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ASSESSMENT OF ANTIOXIDANT PROPERTY OF *QUISQUALIS INDICA*

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ABSTRACT

The methanolic crude extracts of *Quisqualis indica* flower was used for their free radical scavenging properties using ascorbic acid as standard antioxidant. The antioxidant activities of *Quisqualis indica* flower was involved in three antioxidant activity assays based on their different mechanisms, namely DPPH, superoxide radical and Radical cation (ABTS+). The study reveals that the *Quisqualis indica* flower have several beneficial effects by virtue of their antioxidant activity.

Key words: *Quisqualis indica*, Antioxidant, Free Radicals.

INTRODUCTION

Antioxidants are one such substance, which have the capability to neutralize free radicals or their actions. The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades in hope of finding an efficient remedy for several present-day diseases and as means to delay aging symptoms [1]. Free radicals, which have one or more unpaired electrons, are produced during normal and pathological cell metabolism. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves.

Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA stand breaking [2]. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There is a plethora of plants that have been found to possess strong antioxidant activity [3].

Free radicals cause damage to lipids, proteins, enzymes and nucleic acids leading to cell or tissue injury implicated in the process of aging as well as in wide range of degenerative or pathological processes, such as coronary heart diseases, Alzheimer's disease, neurodegenerative disorders atherosclerosis, cataract, inflammation, brain dysfunction, connective tissue disorders, physical injury, acquired immunodeficiency syndrome, stroke, diabetes, cancer, Parkinson's diseases, multiple sclerosis, Down's syndrome, viral infections and digestive system disorders such as gastrointestinal inflammation and ulcer [4-6].

Recently, there has been growing interest in natural antioxidants of plant origin because they have greater application in the food industry for increasing the stability and shelf life of food products. Moreover, they also used as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention [7]. Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to nonradical forms and function as natural antioxidants in human body.

Recently, there are numerous methods that have been developed to evaluate antioxidant activities of compounds and of complex mixtures such as plant extract [8]. Despite the existence of these various methods, just one procedure cannot identify all possible mechanisms characterizing an antioxidant activity [9].

In the present study the methanolic extract of the flower *Quisqualis indica was* employed for the assessment of antioxidant property since there is no work are available in the antioxidant property of these flowers.

MATERIALS AND METHODS

Preparation of flower extracts

The flower powder of *Quisqualis indica* was (1 gram) successively extracted with methanol (10ml) and the extract was filtered, concentrated using vacuum distillation and the said extract were used for the assessment of antioxidant property.

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DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the nonradical form DPPH-H [10].

The free radical scavenging activity of the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method [10]. Briefly, an 0.1mm solution of DPPH in methanol was prepared, and 1ml of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (100, 200, 400, 800 and 1600µg/ml).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbances were measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Superoxide radical scavenging activity

Superoxide anion scavenging activity was measured according to the method of Robak [11] with some modifications. All the solutions were prepared in 100mM phosphate buffer (pH 7.4)1ml of reduced Nicotinamide adenine dinucleotide (NADH, 468 µm) 3ml of HEPD solution of different concentration (100, 200, 400, 800 and 1600µg/ml) were mixed. The reaction was initiated by adding 100ml of phenanzine methosulphate (PMS, 60µm). The reaction mixture was incubated at 25°C for 5 min, followed by measurement of absorbance at 560nm and the percentage inhibition was calculated by using the following equation

Superoxide radical scavenging activity = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the test were performed in triplicates and the results were averaged

Antioxidant Activity by Radical Cation (ABTS.+)

ABTS assay was based on the slightly modified method of Re [11]. ABTS radical cation (ABTS.+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS.+ solution was diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100µL of sample or trolox standard to 3.9 ml of diluted ABTS.+ solution, absorbance was measured at 734 nm by Genesis 10s UV-VIZ (Thermo scientific) exactly 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Table 1. shows the assessment of antioxidant property of methanolic flower extract

Concentration (µg/ml)	Percentage of activity (±SD)					
	DPPH radical scavenging activity		Superoxide radical scavenging activity		Radical cation (ABTS+) scavenging activity	
	Quisqualis indica	Standard (Ascorbic acid)	*Quisqualis indica*	Standard (Ascorbic acid)	*Quisqualis indica*	Standard (Ascorbic acid)
100	29.34±0.098	26.55±0.065	28.45±0.067	39.44±0.065	65.35±0.083	49.65±0.028
200	34.67±0.087	34.54±0.076	32.57±0.056	46.35±0.037	73.99±0.074	54.12±0.038
400	40.34±0.076	47.63±0.087	38.34±0.045	52.37±0.016	78.46±0.075	59.47±0.047
800	49.11±0.065	54.22±0.098	42.11±0.023	64.34±0.028	81.78±0.074	66.34±0.056
1600	52.38±0.054	69.56±0.009	47.64±0.012	72.87±0.037	86.11±0.036	72.89±0.028
IC 50	**40.92**	**44.99**	**38.76**	**49.69**	**54.22**	**39.28**

RESULT AND DISCUSSION

Free radical assay is one of the most widely used methods and has become routine in establishing the antioxidant activity of herbal extracts and phytochemicals. Hydrogen donating ability is an index of primary antioxidants [13]. The antioxidant activities of *Quisqualis indica* flower was involved in three antioxidant activity assays based on their different mechanisms, namely DPPH, superoxide radical and Radical cation (ABTS+).

29.34 to 52.34% of DPPH scavenging activity was found in *Quisqualis indica* in all the concentration increased from 100µg/ml to 1600µg/ml the scavenging activity. The electrons become paired off and the solution loses colour stochiometrically depending on the number of electrons

taken up [10]. The scavenging activity towards the superoxide radical is measured in terms of inhibition of generation of O_2^- The superoxide radical reduces NBT to a blue coloured Formosan that is measured at 560 nm [14]. As far as the superoxide radical scavenging activity was concerned *Quisqualis indica* showed 21.54% (100 µl) to 41.55% (1600 µl).

The ranking of the three antioxidant assessments conducted were similar to the trend of total phenolic and flavonoid contents. Phenolic compound have been reported to defend the plant against microorganisms and herbivores. The decolorization of $ABTS^+$ cation radical is an unambiguous way to measure the antioxidant activity of phenolic compounds [15]. The results of the present study indicated that the *Quisqualis indica* revealed appreciable radical scavenging activity at a range from 65.35 to 86.11% (100-1600 µg/ml).

David Campos [16] reported that the high antioxidant capacity among tubers might be due to the maximum content of phenols, anthocyanin and carotenoids components. The hydroxyl radical scavenging activity is measured as the percentage of inhibition of hydroxyl radicals generated in Fenton's reaction mixture [17]. The decomposition of H_2O_2 by hydro-alcoholic extract of EN may at least partly result from its antioxidant and free radical scavenging activity [13].

CONCLUSION

The study concluded that the flowers *Quisqualis indica* possess significant antioxidant activity compared to other flowers and other well characterized, standard antioxidant systems, *in vitro* and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants, which might be due to the presence of alkaloids, tannins, flavonoids and saponin. Further studies are warranted for the isolation and characterization of antioxidant components and also *in vivo* studies are needed for understanding their mechanism of action as an antioxidant in a better manner.

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