Research Article

Quantitative Estimation of Antioxidant Activity in Various Vegetables by DPPH Method

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ABSTRACT

The purpose of this paper is to analyse the estimation of antioxidant value in different vegetable by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay to better understand the complex interaction between dietary choices and human health. Vegetables are excellent sources of antioxidants, which are essential for battling free radicals linked to a variety of ailments. This has led to an increase in study on antioxidants and their potential benefits for health. The DPPH test is a crucial tool in these endeavours because to its dependability and simplicity. DPPH provide free radicals to react with electron of antioxidant and shows absorbance at 517nm which is highest at minimum antioxidant activity due to free radicals present in solution of DPPH and plant extract. our observation analyse that Phyllanthus (Indian gooseberry aka amla) has highest antioxidant activity around 82%. This activity followed by *Mentha spiata* (spearmint), *Zingiber officinale* (ginger), *Trigonella foenum graecum* (fenugreek), *Coriandrum sativum* (coriander), shows more than 50 % activity so these vegetables can show a promising reference for future exploration. This paper highlights the potential health consequences and highlights the protective effects of antioxidants against diseases linked to oxidative stress, underscoring the significance of eating vegetables. To fully realize the potential of vegetables in enhancing human well-being, future research topics include developing standardized protocols and exploring new antioxidants. To sum up, this paper acts as a compass for scholars and professionals, pointing them in the direction of a more thorough comprehension of the complex interactions that exist between vegetable antioxidants and human health.

Keywords: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Antioxidants as catalase (CAT), superoxide dismutase (SOD), scavenger.

INTRODUCTION

It is readily evident that since ancient times we are using plants products (i.e., leaves, fruits, shoots, and roots et al.) as much medicinal purposes. Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules (i.e., free radicals)[1]. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent which is cause invented free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves[2].

It is well known that mismanagement of reactive oxygen species generates from different metabolic cascades directly induce cancer progression It has been demonstrated that cancer cells contain oxidative stress-mediated defects in in various nucleotide repair system and mitochondrial nucleoid protection[3].

Oxidation reactions are crucial for life, they can also be damaging. Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases[4].

Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson's dementia, Alzheimer's disease, inflammatory disease, muscular dystrophy, liver disorder, and even aging[5].

There are some antioxidants in the form of micronutrients which cannot be manufactured by the body itself such as vitamin E, β-carotene, and vitamin C, and hence these must be supplemented in the normal diet[6].

Vegetables

The Superfoods for Health and Well-being. In daily means as many types of vegetables we use need (i.e., amla, tomato, onion, carrot etc.). Vegetables are rich in essential nutrients, such as vitamins, minerals, antioxidants, and dietary Fiber, that help prevent and treat various diseases, improve digestion, regulate blood pressure, enhance immunity, protect the skin, and promote overall well-being[7].

A diet rich in vegetables and fruits can lower blood pressure, reduce the risk of heart disease and stroke, prevent some types of cancer, lower risk of eye and digestive problems, and have a positive effect upon blood sugar, which can help keep appetite in check. Eating non-starchy vegetables and fruits like apples, pears, and green leafy vegetables may even promote weight loss[8].

Health benefits of vegetables

Vegetables aim to improve your overall digestive health by providing sufficient dietary Fiber, which is a type of indigestible carbohydrate that helps to pass food through the digestive system. Studies have shown that people who eat more fruits and vegetables are more likely to lose

weight. Soy and cauliflowers are effective for weight loss, whereas potatoes and corn are linked to weight gain[9].

Vegetables rich in potassium prevent the formation of kidney stones. However, people with kidney stones should limit the consumption of foods high in oxalate because of supersaturation formed by oxalate and calcium in kidney this oxalate accumulate in fruits seed and leaves so large amount ingested every day there is no use n metabolism so excrete out with kidney form complex with calcium present in kidney[10].

Potassium-rich veggies, such as beets and spinach, can help reduce the damage of a high-sodium diet, effectively lowering your blood pressure.

Two carotenoids, lutein and zeaxanthin, found in veggies, such as basil, corn, spinach, red peppers, and broccoli, help reduce the risk of age-related macular degeneration (AMD)[11].

Nearly all vegetables are full of cancer-fighting antioxidants that may reduce your risk of certain types of cancers. These veggies include cruciferous vegetables, like brussels sprouts and Brassica oleracea[12].

Vitamin C is a key nutrient that is found in many different vegetables. Due to its property of donate free electron This vitamin helps boost our immune system by mang epithelial barrier strong against pathogen; through accumulation in phagocytic cells, it can enhance chemotaxis, phagocytosis and ultimately microbial killing. It is also needed for apoptosis and clearance of the use neutrophils from sites of infection by macrophages, as this decreasing necrosis and potential tissue damage[13].

Leafy greens are full of antioxidants and folate that help reduce your risk of developing Alzheimer's disease and dementia.

Lycopene found in tomatoes can help protect your skin from sunburn. Additionally, avocados and kale can keep your skin more elastic.

DPPH(2,2-diphenyl-1-picrylhydrazyl)

The organic chemical compound 2,2-diphenyl -1-picrylhydrazyl is commonly abbreviated as DPPH. It is a crystalline powder with a dark tint that is made up of stable molecules of free radicals. Two main uses for DPPH are in laboratory research: one is as a standard for the position and strength of electron paramagnetic resonance signals, and the other is as a monitor of chemical events involving radicals, most notably as an antioxidant assay[14].

One well-known radical that acts as a "scavenger" or trap for other radicals is DPPH. As a result, the rate at which a chemical reaction slows down after DPPH is added is used to determine how radical the reaction is. The DPPH radical is deep violet in solution due to a broad absorption band that is around 520 nm; when neutralized, it turns pale yellow or colourless. This feature makes it possible to visually observe the reaction, and it is possible to determine how many starting radicals there are by counting the change in optical absorption at 520 nm or in the DPPH's EPR signal[15].

MATERIALS AND METHODS

This paper carefully looks at the techniques used in the DPPH assay, including the nuances of sample preparation, DPPH solution formulations, and important measurement parameters. To guarantee reliable and similar results, standardization is emphasized, highlighting its significance in bolstering the assay's trustworthiness.

MATERIALS

All materials were used is of A grade compound- 2,2-diphenyl -1-picrylhydrazyl, fresh leaf extracts of *zingiber officinale, chinopodium album, allium cepa, daucus carota, capsicum frutescens, solanum tubersum,citrus limon, memordicacharantia, pisum sativum, beta vulgaris, coriandrum sativum, Phyllanthus emblica, trigonellafoehum graecum, Raphanus sativus, brassica oleracea*,distilled water,smethanol and ethanol.

Vegetables we were use:

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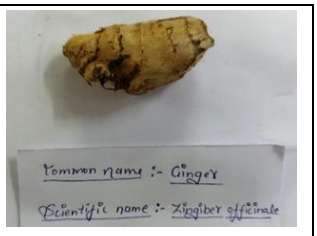
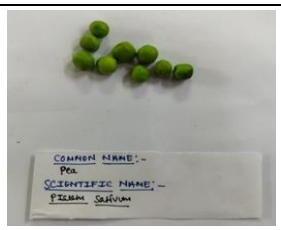
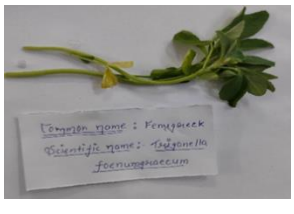
<tr><td>  </td><td>  </td></tr>
<tr><td>Fig: (a) Solanum tubersum, Potato</td><td>Fig: (b) Zingiber officinale, Ginger</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>Fig: (c) Solanum lycopersicum, Tomato</td><td>Fig: (d) Pisum sativum, Pea</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>Fig: (e) Daucus carota, Carrot</td><td>Fig: (f) Allium sativum, Garlic</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>Fig: (g) Beta vulgaris, Beetroot</td><td>Fig: (h) Trigonella foehum, Fenugreek</td></tr>
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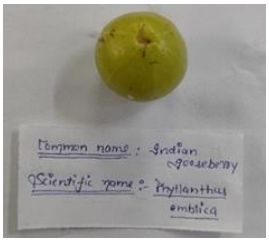	
Fig: (i) *Phyllanthus emblica,* Indian gooseberry	Fig: (j) *Mamordica charantia,* Bittergrout
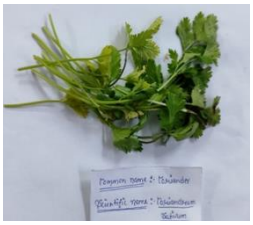	
Fig: (k) *Coriandrum sativum,* Coriander	Fig: (l) *Raphanus sativus,* Radish
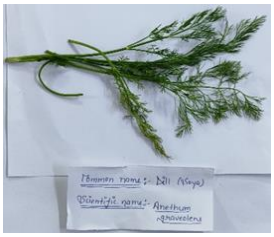	
Fig: (m) *Anethum graveolans,* Soya	Fig: (n) Mentha spiata, mint, pudina
	
Fig: (o) *Capsicum frutescans,* Green chilly	Fig: (p) *Allium cepa,* Onion
	
Fig: (q) *Brassica oleracea, Cauliflower*	Fig: (r) *Citrus limon,* Lemon

METHODS

Preparation of plant extracts:

Take fresh leaves of vegetables and checkout any kind of microbial infection, damage and distortion. Wash undamaged leaves firstly with tap water and then wipe it with ethanol to remove any kind of microbe which could not be washed off with tap water. Leave it for dried off at 37°C or room temperature. Prepare a mortar paste of all plant leaves. Filter this paste and transfer pure plant extract in 2 ml Eppendorf tube.

Prepare a stock solution of DPPH:

Dissolve 20 mg DPPH in 100 ml of methanol to prepare stock solution, 0.2 mg/ml to obtain a concentration of 0.5mM. The exact concentration may vary depending on the expected activity of the sample being tested.

Set up the control:

Prepare a control solution without the sample, using only the solvent exactly 3ml DPPH solution. This will serve as a baseline for comparison.

Prepare test solutions:

Take aliquots of 100microliter of the sample solutions in 2 ml Eppendorf tube and mix with 2.9 ml DPPH solution.

Incubation:

Vortex this solution until solution show colour changes from purple to yellow. If colour does not change immediately allow this test solution and control to incubate in the dark at room temperature for a specific period, usually around 30 minutes to allow the reaction between DPPH and the sample to occur.

Measure the absorbance:

After incubation, measure the absorbance of each test solution at a specific wavelength, typically around 517 nm, using a spectrophotometer. Also, measure the absorbance of the control solution.

Calculate the DPPH activity:

The DPPH activity of the sample can be determined by comparing the absorbance of the test solutions with the control. The decrease in absorbance indicates the scavenging activity of the sample towards DPPH radicals. Higher decreases in absorbance correspond to higher antioxidant activity

Absorbance can be calculated by using formula;

Antioxidant % scavenging activity= (Ac−As)/Ac) × 100

where: Ac—Absorbance of control; As—absorbance of each sample prepared in DPPH

Observation:

Antioxidant activity of different vegetables which we were use given in table 1

Mean and standard deviation data are calculated by standard deviation calculator present at calculator.net.

Table 1: Antioxidant activity of different samples.

S.No	Plant Scientific name	Absorbance at 517 nm	% of Antioxidant Activity
Control	DPPH solution	0.728	----------
1	*Chinopodium album*	0.462	36.53
2	*Allium cepa*	0.691	5.08
3	*Daucus carrota*	0.710	2.47
4	*Capsicum frutescens*	0.696	4.39
5	*Soalnumlycopersicum*	0.602	17.30
6	*Anethum graveolans*	0.533	26.78
7	*Solanum tubersum*	0.699	3.98
8	*Citrus limon*	0.542	25.54
9	*Mamordicacharantia*	0.590	18.95
10	*Mentha spiata*	0.201	72.39
11	*Pisum sativum*	0.732	0.549
12	*Beta vulgaris*	0.500	31.31
13	*Zingiber officinale*	0.250	65.65
14	*Coriandrum sativum*	0.323	55.63
15	*Phyllanthus emblica*	0.130	82.14
16	*Trigonella foenum graecum*	0.270	62.99
17	*Raphanus sativus*	0.549	24.58
18	*Brassica oleracea*	0.370	49.17

RESULTS AND DISCUSSION

As it is observed from above table that *Phyllanthus emblica* commonly called Indian gooseberry aka amla shows 0.130 absorbance at 517 nm which shows highest scavenging activity 82.1% to neutralize free radicals produced by DPPH in DPPH essay of quantitative estimation. Along with *Phyllanthus mentha spiata* (spearmint), *Zingiber officinale* (ginger), *Trigonella foenum graecum* (fenugreek), *Coriandrum sativum* (coriander) Shows more than 50% antioxidant activity i.e. 72.39, 65.65 ,62.99and 55.63 respectively following Phyllanthus.

In this growing world we need to depend on plant products for medicinal purpose. Most of the population currently being use plant-based medicine or herbal drug in majority of cases due to its less toxic effect and low expenditure and this dependence continuously growing in upcoming years. Since ancient ages it is known that plant have anticancer effects. Lots of unfavourable negative impacts shown by several cancer chemotherapeutic agents may have been prime inspiration for using different strategies to finding a better and safer cure for cancer one of the most preferable ways to compensate this problem is plant products.

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