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Free radical scavenging activity of plant extracts of *Chlorophytum tuberosum B*

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ABSTRACT

Roots of Chlorophytum tuberosum are very popular and well known for its aphrodisiac, immune-modulatory and tonic properties. In this study the antioxidant effect ethanolic and aqueous extract of dried roots of Chlorophytum tuberosum Baker was evaluated by 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) radical scavenging, Nitric oxide radical scavenging assay and reducing assay methods and compared. Result indicated that ethanolic extract of the dried roots exhibited potent antioxidant activity.

Keywords*: Chlorophytum tuberosum, Antioxidant, saponin,* DPPH scavenging activity, Nitric oxide scavenging activity, reducing power etc.

INTRODUCTION

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive, oxygen-containing molecules. Types of ROS include the hydroxyl radical, the super oxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid eroxides. These free radicals may either be produced by physiological or biochemical processes or by pollution and other endogenous sources. All these free radicals are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage[1]. Reactive oxygen species [ROS], sometimes called as active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions and hydroxyl radicals (OH.) as well as non-free radical species such as hydrogen peroxide (H^2O^2)[2]. These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations[3]. Living organisms have antioxidant defence systems that protects against oxidative damage by removal or repair of damaged molecules[4]. The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS[5]. A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems[6]. The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury[7]. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in

biological systems from a wide variety of sources. These free radicals may oxi dize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [8]. Antioxidants are added to food to slow the rate of oxidation and, if used properly, they can extend the shelf life of the food in which they have been used. The protection afforded by natural products has been attributed to various phenolic antioxidants which are increasingly becoming of interest in the food industry because they retard oxidative degradation of lipids and thereby improve food quality [9].

Roots of *Chlorophytum tuberosum (*commonly known as Safed musli*) are* very popular and well known for its aphrodisiac, galactogougue, immune-modulatory and tonic properties. More than thirteen species of *Chlorophytum*, reported from India, sold as '*safed musli'* in the crude drug market. This paper reports antioxidant potential of ethanolic and aqueous extracts of roots of *Chlorophytum tuberosum* evaluated by three in-vitro methods i.e. DPPH, Nitric oxide and reducing power methods.

MATERIALS AND METHODS

Plant collection

Roots and tubers were collected from Melghat region of Amravati district of Maharashtra. Botanical identification was made from and voucher specimen was submitted in the herbarium.

Preparation of extracts

The roots of plant were thoroughly washed with tap water, dried at room temperature and transformed to coarse powder. The roots were extracted with two solvents i.e water and ethanol separately by Soxhlet extraction method. Finally, the extract was evaporated and dried under vacuum to obtain thick sticky extract. Preliminary phytochemical screening was done to identify presence of photochemical classes in both extract.

Chemicals

2-2 diphenyl-1 picryl hydrazyl (DPPH), Methanol, Sodium nitroprusside, Sulphanilamide, Potassium ferricyanide, Trichloroacetic acid, Ascorbic Acid, Ferric chloride, N-(1- naphthyl) ethylenediamine dihydrochloride) and all other reagents were of analytical grade.

Instrument

UV- spectrophotometer (Systronic double beam-UV-2201)

DPPH radical scavenging assay

The antioxidant activity of the ethanolic and aqueous extracts of dried roots of the plant *Chlorophytum tuberosum* was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH carried out by using the method of Molyneux [10]. About 1 ml of 100 µM DPPH solution in methanol, equal volume of the extract in methanol of different concentrations of the extract in methanol was added and incubated in dark for 30 min and 1ml of methanol served as control. The change in colour was observed in terms of absorbance using a spectrophotometer at 517 nm. The different concentrations of ascorbic acid were used as reference compound. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging activity was measured spectrophotometrically according to the method described by Govindharajan *et al*. [11] When sodium nitroprusside was mixed with aqueous solution at physiological pH, suddenly it generates nitric oxide, which reacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Nitric oxide scavengers compete with oxygen leading to reduced production of nitrite ions. About 1 ml of Sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4, 0.1 M) was mixed with different concentrations of the *ethanolic and aqueous* extract (200 - 1000 µg/ml) in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25°C for 2 h. After incubation 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride]. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-(1- naphthyl) ethylenediamine dihydrochloride) was measured spectrophotometrically at 546 nm. Control tube was maintained with all chemicals excluding *Chlorophytum*

tuberosum extract. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Reducing power assay

The reducing power was determined according to the method of Berker *et al [12]*. The ethanolic and aqueous extract (100-500 µg/ml, 2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50ºC for 20 min. After the addition of 2.5 ml of 10% trichloroacetic acid the reaction mixture was centrifuged at 3000 rpm for 10 min. About 5 ml of the upper layer was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power.

RESULTS AND DISCUSSION

***In vitro* free radical scavenging assays**

Ethanolic extract showed presence of saponins, alkaloids and phenolic compounds. Aqueous extract showed presence of sugars and saponins. The ethanolic and aqueous extract of *Chlorophytum tuberosum* was investigated for the effects on the *in vitro* generation of free radicals and antioxidant profile. The results of the study showed that the maximum extent inhibition of free radical generation and maximum amount of antioxidant capacity, identification of nature of the active principle and The ethanolic and aqueous extract of *Chlorophytum tuberosum* was analyzed using antioxidant profile against a battery of oxidant moieties that included radicals like DPPH, NO, OH and reducing power assay.

DPPH radical scavenging assay

In the free radical scavenging assays, DPPH is stable and possesses a distinctive absorbance at 517 nm, which significantly decreases on exposure to radical scavengers by donating a hydrogen atom to become a stable diamagnetic molecule. DPPH radical has certain advantage of being unaffected by side reactions *i.e.,* enzyme inhibition and metal chelation [13]. The principle of the reduction of DPPH free radical is that the antioxidant reacts with the stable free radical DPPH and converts it to 1, 1- diphenyl-2-picryl hydrazine [14]. The reducing capacity of extract may be serving as a significant indication of its probable antioxidant capacity and the reducing properties of antioxidants are generally associated with the presence of reductones, e.g., ascorbic acid [15].

DPPH free radical compound has been widely used to test the free radical scavenging ability of the ethanolic and aqueous extract of *Chlorophytum tuberosum* the antioxidant present neutralizes the DPPH by the transfer of an electron or hydrogen atom. The reduction capacity of DPPH could be determined by colour changes from purple to yellow by read at 517nm. The ethanolic and aqueous extract of *Chlorophytum tuberosum* demonstrated H-donor activity in our study. The DPPH radical scavenging activity of extracted material was detected and compared with standard antioxidant - vitamin C. The extract of *Chlorophytum tuberosum* tested against DPPH stable radicals spectrophotometrically which reveals that the radical scavenging activity of ethanolic extract possessed excellent antioxidant capacity by increased with the increasing concentration of the extract as compared to aqueous extract. At a concentration of 25, 100 and 200 µg/ml of ethanolic extract the percentage of inhibition was found to be 19.58, 49.65 and 85.98% respectively while aqueous extract showed percentage of inhibition 13.51, 39.34 and 70.85% respectively. However, the scavenging activity of ascorbic acid at the same concentration was 33.12, 71.12, and 97.79%. The IC50 values of Standard ascorbic acid, ethanolic and aqueous extract of *Chlorophytum tuberosum* was found at the concentration of 85.61µg/ml, 109.17µg/ml and 132.62 µg/ml.

Nitric oxide radical scavenging assay

Nitric oxide is an unstable free radical involved in many biological processes which is associated with several diseases. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be toxic and inhibition of over production is an important goal [16]. The ethanolic extract of *Chlorophytum tuberosum* effectively reduced the generation of nitric oxide from sodium nitroprusside. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. The absorbance of the chromophore was measured at 546 nm in the presence of the extract. At a concentration of 25, 100 and 200 µg/ml of ethanolic extract the percentage of inhibition was found to be 26.30, 55, and 86.31% respectively while aqueous extract showed percentage of inhibition 15.96, 42.42, and 70.97%. However, the scavenging activity of ascorbic acid at the same concentration was 33.03, 69.23, and 95.55%. The IC50 values of Standard ascorbic

acid, ethanolic and aqueous extract of *Chlorophytum tuberosum* was found at the concentration of 88.81µg/ml, 106.38µg/ml and 132.97µg/ml.

Reducing power assay

To find the active species which is capable of donating hydrogen and subsequently its leads to the reducing power activity was determined. The high reducing power is indicative of the hydrogen donating ability of the active species present in the extract. Antioxidant potential of *Chlorophytum tuberosum* ethanolic and aqueous extract was estimated by using potassium ferric cyanide reduction method. In the present study, the reducing power of the ethanolic extract of *Chlorophytum tuberosum* was found to be excellent and steadily increase in direct proportion to the increasing concentrations of the extract. The absorbance reducing power of ethanolic extract at 25, 100 and 200 µg/ml concentration was found to be 0.035, 0.072 and 0.107 respectively, which was relatively more pronounced than that of aqueous extract at 25, 100 and 200 µg/ml concentration was found to be 0.027, 0.052 and 0.089 respectively. As compared to standard ascorbic acid at 25, 100 and 200 µg/ml concentration was found to be 0.028, 0.075, and 0.169 respectively.

Table 1: Results of *in-vitro* antioxidant activity by DPPH method

Sr.no	Concentration (µg/ml)	(% Inhibition)		
		Ascorbic Acid	Ethanolic Extract	Aqueous Extract
1	25	33.12 ± 0.21	19.58 ± 0.03	13.51 ± 0.43
2	50	49.28 ± 0.34	32.88 ± 0.04	18.37 ± 0.54
3	75	63.36 ± 0.52	42.43 ± 048	27.76 ± 0.21
4	100	71.12 ± 1.23	49.65 ± 0.75	39.34 ± 0.32
5	125	79.13 ± 0.05	58.76 ± 0.32	48.04 ± 0.40
6	150	90.63 ± 0.04	69.83 ± 0.43	60.76 ± 0.54
7	175	93.38 ± 0.07	74.89 ± 0.62	65.87 ± 0.44
8	200	97.79 ± 0.04	85.98 ± 0.92	70.85 ± 0.65

Table 2: Results of *in-vitro* antioxidant activity by Nitric oxide method

Sr. No	Concentration (µg/ml)	(% Inhibition)		
		Ascorbic Acid	Ethanolic extract	Aqueous extract
1	25	33.03 ± 0.65	26.30 ± 0.67	15.96 ± 0.55
2	50	47.33 ± 0.54	38.45 ± 0.33	25.63 ± 1.93
3	75	59.89 ± 1.66	45.20 ± 0.87	33.96 ± 0.54
4	100	69.23 ± 0.87	55.00 ± 0.49	42.42 ± 0.87
5	125	74.26 ± 0.05	58.07 ± 0.31	48.07 ± 0.43
6	150	85.63 ± 0.65	67.85 ± 1.76	55.97 ± 0.23
7	175	91.56 ± 0.32	78.68 ± 0.92	62.54 ± 0.12
8	200	95.55 ± 0.47	86.31 ± 0.82	70.97 ± 0.17

Table 3: Results of *in-vitro* antioxidant activity by reducing power method

Sr.no	Concentration (µg/ml)	Absorbance		
		Ascorbic acid	Ethanolic extract	Aqueous extract
1	25	0.028 ± 0.23	0.035 ± 0.43	0.027 ± 0.22
2	50	0.037 ± 0.14	0.046 ± 0.66	0.035 ± 0.43
3	75	0.058 ± 0.71	0.058 ± 0.75	0.043 ± 0.33
4	100	0.075 ± 0.18	0.072 ± 0.32	0.052 ± 0.63
5	125	0.089 ± 0.27	0.078 ± 0.21	0.063 ± 0.52
6	150	0.098 ± 0.92	0.091 ± 0.18	0.076 ± 0.19
7	175	0.135 ± 0.34	0.096 ± 0.19	0.081 ± 0.62
8	200	0.169± 0.55	0.107 ± 0.32	0.089 ± 0.43

CONCLUSION

From the above result, it is concluded that both aqueous and ethanolic extracts of roots of *Chlorophytum tuberosum* showed potent antioxidant activity. But ethanolic extract showed more antioxidant activity as compared to water extract and standard ascorbic acid. The antioxidant activity of both extract might be due to the presence of chemical constituent/s like saponins, phenolic compounds and alkaloids in roots. So there is further need to isolate and study antioxidant chemical constituent/s from the roots.

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