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RESEARCH

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DETERMINATION OF ANTIOXIDANT ACTIVITY OF *HIBISCUS SABDARIFFA* L. AND *RUMEX NEPALENSIS* SPRENG.

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ABSTRACT

As plants produce significant amount of antioxidants to prevent the oxidative stress caused by free radicals and oxygen, they represent a potential source of new compounds with antioxidant activity. Now a days there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry. In India and across the world the Traditional herbal medicine has its own importance in human health care and prevention of many diseases. Ayurveda is the oldest medical system in the world, provides potential leads to find active and therapeutically useful compounds from plants .Considering the growing interest in possessing the antioxidant capacity of medicinal plants, we tried to recognize the effect of antioxidants in *Hibiscus sabdariffa* and *Rumex nepalensis*. The leaves of both taxa investigated are edible as vegetables. Thus they can be used as an easily accessible source of natural antioxidant and as possible food supplement or in pharmaceutical industry.

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KEYWORDS

Antioxidant activity, *Hibiscus sabdariffa, Rumex nepalensis*

INTRODUCTION

Free radicals are constantly generated in vivo for physiological purposes (1). They can be overproduced in pathological conditions, causing oxidative stress (2). A large number of civilization associated diseases such as autoimmune diseases, inflammation, cardiovascular-neurological diseases, cancer and aging are attributed to oxidative stress (3, 4, 5, 6, 7). An adequate intake of natural antioxidants could protect macromolecules against oxidative damage in cells (8, 9). The term antioxidant refers to free radical scavengers, inhibitors of lipid peroxidation and chelating agent (10). Phenolic compounds possess a wide spectrum of biological effects including antioxidant and free radical scavenging (11, 12).

Antioxidants help the organisms in dealing with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and causes damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) formed in vivo, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply and detoxification.

It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants. This is one of the reasons why discovery and synthesis of novel antioxidants is a major active area. In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value (13). Antioxidants derived from fruits, vegetables, spices and cereals are very effective and have reduced interference with the body's ability to use free radicals constructively (11, 14). Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids.

The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last two decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase. Many Indian plants have been investigated for their beneficial use as antioxidants or source of antioxidants using presently available experimental techniques. Numerous other plants used in Indian traditional medicine are reported to show antioxidant activity. The present work deals

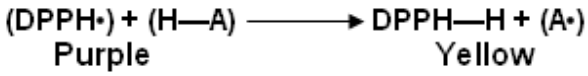
MATERIALS AND METHODS

2.1. Materials: Leaves are used to determine the antioxidant activity of *H. Sabdariffa* and *R. nepalensis.*

2.2. Methods: Quantitative antioxidant assay:-DPPH ASSAY

Working Principle: Quantitative measurements were done by modified DPPH assay (15). DPPH (1, 1-diphenyl-2-picrylhydrazy) is one of a few stable and commercially available organic nitrogen radicals and has a UV-Vis absorption maximum at 515 nm. The presence of antioxidants decolorize/reduce DPPH and extent of decolorization is a function of the concentration of antioxidant component in the respective extract. The absorbance progress of the reaction mixture is monitored at 515 nm for 30 min or until the absorbance is stable. The percentage of the DPPH remaining is calculated as $\%DPPH_{rem}$ is inversely proportional to the antioxidant concentrations, and the concentration that causes a decrease in the initial DPPH concentration by 50% is defined as $IC_{50.}$

The scavenging reaction between (DPPH•) and an antioxidant (H-A) can be written as:



Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPH-H and as consequence the absorbance decreased from the DPPH• radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (16).

2.3 **Experimental setup:** (Table 1.1)

Chemicals/reagents:

Table: 1.1
Experimental set up for DPPH assay.

	Solvent Blank	Control	1	2	3	4	5
Extract Concentration**(mg/ml)**	0	0	0.04	0.08	0.12	0.16	0.20
Vol. of Extract/ vitamin C **(µl)**	0	0	20	40	60	80	100
Methanol **(µl)**	500	0	0	0	0	0	0
Ethanol **(µl)**	1500	500	480	460	440	420	400
DPPH **(µl)**	0	1500	1500	1500	1500	1500	1500
Mix well incubate for **30 min** at R.T and measure the absorbance at **515 nm**							

1. DPPH solution: 0.001% methanolic solution
2. Extract stock solution: 1 mg/ml ethanolic solution.
3. Vitamin C solution: 1 mg/ml ethanolic solution.

DPPH and other stock solutions has been prepared and kept at 4^0 C after wrapping with aluminum foil to protect from light. Before the experiment starts, the solutions were brought into room temperature. A stock solution of the test sample is prepared with concentration of 1mg/ml of the extract in ethanol. Vitamin C (1mg/ml) is used as standard positive control. Spectrophotometer is calibrated and zero absorbance is adjusted with solvent blank. Absorptions at each concentration of both extract and standard solutions are noted and percentage of inhibition is calculated by comparing the absorbance value of the control and the test sample. A reaction mixture of same composition without the test sample was taken as the control. Samples of each concentration were tested in triplicate and the average absorption was taken. Percentage of inhibition was calculated by comparing the absorbance values of control and test samples.

$$\% \text{ Inhibition of DPPH} = \frac{A(c) - A(s)}{A(c)} \times 100$$

Where A (c) = absorbance of control, A(s) = absorbance of sample. Plot a graph between percentage inhibition and concentrations, IC_{50} value is determined as the concentration of extract corresponding to 50% inhibition. IC50, which is defined as the amount of antioxidant required to inhibit 50% of DPPH free radicals under the experimental conditions (17, 18).

RESULTS

From graph (percentage DPPH scavenging activity), in *Rumex nepalensis*, at minimum concentration of 0.04 mg/ml, leaves extract and Vit. C scavenged 13.3% and 56.52% DPPH respectively. 90.41% and 89.02% DPPH scavenging activity at maximum concentration of 0.2 mg/ml was observed for leaves and Vit.C respectively. The half maximal inhibitory concentration (IC50) values of DPPH scavenging activity of leaves extract and Vit. C was 0.11 mg/ml. and 0.14 mg/ml. for *Rumex nepalensis* and *Hibiscus sabdariffa* respectively. Results of the scavenging activity of leaves extract on DPPH are shown in Table 1.2 and Graph 1.3 and 1.4

Table 1.2
DPPH scavenging activity of leaf extract

Conc. Of extract (mg/ml)	Absorbance (515nm)		
	% scavenging activity of *Rumex nepalensis*	% scavenging activity of *Hibiscus sabdariffa*	% scavenging activity of Ascorbic acid
0.04	13.3	17.05	56.52
0.08	16.94	23.04	71.66
0.12	54.94	45.00	82.91
0.16	88.05	80.07	85.83
0.20	90.41	88.03	89.02
Absorbance of control = 0.720			

In the present investigation ethanol extract of leaves was tested for antioxidant activity. In which leaf of *Rumex nepalensis* shows IC 50 value 0.11 mg/ml and leaf of *Hibiscus sabdariffa* shows 0.13 mg/ml. It has been determined that the antioxidant effect of plant products is mainly due to radical scavenging

activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes (19). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (20). The presence of phenols in leaf extracts may be responsible for the antioxidant activity of *Rumex nepalensis* and *Hibiscus sabdariffa.*

The oil of *Hibiscus sabdariffa* belongs to the linoleic/oleic category; the global characteristics of oil suggest that it could have important industrial applications (21, 22). The acute and sub-chronic toxicity studies characterize the plant to have low toxicity which makes it safe for human consumption (23).

Fig.1.3
DPPH Scavenging activity of leaf extract of Rumex nepalensis

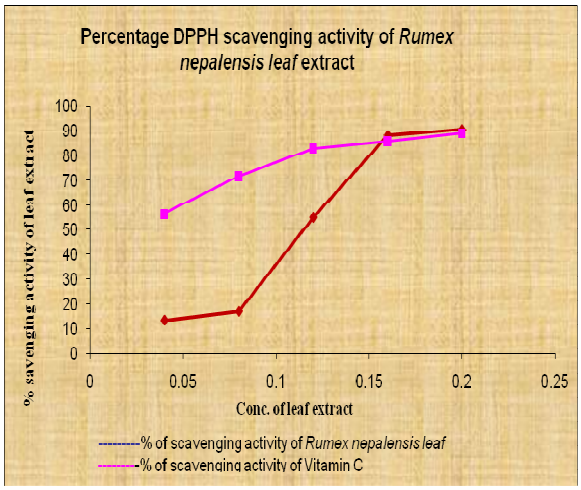
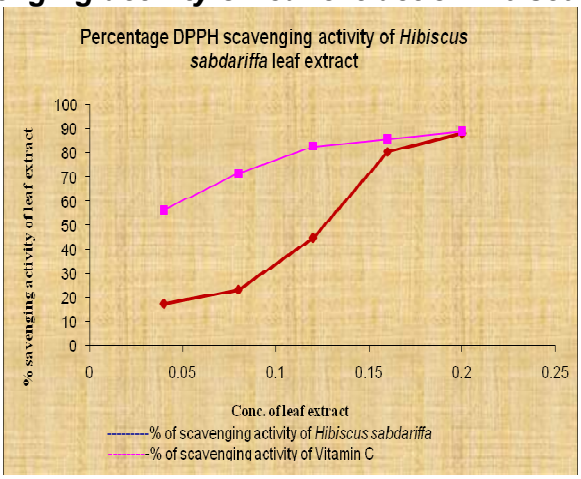


Fig. 1.4
DPPH Scavenging activity of leaf extract of Hibiscus sabdariffa



Oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders such as inflammation, viral infections, autoimmune pathologies, and digestive system disorders including gastrointestinal inflammation and ulcer (24). For instance in diabetes, increased oxidative stress which co-exist with reduction in the antioxidant status has been postulated: Oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role I n the long term complication of diabetes (25, 26). Similarly, in carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process starting with DNA damage and accumulation of genetic events in one or few cell lines which leads to progressively dysplastic cellular appearance, deregulated cell growth, and finally carcinoma (27).

Hence, therapy using free-radical scavenging antioxidants has potential to prevent, delay or ameliorate many of these disorders (28). Over the past two decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole, or their identified ingredients with antioxidant properties have substantial protective effects on human carcinogenesis (29, 27, 30,31).

Several workers have reported antioxidant activity of different plant from different parts. Significant antioxidant activity of *Hibiscus sabdariffa* and 0.33 mg/ml, IC 50 value of *Ipomoea obscura* leaves for DPPH (32). Antioxidant activity of *Tribulus terrestris, Glycyrrhiza glabra, Curcuma longa, Boswellia serrata, Tylophora indica*
are reported (33). The experimental data of these species reveal that extracts of these plants are likely to have the effect of scavenging free radical.We observe that a dose–response relationship is found in the DPPH radical scavenging activity; the activity increased as the concentration increased for each individual. The involvement of free radicals, especially their increased production, It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (p-phenylene diamine, p-aminophenol, etc.), reduce and decolourise DPPH by their hydrogen donating ability (34). appears to be a feature of most, if not all human diseases, including cardiovascular disease and cancer (35[). The leaves of both taxa investigated are edible as vegetables. Thus they can be used as an easily accessible source of natural antioxidant and as possible food supplement or in pharmaceutical industry.

CONCLUSION

From the present investigation it is concluded that, both the plant taxa having good antioxidant activity. The leaves of both taxa investigated are edible as vegetables. Thus they can be used as an easily accessible source of natural antioxidant and as possible food supplement or in pharmaceutical industry.

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