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Comparison of Anthocyanin Pigment Extraction Techniques to Evaluate the Free Radical Scavenging Capacity of Butterfly Pea (*Clitoria ternatea* L.) Flower

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ABSTRACT: The Fabaceae plant species *Clitoria ternatea* L., often known as butterfly pea, contains edible flowers that are a rich source of anthocyanins (water soluble plant pigment) called ternatins having bright blue colour. The objective of present study was to evaluate the free radical scavenging activity of the anthocyanin pigment concentrate obtained by different extraction methods using hydrogen peroxide (H_2O_2) scavenging activity (%) assay. The extraction methods followed were aqueous (distilled water), acidified aqueous (distilled water with 1% citric acid), solvent (50% ethanol), acidified solvent (50% ethanol with 1% citric acid) and microwave assisted extraction (MAE) with aqueous solvent. The results revealed that significantly higher (79.28±0.47%) scavenging activity was observed in MAE with aqueous medium and lowest (64.39±1.75%) in aqueous extraction method. Using MAE method with aqueous medium as solvent resulted in the pigment concentrate with an intense blue colour and high antioxidant properties.

Keywords: Butterfly pea flower, anthocyanin, pigment extraction, free radical, hydrogen peroxide, antioxidants.

INTRODUCTION

Reactive oxygen species (ROS) are a class of oxygen containing free radicals produced by oxidation reactions in organisms. These 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). Free radical production can result in thousands of reactions and significant tissue damage, which in turn leads to harm DNA, proteins, and lipids (Sreejayan, 1997; Basile *et al*., 1999). Oxidative stress (OS) is the imbalance between cellular ROS production and cellular ROS scavenging ability (Khan *et al*., 2013). Antioxidants are crucial in providing resistance to OS by scavenging free radicals. The ability to contribute hydrogen atoms or electrons to free radicals and displace them is what is known as the antioxidant property. This prevents the damage that free radicals would otherwise inflict (Tan and Lim 2015). Recent studies have shown that the abundance of antioxidants present in a variety of foods and beverages such as fruits, vegetables, medicinal herbs, tea, coffee *etc.,* have a positive impact on human health (Gulcin, 2012).

Anthocyanins are blue, red, or purple pigments that are present in plants, particularly in their flowers, fruits, and tubers, while it is blue and red in alkaline and acidic conditions, respectively. Despite having a positive charge on the oxygen atom of the C-ring of the basic flavonoid structure; nevertheless, it is regarded as one of the flavonoids (Khoo *et al*., 2017). 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). In addition, it provides more health advantages in terms of antimicrobial, antiproliferative, hypoglycemic, and others (Yoon *et al*., 2018; Li *et al*., 2019; Yue *et al*., 2019; Gamage *et al*., 2021).

One of the crops that contains abundance of anthocyanin pigment is *Clitoria ternatea* L., known as butterfly pea, blue pea, Cord of an pea, and Asian pigeon wings belongs to the Fabaceae family, which yield edible flowers with colours ranging from dark blue, light blue to white and it is one of the important plants widely cultivated in tropical and temperate regions worldwide including Asia, Southeast Asia, the Caribbean, and Central and South America (Adisakwattana *et al*., 2020). Anthocyanins in butterfly pea flowers are distinctive for their profusion of polyacylated anthocyanins, or "ternatins" which are polyacylated derivatives of delphinidin 3,3′,5′-triglucoside (Gamage *et al*., 2021). As a result butterfly pea flowers with greater potential to be used as a natural source of anthocyanin pigment with blue hue besides health benefits as a supplement in the food and pharmaceutical industries (Jeyaraj *et al*., 2020).

The first crucial stage in recovering active compounds from plant materials is extraction (Panda *et al*., 2022; Jeyaraj *et al*., 2020). The goal of choosing an effective extraction technique is to produce the highest yield with the highest concentration of the target chemicals (Gamage *et al*., 2021). It is crucial to use an appropriate extraction technique to obtain the most number of anthocyanins without degrading them because these are sensitive to heat, light, acids and alkalis (Chandrasekhar *et al*., 2012; Jeyaraj *et al*., 2020). In this regard, the experiment was conducted to evaluate the free radical scavenging activity of the anthocyanin pigment concentrate obtained by different extraction methods.

MATERIALS AND METHODS

The laboratory experiment was conducted at Department of Post Harvest Technology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, Kerala during 2021-22.

Sample preparation. Fresh flowers were gathered in the early morning from Medicinal and Aromatic field block, College of Agriculture, Vellanikkara. The flowers were rinsed under running water from the tap and then drained free of water. The petals were separated from the sepals and dried in a cabinet dryer at 40±2 °C until they reached a consistent weight. The dried petals were then pulverised in a commercial blender and sieved through an 80 mesh size sieve. The petal powder was then sealed in a laminated pouch of aluminium foil and kept in the freezer until pigment extraction.

Extraction of anthocyanin pigment. The experiment was laid out in a Completely Randomized Design with five treatments. The extraction was carried out by using the following methods: T_1 - Aqueous (distilled water) extraction, T_2 - Acidified aqueous (1% citric acid) extraction, T_3 - Solvent extraction (50% ethanol), T_4 - Acidified solvent extraction (50% ethanol with 1% citric acid) and T_5 - Microwave assisted extraction with aqueous solvent (distilled water). 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 microwave-assisted extraction method, the sample was combined with distilled water, and the tube containing the suspension was heated between 45 and 50 °C by being exposed to a 300-watt microwave for 120 seconds. Filter paper was used to filter the extract and a rotary vacuum evaporator (Heidolph rotary evaporator, Germany) was used to evaporate the filtrate at 60 °C and 114 mbar (Azima *et al*., 2017). The concentrated filtrates were preserved in glass vials, enclosed inside a laminated aluminium foil bag and kept refrigerated (4–7 °C) condition until analysis.

Hydrogen peroxide scavenging activity (%). The free radical scavenging activity of the anthocyanin pigment concentrate was determined by hydrogen peroxide assay (Mahendran *et al*., 2021). 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.

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

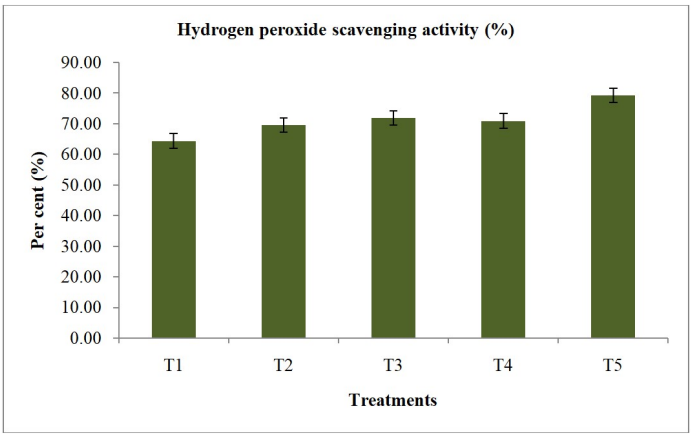
In which, A_o and A_1 is Absorbance of control and sample, respectively.

Statistical analysis. The experiment was carried out in triplicates and results were expressed as mean values with standard deviation (±SD) (Panse and Sukhatme, 1989). 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.

RESULTS AND DISCUSSION

The free radical scavenging activity of anthocyanin pigment concentrates of butterfly pea flowers was determined by hydrogen peroxide scavenging activity (%) and the results pertaining to it are shown in Fig. 1. The anthocyanin pigment concentrates obtained using different extraction methods have demonstrated their ability to diminish the free radicals. Significantly higher (79.28±0.47%) scavenging activity was noted in

microwave assisted extraction method using aqueous solvent (T_5) and significantly lower (64.39±1.75%) scavenging activity was observed in aqueous method of extraction (T_1). The hydrogen-donating activity, measured utilizing hydrogen peroxide radicals as the hydrogen acceptor, demonstrated that a strong association could be found between anthocyanin pigment concentrates obtained using different extraction methods and their rate of inhibition (Al-Amiery *et al*., 2012). In addition, during the process of pigment extraction, the extract will not only contain the anthocyanin pigment but a mixture of bioactive components. Since the butterfly pea flowers are known to contain higher amount of polyphenols (Tuan Putra *et al*., 2021), the main polyphenol constituent being the anthocyanin itself (Pasukamonset *et al*., 2016) might have contributed to its higher free radical scavenging activity depending upon the method of extraction and solvent used. The phenolics play an important role in the absorption or neutralization of free radicals (Basile *et al*., 1999).



(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 aqueous solvent

Fig. 1. Hydrogen peroxide scavenging activity (%) of anthocyanin pigment concentrates of Butterfly pea flower petals.

CONCLUSION

The butterfly pea bears edible flowers that are a rich source of anthocyanins called "ternatins" with bright blue colour. Phytochemicals having anti-diabetic, antioxidant, anti-microbial, anti-inflammatory, and anti-proliferative/anti-cancer activities are also abundant in the flower. From the current study, it could be concluded that aqueous medium with and without MAE method resulted in the pigment concentrate with a bright blue colour and high free radical scavenging activity. More research is required to determine the pigment's antioxidant property using other assays given the present demand for plant based blue colourants.

FUTURE SCOPE

Future research should concentrate on antioxidant property retention and pigment stability when applied to various processed products *etc*.

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Conflict of Interest. None.

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