

Hydroxyl Radical Scavenging Activity

A volume of 500 µl of 2-deoxyribose, 200 µl of premixed ferric chloride and ethylenediaminetetraacetic acid (EDTA) (1:1; v/v), 100 µl of hydrogen peroxide (H_2O_2) were added to 100 µl of each concentration of the sample. The reaction was triggered by adding 100 µl of ascorbic acid and the reaction mixtures were incubated for 1 hour at 37℃. A volume of 0.5 ml of the reaction mixture was added to 1 ml of Trichloroacetic acid and then 1 ml of Thiobarbituric acid was added into the same mixture. The mixtures were heated for 15 min on a boiling water bath. After the mixture being cooled, the absorbance at 532 nm was measured. The control was prepared using a mixture of 2-deoxyribose, phosphate buffer, premixed ferric chloride, EDTA and H_2O_2. Distilled water was used as the blank. The same procedure was carried out for standard concentration series using 100 µl of ascorbic acid instead of the sample. Percentage of SA was calculated using the following equation where A^o is the absorbance of the control and A^s is the absorbance of the sample.[11]

$$SA\ (\%) = \left(\frac{1 - A^s}{A^o}\right) \times 100$$

Iron Reducing Power

A volume of 2 ml of pH 6.6 phosphate buffer, 2 ml of potassium ferricyanide were added to 2 ml of each concentration of the sample. The reaction mixtures were incubated at 50℃ for 20 min followed by addition of 2 ml of trichloroacetic acid. The mixtures were centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 2 ml of each of the mixture was mixed with 2 ml of distilled water and 0.4 ml of fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm against distilled water blank. The same procedure was carried out for standard concentration series using 2 ml of ascorbic acid instead of the sample.[11]

Estimation of Anti-inflammatory Activity

Nitric Oxide Radical Scavenging Activity

A volume of 0.5 ml of sodium nitroprusside (SNP) was added to 1 ml of each concentration of the sample and the reaction mixtures were incubated at 25℃ for 180 min. Griess reagent was prepared by mixing equal volumes of 1% sulphanilamide and 0.1% naphthylethylene diamine dichloride immediately before use. A volume of 1 ml of freshly prepared Griess reagent was added into each of the reaction mixture and the absorbance was measured at 546 nm. The control was prepared using SNP, phosphate buffered saline (PBS) and Griess

reagent. Distilled water was used as the blank. The same procedure was carried out for standard concentration series using 1 ml of ascorbic acid instead of the sample. Percentage SA was calculated using the following equation where A^o is the absorbance of the control and A^s is the absorbance of the sample.[12]

$$SA\ (\%) = (\frac{A^o - A^s}{A^o}) \times 100$$

Human Red Blood Cell Assay

A healthy volunteer who had not taken anti-inflammatory drugs for a period of 2 weeks before obtaining blood sample was selected as the donor of human blood. A volume of 5 ml of blood was collected and it was centrifuged at 3000 rpm for 20 minutes. Resulted supernatant was discarded and the pellet was washed using a volume of normal saline equal to the pellet volume. Centrifugation was carried out until yellowish colour of the supernatant went off and became clear.

A volume of 1 ml of each concentration of sample and 0.1 ml of red blood cell suspension were combined. The reaction mixtures were incubated at 56℃ for 30 minutes. The tubes were cooled and the reaction mixtures were again centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 540 nm. The control was prepared using red blood cell suspension only. Distilled water was used as the blank. The same procedure was carried out for standard concentration series using 1 ml of aspirin instead of the sample. Percentage of protection was calculated using the following equation where A^o is the absorbance of the control and A^s is the absorbance of the sample.[13]

$$Protection\ (\%) = 100 - (\frac{A^s}{A^o} \times 100)$$

Data Analysis

All tests were carried out in triplicate and the data shown in the table and the graphs are the mean together with standard deviation.

RESULTS AND DISCUSSION

The main objective of this study was to determine the antioxidant capacity and anti-inflammatory activity of the aqueous extract of the peel of a Sri Lankan variety of rambutan; Malwana special which was prepared according to the method of kasaya preparation in Ayurvedic medicine.

Phenols and flavonoids are known to be responsible for antioxidant activity in many plant species.[6,11] Total phenol content was estimated using Folin Ciocalteu assay. Under alkaline conditions phenolic compounds dissociate to an anionic form which reacts with Folin reagent. If the test is positive, the reagent gives a colour change from yellow to dark blue due to the reduction of phosphomolybdate-phosphotungstate complex forming Mo (v) species.[4] The AEPR gave a value of 463.5±5.2 mg (PGE)/g for the phenol content. Total flavonoid content was estimated by Aluminium chloride colourimetric assay. The colour of the reaction mixture turns to brown upon addition of $AlCl_3$ due to formation of acid stable complexes between $AlCl_3$ and flavonoids.[4] The AEPR gave a value of 375.0±13.2 mg (QE)/g for the flavonoid content.

The DPPH assay is based on the ability of natural products to donate electron to the DPPH radical. The deep purple colour of the DPPH radical is reduced depending on the activity of radical scavenging activity of the test sample. The degree of colour change is proportional to the potency and the concentration of antioxidants.[11] Figure 1 shows the percentage scavenging activity (SA) vs concentration of AEPR and the percentage SA vs concentration of the standard.

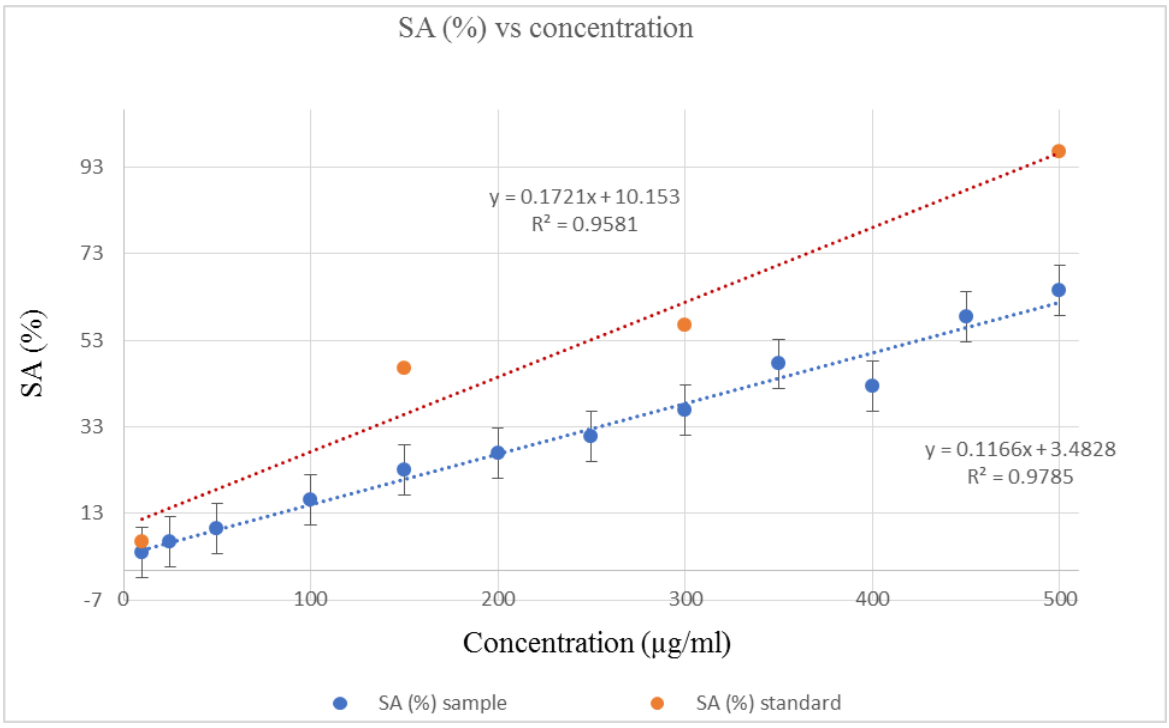

Figure 1: Percentage scavenging activity vs concentration of AEPR and percentage scavenging activity vs concentration of ascorbic acid standards in DPPH radical scavenging activity assay.

The DPPH radical scavenging activity of AEPR was 3.9 - 64.5% for concentrations of 10 – 500 µg/ml. The positive control ascorbic acid gave values of 6.5 – 96.4%.

Hydroxyl radical is one of the reactive oxygen species (ROS) in human body which damages to cells by reacting with polyunsaturated fatty acid moieties of cell membrane phospholipids.[11] The hydroxyl radicals also contribute to carcinogenesis, mutagenesis and cytotoxicity.[11] The reaction between iron-ethylenediaminetetraacetic acid complex and H_2O_2 is accelerated by ascorbic acid. Hydroxyl radicals generated by decomposition of H_2O_2 reacts with 2-deoxyribose to form malonaldehyde. It yields a pink chromogen when heated with TBA at low pH conditions. Hydroxyl radical scavenging capacity is proportional to its' antioxidant activity and it is indicated by the low intensity of pink colour.[11] Figure 2 shows the percentage SA vs concentration of AEPR and the percentage SA vs concentration of the standard.

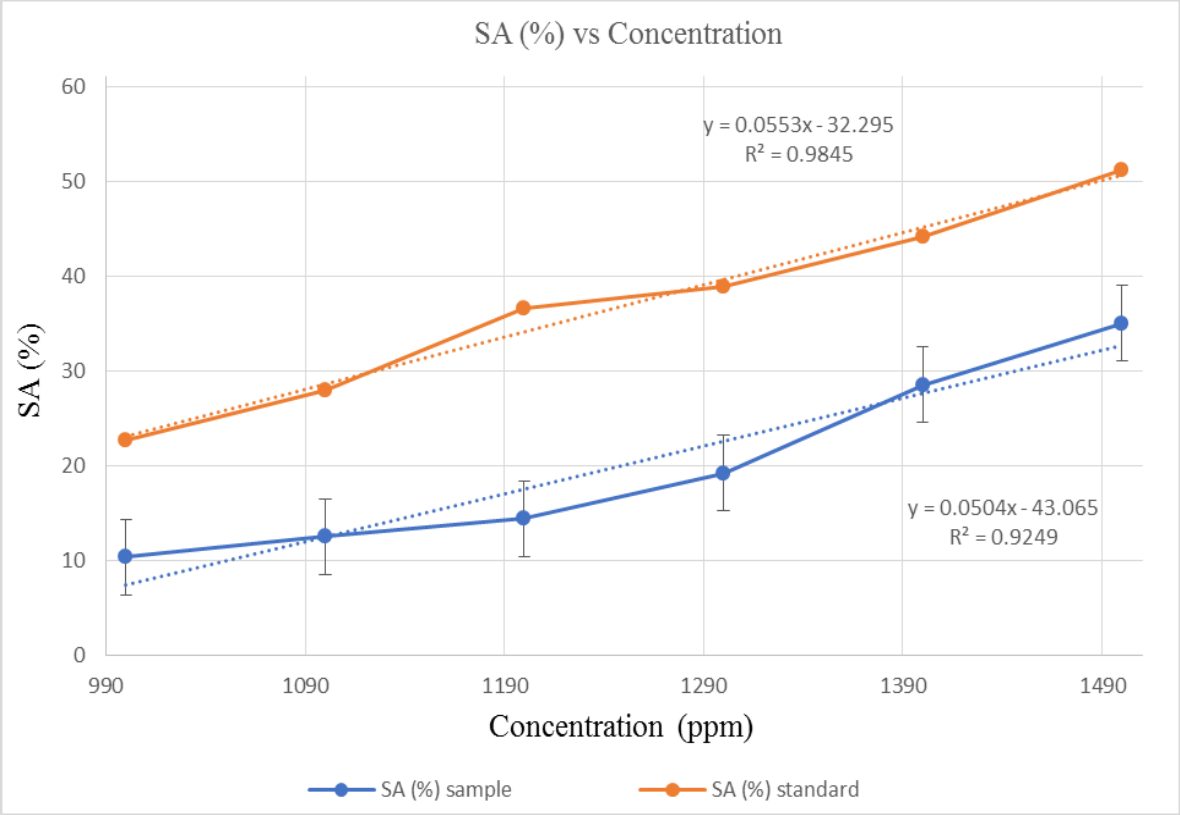

Figure 2: Percentage scavenging activity vs concentration of AEPR and percentage scavenging activity vs concentration of ascorbic acid standards in Hydroxyl radical scavenging activity assay.

The hydroxyl radical scavenging activity of AEPR ranged from 10.3 - 35.0% for concentrations of 1000 - 1500 ppm. The positive control ascorbic acid showed values of 22.6 - 51.1%.

Fe^{3+} is reduced to Fe^{2+} due to presence of antioxidants and the reducing power can be monitored by formation of Perls' Prussian blue complex at 700 nm. The reducing power has been shown to exhibit antioxidant activity by donation of a hydrogen atom to break the free radical chain.[11] As given in Table 1, AEPR exhibited a good reducing power compared to the positive control ascorbic acid.

Table 1: Absorbance values for AEPR and ascorbic acid concentration series.

Concentration (µg/ml)	Absorbance values for AEPR	Absorbance values for the standard
1100	0.128 ± 0.003	0.773 ± 0.025
1150	0.171 ± 0.006	-
1200	0.209 ± 0.011	1.052 ± 0.030
1250	0.237 ± 0.006	-
1300	0.318 ± 0.005	1.149 ± 0.021
1350	0.387 ± 0.005	-
1400	0.448 ± 0.007	1.252 ± 0.013
1450	0.481 ± 0.006	-
1500	0.598 ± 0.005	1.290 ± 0.004

It has been reported that ROS participate in the process of inflammation.[6] Excess ROS damage cellular components and hence it augments the state of inflammation.[2,6] The compounds showing SA towards ROS and free radicals are expected to have therapeutic potential towards inflammatory diseases.[6] Nitric oxide (NO) is synthesized from L-arginine by three isoforms of nitric oxide synthase (NOS) enzyme in vascular endothelial cells, certain neuronal cells and phagocytes.[12] Chronic exposure to NO radical causes various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis.[12]

NO is generated by SNP in aqueous medium at physiological pH.[14] Interaction of NO with oxygen produces nitrite ions which convert sulfanilic acid to a diazonium salt under acidic conditions.[14] The red pink azo dye is formed by the reaction of diazonium salt with napthylethylene diamine dichloride.[14] During this method nitric oxide radical gets scavenged by antioxidants and the scavenging effect is indicated by the reduction of the

colour intensity of the reaction mixture.[14] Figure 3 shows the percentage SA vs concentration of AEPR and the percentage SA vs concentration of the standard.

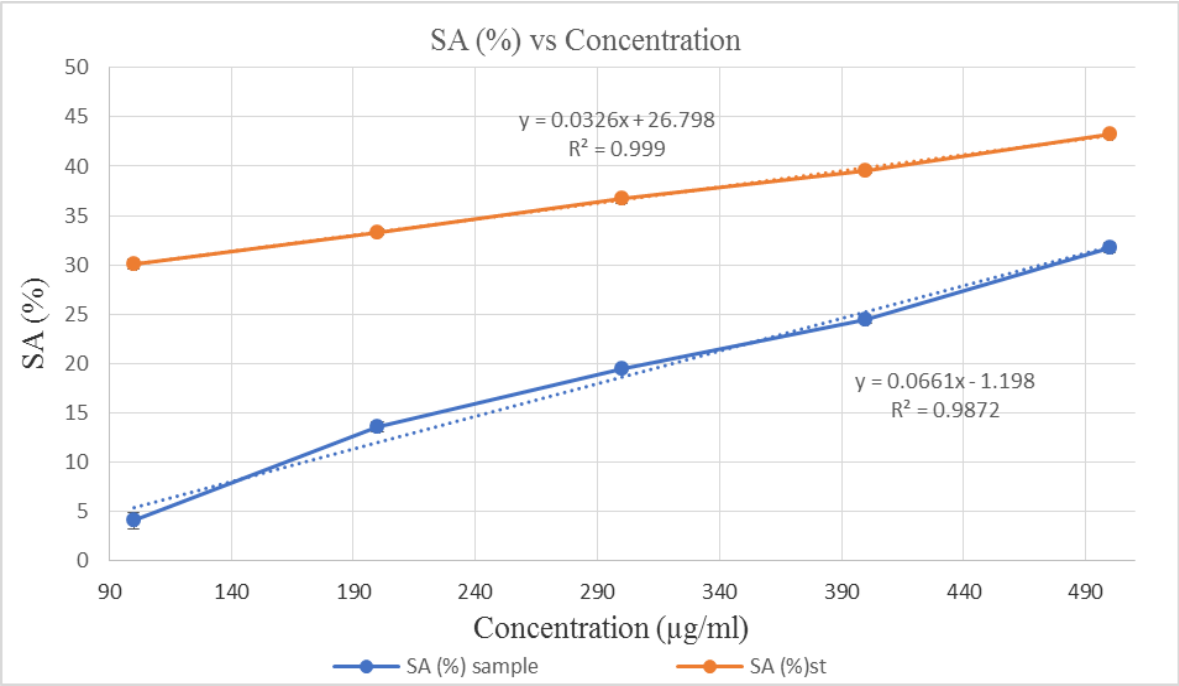

Figure 3: Percentage scavenging activity vs concentration of AEPR and percentage scavenging activity vs concentration of ascorbic acid standards in Nitric oxide radical scavenging activity assay.

Nitric oxide radical scavenging activity of AEPR was 4.1 - 31.7% for concentrations of 100 – 500 µg/ml. Ascorbic acid gave values of 30.1 - 43.3%.

Lysosomes in eukaryotic cells involve in the degradation of macromolecules delivered by endocytosis and auto phagocytosis.[15] Inflammatory cytokines can be secreted or degraded by lysosomes in order to regulate the release of cytokines such as IL-1β, IL-18 and TNF-α at an immune response.[15] Thus, lysosomes can positively regulate inflammation and lysosomal compartments play a main role in the inflammatory signaling network due to the involvement of lysosomal membrane proteins such as TMEM9B in the activation of NF−κB and MAPκ pathways.[15]

Human Red Blood Cell assay is based on inhibition of heat induced lysis of erythrocytes by stabilizing the cell membrane.[13] The erythrocyte membrane is considered as a model of the lysosomal membrane which plays an important role in inflammation.[13] Figure 4 shows the

percentage protection vs concentration of AEPR and the percentage protection vs concentration of the standard.

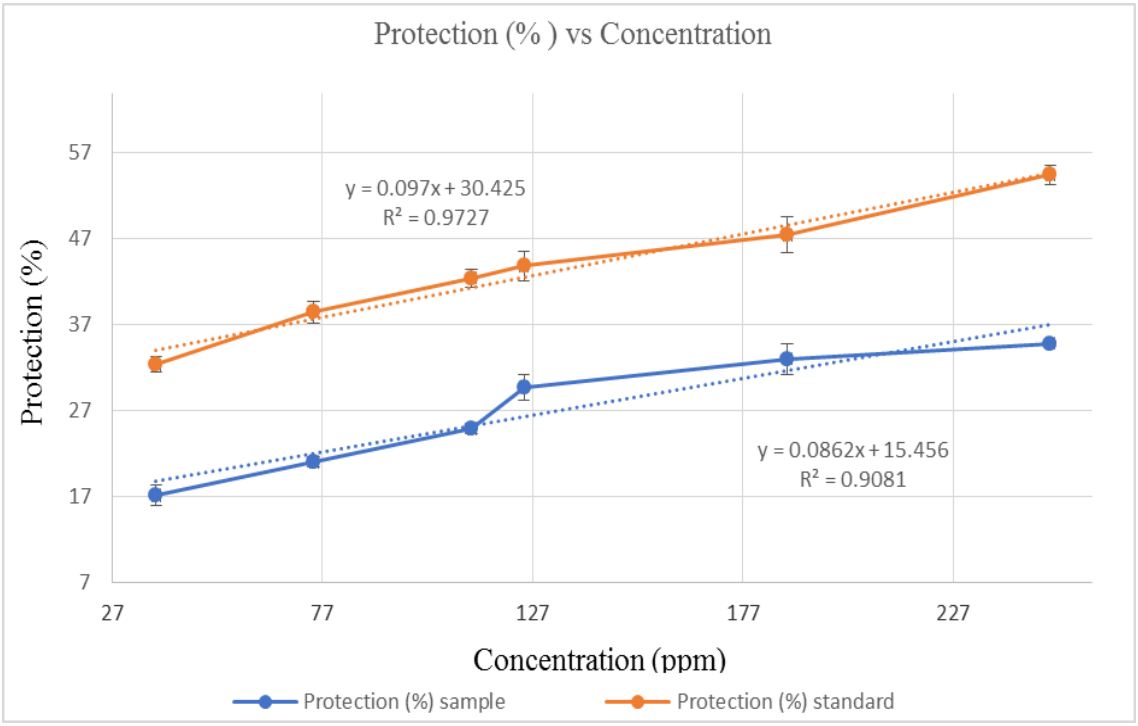

Figure 4: Percentage protection vs concentration of AEPR and percentage protection vs concentration of aspirin standard series in Human Red Blood Cell assay.

The protection reported for HRBC assay was 17.1 – 34.8% for concentrations from 35.5 – 250.0 ppm. Aspirin protection ranged from 32.3 - 54.5%.

CONCLUSION

Our results show that the AEPR prepared according to the method of kasaya preparation in Ayurvedic medicine has a positive activity towards DPPH radical scavenging activity, hydroxyl radical scavenging activity, iron reducing power, nitric oxide radical scavenging activity and HRBC assays.

Therefore, it can be concluded that the decoction of the peel of Malwana special rambutan is a rich source of natural antioxidants and anti-inflammatory compounds and can be further modified as a potential therapeutic agent due to its' significant activity towards *in vitro* studies.

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REFERENCES

1. Handunnetti SM, Kumara RR, Deraniyagala SA, Ratnasooriya WD. Anti-inflammatory Activity of *Ixora coccinea* Methanolic Leaf Extract. Phcog Res, 2009; 1(2): 80-90.
2. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev, 2010; 4(8): 26-118.
3. Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal, 2012; 24(5): 90-981.
4. Dissanayake DMRH, Deraniyagala SA, Hettiarachchi CM, Thiripuranathar G. The Study of Antioxidant and Antibacterial Properties of Skin, Seeds and Leaves of The Sri Lankan Variety of Pumpkin. J Pharm, 2018; 8(2): 43-48.
5. Weerasinghe WPNW, Deraniyagala SA. Antioxidant activity of some Sri Lankan endemic medicinal plants. Pharm J Sri Lanka, 2016; 6(1): 9-14.
6. Ganesh Gadamsetty, Saurabh Maru, Sarada NC. Antioxidant and Anti-inflammatory Activities of the Methanolic Leaf Extract of Traditionally Used Medicinal Plant Mimusops elengi L. Pharm Sci & Res, 2013; 5(6): 125-130.
7. Suganthi A, Marry Josephine R. *Nephelium Lappaceum* (L.): An overview. Pharm Sci & Res, 2016; 1(5): 36-39.
8. Nethaji R, Thooyavan G, Mullai Nilla K, Ashok K. PHYTOCHEMICAL PROFILING, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACT IN RAMBUTAN FRUIT (*NEPHELIUM LAPPACIUM*) EPICARP AGAINST THE HUMAN PATHOGENS. Int J Curr Innov Res, 2015; 1(9): 201-206.
9. Rohman A. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Res. J Phytochem, 2017; 11(2): 66-73.
10. Perera PRD, Sagarika Ekanayaka S, Ranaweera KKDS. *In Vitro* Antiglycation Activity of Some Medicinal Plants Used in Diabetes Mellitus. Med Aromat Plants, 2013; 2(6): 1-3.
11. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med, 2012; 12: 221.

12. Boora F, Chirisa E, Mukanganyama S. Evaluation of Nitrite Radical Scavenging Properties of Selected Zimbabwean Plant Extracts and Their Phytoconstituents. J Food Process, 2014; 1-7.
13. Kuganesan A, Thiripuranathar G, Navaratne AN, Paranagama PA. ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF PEELS, PULPS AND SEED KERNELS OF THREE COMMON MANGO (*MANGIFERA INDICAL* L.) VARIETIES IN SRI LANKA. Pharm Sci & Res, 2017; 8(1): 70-78.
14. Menikpurage IP, Soysa SSSBDP, Abeytunga DTU. Antioxidant activity and cytotoxicity of the edible mushroom, *Pleurotus cystidiosus* against Hep-2 carcinoma cells. J Natn Sci Foundation Sri Lanka, 2012; 40(2): 107-114.
15. Ge W, Li D, Gao Y, Cao X. The Roles of Lysosomes in Inflammation and Autoimmune Diseases. Int Rev Immunol, 2014; 1-17.