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Antioxidant Capacity of Some Medicinal Leaves and Flowers of Jharkhand Using Spectrophotometer

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Abstract

Ayurvedic form of medicine is believed to be exist in India for thousands of years. Ayurvedic medication has been practiced either through diet or other techniques. Different parts of plants (leaves, flowers, bark, fruit, root) are rich in antioxidants. Hence, the extracts of leaves and flowers can be used for antioxidant assay. The DPPH, ORAC, FRAP, CUPRAC, etc. are some of the methods which can be used for the antioxidant assay. In our study, the antioxidant capacity of aqueous extract of leaves and flowers are studied using Ce (IV) and FRAP method and are compared. However, the antioxidant capacity values studied in both methods are not found to be comparable.

Keywords: Antioxidant Capacity, Reactive Oxygen Species, medicinal plant leaves, Free Radicals, Superoxide Anion.

Introduction

Any substance that has the ability to slow down or stop other molecule from oxidizing is called an Antioxidant. Superoxide (O_2^-) anion, hydrogen peroxide(H_2O_2), peroxyl (ROO^-) radical and reactive hydroxyl (OH^-) radical is the most prevalent ROS (Reactive Oxygen Species)[1]. The beginning of the development of free radicals is oxygen. For metabolism such as degradation of nutrients for development of energy and growth. Aside from these other external factors that can produce harmful free radicals include tobacco smoke[2], oil fumes[3], UV-Radiation absorbed from the sun[4], air toxins[5], and physical activity[6].

Free radicals are responsible for numerous conditions like Cancer, Coronary Artery disease, Rheumatism, Dementia, Parkinson's disease, fine lines on face, and advancing age[7]. Several scientists contend that nearly every known disease is brought on by harmful free radicals.

Luckily nature has endowed a defense mechanism against the adverse effects of free radicals and ROS with adequate protective mechanism by means of generating antioxidants in each cell. Antioxidants can either be synthesized in the body or consumed through the diet. Vegetables, fruits are very rich in antioxidants and hence their consumption has been associated with lower mortality rate and lower the chances of cancer and heart diseases[8].

Apart from the dietary sources, flowers and various parts of medicinal plants also provide antioxidants. Polyphenols are the major antioxidants found in plants and flowers[8]. The primary source of phenolics antioxidant qualities is their redox characteristics, which makes it feasible for them to perform their role as singlet oxygen suppressors, hydrogen donors, and reducing agents[9]. Flavonoids are other major antioxidants found in plants and flowers. Flavonoids have potent antioxidant activity in vitro but a number of variables constrain their antioxidant efficiency in vivo. In comparison to other antioxidants found in food like vitamin C and Vitamin E, flavonoids are not well absorbed by people[10].

Consumption of antioxidants is highly beneficial but some antioxidants show adverse effects on health. The deficiency of Iron and Zinc can be caused due to the high reducing ability of the antioxidants[11]. Moreover, people who consume high doses of phytic acid which is present in beans, lentils, corn, whole grain bread uncoated, may have deficits in calcium and iron. In the same way cabbage, beans and tea have tannins, while in the case of cocoa beans, chocolates, turnip and spinach have oxalic acid. A high diet in these hinders the digestion of minerals[11].

Many of the flowers and plants found in India have spiritual values and do also have medicinal properties. The good fragrance of flowers can reduce the mental stress and the wide range of medicinal application of plants and flowers can be seen in *Charaka Samhitha*, an early text on Ayurveda (Indian traditional medicine).

Research on antioxidants has increased considerably during the past ten years. The methods and variations for the assay of antioxidants in Chemistry are being increasing considerably. Most significant characteristics from a pharmacology and medicinal standpoint, antioxidant properties like polyphenols are used for elimination of free radicals and limiting lipid peroxidation.

Some of the methods available for antioxidant assay are ORAC, DPPH, TRAP, FRAP etc. In our study we have used Ce (IV) and FRAP method for the antioxidant assay in the below mentioned flowers and leaves of plants for the first time. The basic principle of Ce (IV) assay is to analyze the oxidization of the antioxidant with Cerium Sulphate at ambient temperature in a mildly acidic environment. The Amaranth Dye reduces the residual unreacted Ce (IV). When evaluated spectrophotometrically at 535nm, the solution takes on a pink hue due to the unreacted dye. The concentration of antioxidant has direct correlation with this. The Ferric to Ferrous reduction is also the basic foundation of the FRAP(1,10-Phenanthroline) assay.

Experimental Analysis

Apparatus

All absorbance were made using Double Beam UV-Vis Spectrophotometer (Ranchi University, Dept. of Chemistry) at a predefined wavelength using an approved spectrophotometric method using two identical Quartz Cuvettes whose thickness is 1cm. For incubation a water bath was used (GSW Water Bath rectangular, double wall, thermostatic control, Ranchi University, Dept of Chemistry) to keep temperature steady.

Reagents and chemicals

The standard antioxidant, Gallic acid was purchased from Sigma Aldrich chemical co. Cerric Sulphate Tetrahydrate was purchased from Janki Traders and Chemicals (Ranchi, India). Ethyl alcohol (92%) of AR grade was from Riedel. concentrated Sulphuric acid (99.999%, Brand: Thermofisher) and amaranth dye (Brand: Pure Chems) used was purchased from Janki traders, Ranchi.

$NH_4Fe\ (SO4)_2 \cdot 12H_2O$, 1,10-Phenanthroline and HCl purchased from Fluka which were utilized for the FRAP assay. Triple distilled water was also used for the complete experiment.

Preparation of extracts

Medicinal plants and flowers were collected from the surrounding environment and some of them were hand plucked from different districts of Jharkhand and they were identified by Department of Botany, Ranchi University. The gathered material was pulverized with a mortar and pestle for after being dried for 1.5 hrs. in hot air oven at 90℃. 50ml of double distilled water have been utilized to reflux each sample powder for 40 min. Whatman 40 filter paper was used to filter the refluxed solution, making sure that the extracts were free of contaminants and turbidity. For the both the methods, the extract solution's clear solution was appropriately diluted. For accurate results all the samples examined were fresh.

Method-1

Cerium (IV)-Amaranth Method

Standardization of Ce (IV) Solution.

At first 0.019 g of $Ce\ (SO4)_2.4H_2O$ were incorporated using 0.25 ml of concentrated sulfuric acid and for total dilution 25ml of water was added and made homogeneous with magnetic stirrer at room temperature. After being moved to 50ml volumetric flask, this solution was diluted using double distilled water to the appropriate level, yielding a final concentration to 0.8mM. The Ferroin indicator was used to standardize the prepared Cerium solution using Arsenic Trioxide[12].

General procedure

49.9 mM of Amaranth dye solution, 0.12 mM of Ce (IV) solution, and various quantities of standard antioxidant solution were added to a final volume of 10 ml of standard flask. Following thorough shaking, the reaction mixture's absorbance at 535 nm was measured in relation to the blank, which included all of the reagents except the antioxidant. Similarly, as previously mentioned, an antioxidant test was performed on an aqueous extract of medicinal plants or flowers. The wavelength at which the absorbance was measured was 535 nm[13].

Method 2

FRAP (1,10-Phenanthroline) Assay

0.190g of Ferric Ammonium Sulphate were taken and dissolved in 0.5M Hydrochloric Acid. 0.9×10^{-2} M concentration containing 1,10-Phenanthroline solution was prepared by taking suitable weight by dissolving it in water. Both these solutions were made miscellaneous in 100ml volumetric flask and diluted to 100ml using distilled water. This solution was sensitive to light; hence it should be kept in dark. In the 10 ml volumetric flask, 1,10-Phenanthroline, antioxidant solution and some amount of Ethyl Alcohol (92%) were added and diluted with distilled water. It was then kept for incubation at 60^0 C for about 40 minutes, cool to room temperature and then absorbance was measured at 515nm against a blank containing all the reagents except antioxidant.[14]

Results and discussion

In this work, an antioxidant experiment was performed to determine the potential of flowers and medicinal plant extracts to eliminate free radicals using the Cerium and FRAP methods. Natural antioxidants substance found in medicinal plants and flowers were accountable for reducing or mitigating the harmful effects of oxidative stress.

Spectral characteristics

Total antioxidant content was determined in extracts from medicinal plants and flowers. Freshly created methods, such as the Cerium method and the FRAP (1, 10-phenanthroline) method, have been applied to determine total antioxidant capacity. The two antioxidant capacity assays employed in this investigation were spectrophotometry-based. Both approaches require a redox reaction, but each has a different mechanism of action under different reaction conditions, resulting in differences in antioxidant readings.

The absorbance of colored solution was measured in a spectrophotometer containing a mixture of 0.119mM of Ce (IV), 49.9 µm amaranth dye and various concentrations of standard Gallic acid solution in the wavelength region of 400-700nm against blank in room temperature. The optimum wavelength with maximum absorption was found to be at 535 nm. The intensity of colored species at 535 nm increases as the concentration of the Gallic acid solution increases.

Optimization conditions

Since the Ce (IV) has the power to oxidize both antioxidant as well as dye solution, an order of Ce (IV) solution, antioxidant and dye solution should be followed during addition of solutions. The absorbance was taken at various temperatures. No significant variations occurred in absorbance value therefore the experiment were done at room temperature.

However, in FRAP method, absorbance greatly varies with temperature. As a result, the mixture used for the reaction went for incubation in a water bath at 60^0 C FOR 40 minutes, and the absorbance was measured after being cooled to room temperature.

Calibration curve of Gallic acid and total antioxidant capacity in flowers and medicinal plants

In spectrophotometric determinations, calibration curve was constructed for standard antioxidant Gallic acid under optimum experimental condition. A linear relationship was observed, when increase in absorbance was plotted against varying antioxidant concentration.

The calibration line of Gallic acid standard with respect to cerium method and FRAP method were drawn (Fig 1 & 2). For the evaluation of these two assay methods, we performed linear regression equation and correlation analysis. The newly developed Cerium method and FRAP method gave a correlation coefficient close to unity. The R^2 values of cerium and FRAP methods were 0.9971 and 0.9922 respectively. The linear regression equation for Gallic acid from cerium method was found to be $y = 0.0432x - 0.1174$, where x was the concentration of Gallic acid in µm/10ml, 0.1174 was the intercept and y was the absorbance of the solution.

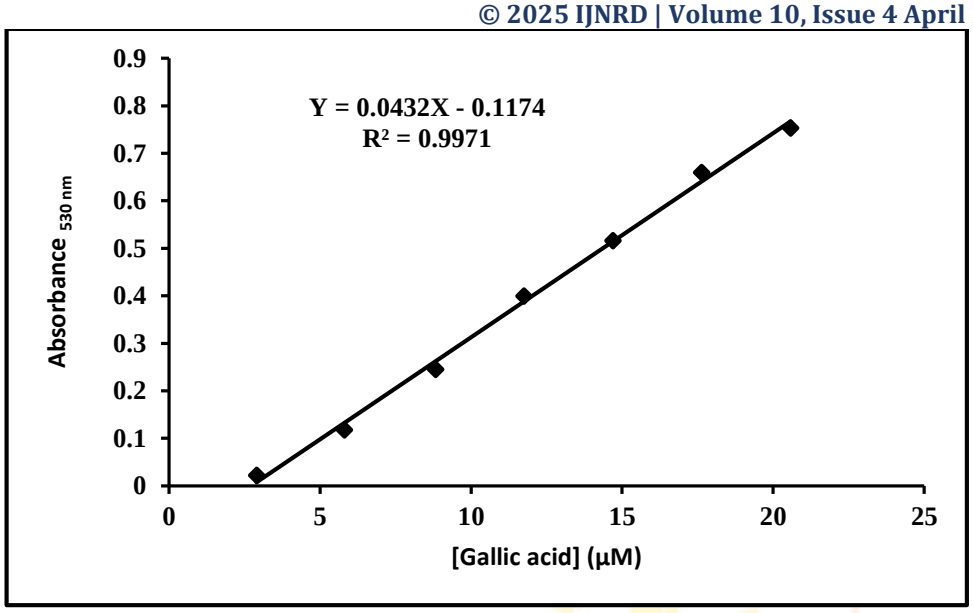

Fig 1: Gallic Acid calibration curve of Cerium-Amaranth dye assay having different concentration.

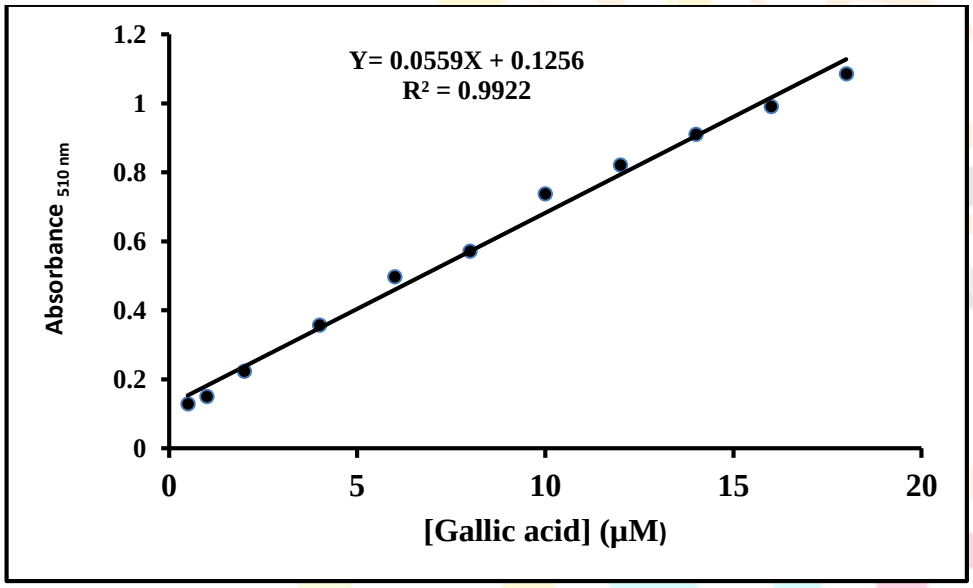

Fig 2: Gallic acid calibration curve of FRAP using different concentration.

APPLICATIONS

The total antioxidant capacity of nearly 15 flowers and 12 medicinal plants were systematically assessed. These plants and flowers were used for the first time for the determination of total antioxidant capacity using Cerium and FRAP method. The results of these two assays for antioxidant capacities determined in flowers were given in table 1. Total antioxidant capacity determined by Ce (IV) method ranges from the value of 0.03910 to 0.0046µm/GA/g and that of FRAP ranges between 0.0166 to 0.0007µm/GA/g. Out of 15 flowers selected for the evaluation of antioxidant activities, Rosaceae, Bougainvillea glabra, Crossandra Infundibuliformis and Peltoforum pterocarpum exhibited greater antioxidant capacity. Due to the presence of high content of ascorbic acid, carotenoids and flavonoids, flowers showed good antioxidant activity. Numerous secondary metabolites, including phenolic acids (like gallic acid and chlorogenic acid), flavonoids (like flavonols and anthocyanins), aromatic components (like essential oils, monoterpenes, and

sesquiterpenes), and hydrolyzable and condensed tannins (like rugosins and procyanidins), have been identified in Rosaceae[15,16,17].

. Bougainville was a thorny wood plant with different colors of flowers used for decorative purpose. They were used for cure of ulcer, diarrhea, anti-microbial activity. It can also be used to reduce blood glucose level[18-19]

Table-1

Antioxidant capacities of some of flowers determined by Cerium and FRAP methods.

Common names	Botanical names	Capacity(µmol/GA/g)	
		Ce (IV) method	FRAP
Rose	*Rosaceae chinensis*	0.03910	0.01666
Paper Flower	*Bougainvillea Glabra*	0.03706	0.01072
Firecracker flower	*Crossandra Infundibuliflorum*	0.02890	0.00554
Yellow poinciana	*Peltoforum Pterocarpum*	0.02646	0.00666
Cornacea	*Dianthus Caryophyllus*	0.01865	0.00266
Oleander	*Nerium Oleander*	0.01411	0.00414
Flamboyant	*Delonix Regia*	0.01366	0.00655
Champaca	*Magnolia Champaca*	0.01295	0.00239
Jasmine	*Jasminum Officinale*	0.01277	0.00264
Vinca rosea	*Catharanthus roseus*	0.01142	0.00188
Gladiola	*Gladiolus grandiflora*	0.00886	0.00161
Sarbara	*Gerbera Jamesonii*	0.00790	0.00078
Chrysanthemum	*Dendranthema X grandiflorum*	0.00561	0.00070
Denzia	*Denzia sp.*	0.00478	0.00149
Hibiscus	*Hibiscus rosa sinensis*	0.00466	0.00261

Table 2: antioxidant capacities of some medicinal plants determined by cerium and FRAP methods.

Common names	Botanical names	Capacity (µmol/GA/g)	
		Ce (IV) method	FRAP
Agave	*Agave tequilana*	0.032025	0.007505
Aloe vera	*Aloe vera*	0.006242	0.000571
Arka	*Calotropis gigantea*	0.052945	0.000868
Basil	*Ocimum basilicum*	0.007379	0.001297
Betel	*Piper betel*	0.007788	0.002656
Castor	*Ricinus communis*	0.040929	0.012500
Fenugreek	*Trigonella foenum-graecum*	0.023560	0.007614
Lemon	*Citrus lemon*	0.005022	0.002626
Mugwort	*Artemisia vulgaris*	0.011995	0.002935
Papaya	*Carica papaya*	0.063233	0.01019
Pongemia	*Millettia pinnata*	0.007654	0.001994
Rue	*Ruta graveleons*	0.023026	0.001627

From the assay it was found that all the samples contain antioxidants. From the Ce (IV) method, the antioxidant capacity was found to be highest for Carica papaya (0.063223) followed by Castor (0.040929).

Whereas from the FRAP method, the Ricinus communis is found to have highest capacity (0.0125) followed by papaya (0.01019). C. papaya leaves are found to have great medicinal uses. C. papaya leaves are found to contain alkaloids, flavonoids, cardiac glycosides, tannins, Anthraquinones (Free), Anthraquinones (Bound), Phlobatinins Saponins , Anthocyanosides[20]. Papaya leaves have milky sap that contains Acetogenin which is useful for preventing and killing cancer cells[21]. C. Papaya leaf tea cures thrombocytopenia and also known for its anti-inflammatory activities[22]. Ricinus communis is a cultivated shrub. Even though its seeds are poisonous due to presence of toxic proteins the castor bean oil is used in ayurvedic system of medicine. Extract of leaves of R. *comminis* is found to exhibit insecticidal activity and hence can become a potential biopesticide for economic and environmental friendly pest controle[23]. From the Ce (IV) method the least capacity was found in C. lemon (0.005022) followed by Aloevera (0.006242). whereas from the FRAP method the least capacity was found in Aloevera (0.000571) followed by Calotropis Gigantea (0.000862).

Conclusion

In this present work, a newly developed simple, inexpensive spectrophotometric method like Cerium method was used for the determination of total antioxidant capacity of simple antioxidant compounds. The obtained results of antioxidant assay were correlated to those obtained by reference method such as FRAP. All the tested flowers and medicinal plant samples possessed antioxidant activity. The advantage of this cerium method is that it did not require any high-level instrumentation, and the required instrument is a spectrophotometer. The reagents used in this method were easily available, they were stable and it was fast enough to oxidize Gallic acid, a standard oxidant used in these methods. It can be carried out at room temperature and only aqueous extractions were essential.

From methodological point of view, cerium method was recommended as easy and accurate, whereas FRAP method was time consuming, needed 60^0 C incubation for 40 minutes. From all these points of view, Cerium assay was considered to be a viable method.

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