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Assessment of free radical scavenging activity of Mangosteen (*Garcinia mangostana* L.) fruit peel anthocyanin pigment obtained through different extraction methods

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Abstract
Mangosteen (*Garcinia mangostana* L.) belonging to the family Clusiaceae, a tropical fruit that is highly prized for its deliciously tasting edible aril, which is enclosed in an inedible dark-purple rind that is considered as waste but is as rich source of anthocyanin pigment. The experiment was conducted with an objective to evaluate the free radical scavenging activity of the anthocyanin pigment concentrate obtained by different extraction methods of Mangosteen fruit peel, which included aqueous (distilled water) extraction, acidified aqueous (1% citric acid), solvent extraction (50% ethanol), acidified solvent (50% ethanol with 1% citric acid) extraction and Microwave Assisted Extraction (MAE) with acidified solvent (50% ethanol with 1% citric acid). The results revealed that significantly higher (86.37±0.77) free radical scavenging activity was seen in MAE extraction method using acidified solvent (50% ethanol with 1% citric acid), whereas it was lower (56.25±0.77) in aqueous extraction method. Using MAE and acidified ethanol as the solvent, antioxidants can be effectively extracted in the anthocyanin pigment concentrate from Mangosteen peel resulting in higher free radical scavenging activity with hydrogen peroxide (H_2O_2) assay. Given the current need for plant-based colourants with more health benefits, further studies are needed to ascertain the pigment's antioxidant property utilizing various assays.

Keywords: Mangosteen fruit peel, anthocyanin pigment, extraction, hydrogen peroxide, scavenging activity

1. Introduction
The search for natural sources that could deliver active compounds to counteract the effects of free radicals on cells has grown in recent years. Hence, there have been a lot more studies on natural antioxidants (Ghasemzadeh *et al*., 2018) [7]. Mangosteen is a tropical fruit that is highly prized for its deliciously tasting edible aril. But, the edible portion makes up only 30% of the total fruit, while the remaining pericarp and seed are regarded as waste (Osman and Milan, 2006) [18]. The recent studies have shown that the pericarp extract of the mangosteen is the primary sources of phytonutrients such as anthocyanins, oligometric proanthocyanins and xanthones and so there have been several reports on its antioxidant, antimicrobial, antidiabetic, antiproliferative, and anticancer capabilities (Geetha *et al*., 2011; Ghasemzadeh *et al*., 2018; Kosem *et al*., 2013; Lacombe *et al*., 2010; Moongkarndi *et al*., 2004; Sze Lim *et al*., 2013; Taher *et al*., 2016; Tjahjani *et al*., 2014; Zafra-Stone *et al*., 2007; Zarena and Sankar, 2012) [6, 7, 11, 12, 17, 22, 23, 25, 27, 28].

Anthocyanins are known for their high antioxidant properties which by giving the hydrogen atom to free radicals, they can either directly scavenge free radicals or indirectly prevent them by chelating free metal ions (Mishra *et al*., 2013) [16]. The body needs antioxidants to stop free radicals, enhance health and boost immunity (Kondo *et al*., 2009) [10]. The free radicals *viz*., single oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide radical ($^{\bullet}O^{-}_2$) and hydroxyl radical ($^{\bullet}OH$) are chemical substances that can exist individually with one or more unpaired electrons, produced as undesirable byproducts (Das and Roychoudhury, 2014) [4]. Free radical production can result in thousands of reactions and significant tissue damage, which in turn leads to harm DNA, proteins and lipids (Basile *et al*., 1999; Sreejayan, 1997) [2, 21]. It is believed that the antioxidants in Mangosteen rind have a significant role in promoting health benefits and preventing various ailments (Gondokesumo *et al*., 2019) [8]. In this context, Mangosteen is not only used as food but it is also used as functional food, especially its peel which has the

highest level of phytonutrients compared to the other parts of the fruit (Wittenauer *et al*., 2012) [26]. These phytonutrients which mainly contains anthocyanins can be successfully used as food colourant that increases the aesthetic value of food as well as significantly adds value to the food in terms of nutrients.

Extraction is a vital first step in obtaining the active chemicals from plant materials (Jeyaraj *et al*., 2020) [9] and to generate the best yield with the highest concentration of the target compounds, one must choose an efficient extraction method (Gamage *et al*., 2021) [5]. Because anthocyanins are sensitive to heat, light, acids and alkalis, it is essential to apply the right extraction method in order to recover the maximum quantity possible without compromising their quality (Chandrasekhar *et al*., 2012; Jeyaraj *et al*., 2020) [3, 9]. With this background, the experiment was conducted with an objective to evaluate the free radical scavenging activity of the anthocyanin pigment concentrate obtained by different extraction methods of mangosteen fruit peel.

2. Materials and Methods

The study was conducted in the year 2021-22 at Department of Post-Harvest Technology, College of Agriculture, Vellanikara, Kerala Agricultural University, Thrissur, Kerala, India.

2.1 Sample preparation

Based on a colour index that displays a purple-black colour on the mangosteen fruit skin at the sixth stage of development, fresh fruits with uniform colour were chosen (Palapol *et al*., 2009) [19]. The fruits were properly cleaned with distilled water before being shade-dried to eliminate extra moisture. Using a stainless steel peeler, the exocarp was peeled. The peel was then dried in a cabinet dryer at 40±2 °C until it reached a consistent weight, and then it was powdered in a commercial blender, and sieved through an 80 mesh size sieve. The peel powder was then sealed in a laminated pouch of aluminium foil and kept in the freezer until pigment extraction.

2.2 Extraction of anthocyanin pigment

The extraction was done using the traditional solid-liquid extraction method, which included: T_1 - Aqueous (distilled water) extraction; T_2 - Acidified aqueous (1% citric acid); T_3 - Solvent extraction; T_4 - Acidified solvent extraction; and T_5 - Microwave assisted extraction with acidified solvent (50% ethanol with 1% citric acid). At a temperature of 45 °C, the sample was agitated in the solvent for 45 min.

The solvent and plant samples were mixed in a 1:20 (w/v) ratio. In the case of microwave-assisted extraction, the sample was combined in the same ratio with an acidified solvent (50% ethanol with 1% citric acid), and the tube containing the suspension was exposed to microwave radiation at 300W for 120s while the temperature was kept between 45 and 50 °C. Filter paper was used to filter the extract. A rotary vacuum evaporator (Heidolph rotary evaporator, Germany) was used to collect and evaporate the filtrate at 60 °C and 114 mbar (Azima *et al*., 2017) [1]. The concentrated filtrates were stored under refrigeration (4–7 °C) in glass vials with an aluminium foil laminated pouch until analysis.

2.3 Hydrogen peroxide (H_2O_2) scavenging activity (%)

The free radical scavenging activity of the anthocyanin pigment concentrate was determined by hydrogen peroxide assay (Mahendran *et al*., 2021) [13]. Hydrogen peroxide (10 mM) solution was prepared in phosphate buffered saline (0.1 M, pH 7.4). One mL of the pigment sample was rapidly mixed with two mL of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer (Agilent Cary 60 Spectrophotometer, Australia) after 10 min of incubation at 37 °C against a blank (distilled water with hydrogen peroxide solution). The percentage of scavenging of hydrogen peroxide was calculated using the following formula.

$$\text{Perccentage scavenging } (H_2O_2) = \frac{A_0 - A_1}{A_0} \text{ x } 100$$

In which, A_o and A_1 is the Absorbance of control and sample, respectively.

2.4 Statistical analysis

The experiment was carried out in triplicates and results were expressed as mean values with standard deviation (±SD) (Panse and Sukhatme, 1989) [20]. One-way analysis of variance (ANOVA) was carried out to determine significant group differences ($p \leq 0.05$) between means. Duncan Multiple Range Test (DMRT) was used to compare mean values.

3. Results and discussion

The free radical scavenging ability of anthocyanin pigment concentrates of Mangosteen fruit peel was measured using hydrogen peroxide scavenging activity (%). The results related to the H_2O_2 scavenging activity are presented in Figure 1.

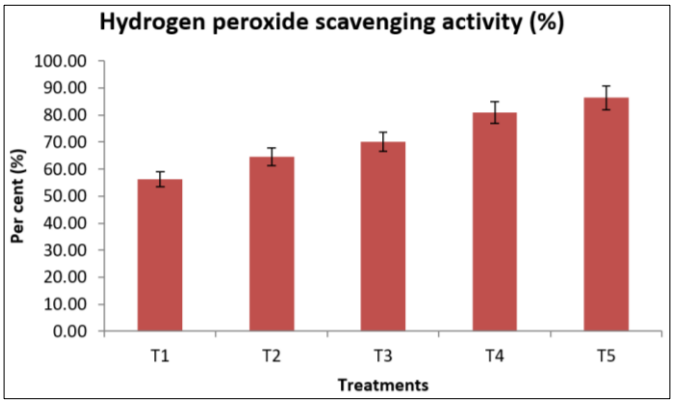

Fig 1: Hydrogen peroxide scavenging activity (%) of anthocyanin pigment concentrates of Mangosteen fruit peel

(T_1) Aqueous (T_2) Acidified aqueous with 1% citric acid (T_3) Solvent – 50% ethanol (T_4) Acidified solvent – 50% ethanol with 1% citric acid (T_5) Microwave assisted extraction with acidified solvent – 50% ethanol with 1% citric acid

In the present study, all the extraction techniques yielded anthocyanin pigment concentrates that showed effective at reducing free radicals. Significantly higher (86.37±0.77) free radical scavenging activity was observed in MAE extraction method using acidified solvent (50% ethanol with 1% citric acid), whereas it was lower (56.25±0.77) in aqueous extraction method. In microwave assisted extraction method, microwave radiation is used as a heating medium and this method provides better temperature control than other techniques, making it more practical for extracting

thermolabile antioxidant chemicals (Megawati *et al*. 2019; Thirugnanasambandham and Sivakumar 2017) [14, 24]. Similarly phenolic concentration of Mangosteen peel extract was also affected by microwave power, wherein longer the extraction time, more is the phenolic concentration in the extract (Meghawati *et al*., 2020) [15].

4. Conclusion

Mangosteen fruit is well-known for its deliciously tasting fleshy aril which is covered with inedible dark purple rind that is rich in anthocyanin pigment. Using MAE and acidified ethanol as solvent, antioxidants can be effectively extracted in the anthocyanin pigment concentrate from Mangosteen peel resulting in higher free radical scavenging activity using H_2O_2 assay. Given the current need for plant-based colourants with more health benefits, further studies are needed to ascertain the pigment's antioxidant property utilizing various assays.

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