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EVALUATION OF ANTIOXIDANT ACTIVITY OF PURPLE HEART USING IN VITRO METHODS

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ABSTRACT

Purple heart *(Tradescantia pallida)* belongs to the family Commelinaceae. The present study aimed to evaluate the freshly prepared leaf juice for free radical scavenging activity of hydrogen peroxide, DPPH and, nitric oxide at different concentrations and the activity was expressed using IC50. The IC50 values of purple extracts were found to be 194µg/ml, 861.11 µg/ml and 855.0 µg/ml respectively for hydrogen peroxide, DPPH radical and nitric oxide radicals. The antioxidant activity might be due to the presence of sesquiterpenes in the leaves of purple heart.

KEY WORDS: *Tradescantia pallida,* Free radicals, antioxidants.

INTRODUCTION

Free radicals arise from both endogenous and exogenous sources. Immune cell activation, inflammation, ischemia, infection, cancer, excessive exercise, psychological stress, and aging are all sources of endogenous free radical production. Environmental pollutants, heavy metals (Cd, Hg, Pb, Fe, and As), some drugs (cyclosporine, tacrolimus, gentamicin, and bleomycin), chemical solvents, cooking (sausages, used fats and oils), cigarette smoke, alcohol, radiation (Willcox JK., 2004). Once these exogenous compounds enter the body, they are broken down or metabolized, forming free radicals as byproducts.

Superoxide radical (O2•−), hydrogen peroxide (H2O2), hydroxyl radical (•OH), and singlet oxygen are well-defined reactive oxygen species (ROS). They arise as by-products of metabolism in biological systems (Alugoju P., 2015). Processes such as protein phosphorylation, activation of many transcription factors, apoptosis, immunity, and differentiation all depend on the proper production and intracellular presence of ROS, which must be kept at low levels. Increased production of ROS has deleterious effects on important cellular structures such as proteins, lipids, and nucleic acids. Evidence that oxidative stress of varying significance may play a role in the development and/or progression of many diseases, including cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular disease is abundant (Gabriele P., 2017). Phytochemicals are natural molecules obtained from plants. Epidemiological and clinical evidence supports that bioactive phytochemicals (eg, B alkaloids, flavonoids, polysaccharides, terpenoids, stilbenoids, and saponins) may provide important health benefits.

Tradescantia pallida are traditionally used to improve blood circulation and prevent eye pain, in addition to their anti-inflammatory and anti-toxic potential (Ragragio EM., 2013). Huq et al. Phytochemical screening of T. pallida extracts was performed, which detected the presence of alkaloids, tannins, and carbohydrates in high, medium, and trace amounts (Huq S., 2016). Shi et al. T. pallida were found to contain two major anthocyanins with natural food coloring (Lin M., 1992). One of them was cyanidin-3,7,3'-triglucoside, which contained three ferulic acids and extra terminal glucose molecules. Therefore, the current study was designed to evaluate fresh Purple Heart leaf juice for a free radical scavenging assay based on the presence of antioxidant phytochemicals in it.

MATERIALS AND METHODS

Plant material: The fresh leaves were obtained from the campus of Raghu group of institutions. The obtained leaves are crushed and prepared the juice using motor and pestle. The freshly prepared extract was used for *in vitro* antioxidant activity.

Figure 1: Collection and preparation of Purple heart.

Chemicals and Reagents: NaH_2PO_4, Na_2HPO_4 and hydrogen peroxide, Sodium nitroprusside, Sulfanilamide, Napthyl ethylene diamine hydrochloride, Phosphoric acid were purchased from Sisco Research Laboratories, Mumbai. 1, 1 -diphenyl-2-picrylhydrazyl**,** ethanol were purchased from S.D Fine Chemicals, Mumbai.

Methods

Hydrogen Peroxide Scavenging Activity: The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch et al., 1989. The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. A solution of 43 mM H_2O_2 was prepared in 0.1M phosphate buffer (pH 7.4). The purple heart and riboflavain of different concentrations were prepared in 3.4 mL phosphate buffer were added to 0.6 mL of H_2O_2 solution (43 mM) and absorbance of the reaction mixture was recorded at 230 nm. All experiments were performed in triplicates and percent inhibition activity was calculated.

DPPH Radical Scavenging Activity: The potential of extract and riboflavin was determined on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Aliquots of 1ml of a methanolic solution containing each concentration of extract were added to 3 ml of 0.004% MeOH solution of DPPH. Absorbance at 517 nm, against a blank of methanol without DPPH, was determined after 30 min (UV, Perkin-Elmer-Lambda 11 spectrophotometer) and the percent inhibition activity was calculated (Braca et al., 2001).

Nitric Oxide Radical Scavenging Activity: Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction (Ebrahimzadeh MA et al., 2010). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and purple heart and the riboflavin in different concentrations were incubated at 25°C for 150 min. After incubation 1.5 ml of the Griess reagent (1% sulphanilamide and 0.1% naphthyl ethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546nm. The percentage of inhibition was calculated accordingly.

RESULTS

Table 1: % inhibition of Hydrogen peroxide with Riboflavin and Purple heart.

Concentrations (µg/ml)	Hydrogen peroxide	
	Purple heart	Riboflavin
20	27.685±1.005	42.19±0.01
40	29.585±0.215	44.09±0.79
80	32.705±2.775	56.20±0.60
120	40.305±3.035	58.63±2.43
160	46.530±0.190	66.53±0.37
200	50.525±0.175	68.98±0.38
IC50	**194.81**	**64.23**

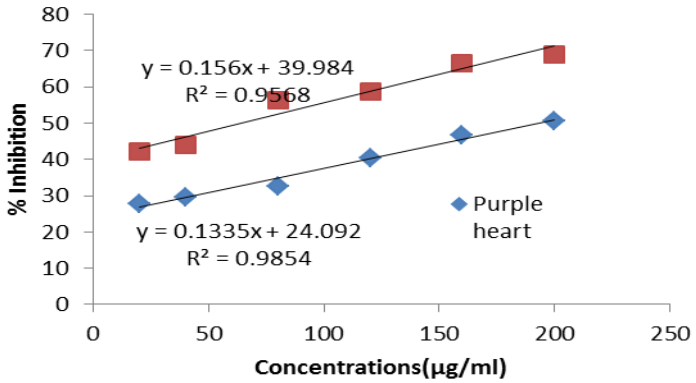

Figure 1: % inhibition of Hydrogen peroxide with Riboflavin and Purple heart.

Table 2: % inhibition of DPPH radical with Riboflavin and Purple heart.

Concentrations (µg/ml)	DPPH	
	Purple heart	Riboflavin
100	21.415±0.515	46.61±0.435
200	22.650±0.185	52.80±0.450
300	29.610±1.045	54.37±0.045
400	38.470±0.350	57.61±0.025
500	40.010±0.645	66.61±0.190
1000	53.185±0.180	76.29 ±0.215
IC50	**861.11**	**141.25**

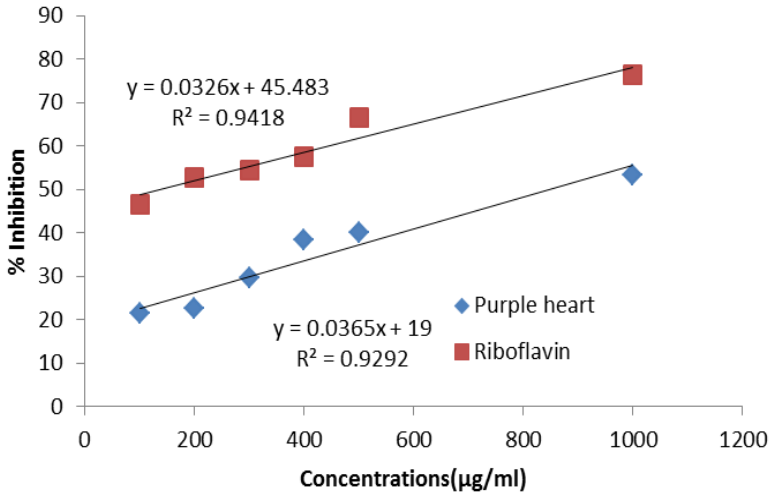

Figure 2: % inhibition of DPPH radical with Riboflavin and Purple heart.

Table 3: % inhibition of nitric oxide radical with Riboflavin and Purple heart.

Concentrations (µg/ml)	Nitric Oxide	
	Purple heart	Riboflavin
100	8.84±0.15	37.20±1.15
200	15.45±2.08	39.54±0.49
400	23.03±2.86	40.76±0.85
600	32.95±0.65	44.60±1.19
800	46.30±0.80	47.07±2.20
1000	55.35±0.55	55.78±3.27
IC50	**855.0**	**908.3**

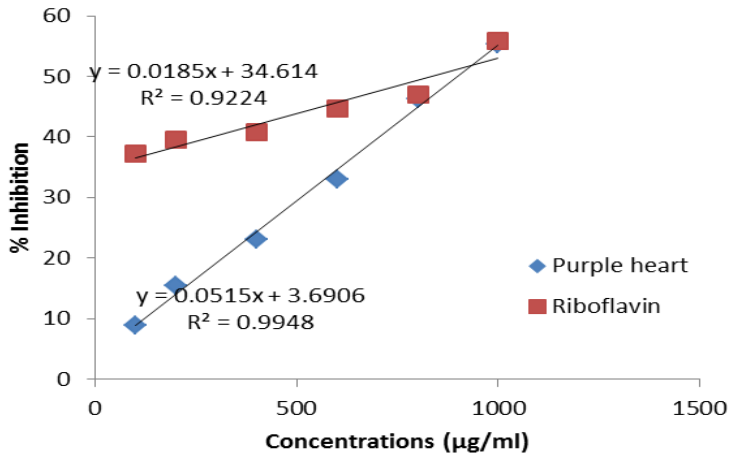

Figure 3: % inhibition of nitric oxide radical with Riboflavin and Purple heart.

DISCUSSION

Free radicals are divided into oxygen and nitrogen free radicals according to their physiological role. The excessive production of free radicals can be caused by uncontrolled stress caused by an imbalance between the body's natural defenses in the formation of stress-induced oxidative stress. Radicals are involved in cellular components and cause injury or death. The production of all kinds of free radicals is stored in normal cells, and overproduction can now be considered the main cause of many diseases (Mehdi S., 2020). Currently, there has been a huge increase in natural product research in the search for natural antioxidants as an alternative to synthetic drugs. Due to its low side effects compared to synthetic drugs. Natural antioxidants show good potential to act as therapeutic agents in their mechanism of elimination of radical chain reactions in biological systems. Growing experimental evidence suggests that these plant products influence many cellular events in terms of their free radical scavenging activity (Lobo V., 2010).

The antioxidant vitamin C has been found in animals and plants. It is not synthesized biologically and most of it can be obtained from food. Ascorbic acid, in combination with glutathione, is stored in a reduced form and supports proteins of disulfide isomerase and glutaredoxin. It acts as a reducing agent like hydrogen peroxide to neutralize free radicals. Therefore, this study was designed to evaluate the effectiveness of selected extract on stress using *in vitro* scavenging activity of hydrogen peroxide, nitric oxide, and DPPH radicals.

Hydrogen peroxide is produced *in vivo* in a disruptive reaction catalyzed by the enzyme superoxide dismutase (SOD). It is not a free radical, it can even cause cell damage at low levels (10µM), but at high levels, it inactivates cellular enzymes that produce enzymes such as glyceraldehyde-3-phosphate dehydrogenase. It can easily penetrate the membrane of biological systems, but it does not directly affect DNA, but it can damage DNA by producing hydroxyl radical (OH-) in the presence of transition metal ions (Cadenas E., 2000). Major antioxidant enzymes that can eliminate H_2O_2 include catalase, glutathione peroxidase, and peroxiredoxins. The purple heart shows inhibitory activity towards hydrogen peroxide at a concentration of 194.19 µg/ml (Table 1).

DPPH is a well-known scavenger of other radicals. DPPH radical scavenging is an acceptable way of testing the antioxidant activity of plant extracts. The reaction of DPPH with an antioxidant or reducing complex produces the corresponding hydrazine DPPH2, which can be followed by a color change from purple to yellow. Purple heart showed a comparable effect (Table 2). The effect of antioxidants on DPPH is thought to be due to their ability to donate hydrogen (Sagar B., 2011).

Nitric oxide is a small molecule produced by tissues by nitric oxide synthases (NOS) that convert L - arginine to L - citrulline NO• is a secondary messenger of guanyl cyclase and protein kinases and helps relax smooth muscle in blood vessels. Scavenging these radicals can help stop the triggering mechanisms initiated by the strong generation of NO that threatens human health. Sodium nitroprusside produces nitric oxide, which is subsequently converted into nitric and nitrous acids with soluble oxygen and water (Josiah BH., 2015). This method is based on the inhibition of nitric oxide formation from sodium nitroprusside. In the presence of antioxidants, the amount of nitric acid will be reduced and the rate of this reduction will indicate the magnitude of the effectiveness. In this study, Purple heart demonstrated scavenging nitric oxide radical scavenging activity (Table 3).

CONCLUSIONS

The antioxidant activity of Purple heart *in vitro* may be due to hydrogen donating ability or electron-donating ability. The oxygenated sesquiterpene such as spathulenol, caryophyllene oxide, β-caryophyllene and α-copaene (Renato FM.., 2022) contribute to the biological activities demonstrated by *Tradescantia pallida* and may be responsible for scavenging hydrogen peroxide, DPPH, and nitric oxide radicals depending on the concentration.

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