Available online on 15.06.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

In-vitro Antioxidant activity assay of *Habenaria longicorniculata* J. Graham wild medicinal tubers

BN Satish [1], Mallya Suma V [2]*

[1] PhD Scholar, Department of Dravyagauna, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, 574118

[2] Associate Professor, Department of Dravyagauna, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, 574118

ABSTRACT

About: *Habenaria longicorniculata*J.Graham are wild medicinal orchids with immunomodulatory and rejuvenating properties. Hence it has been tested to evaluate its antioxidant property. **Materials and methods:** Tubers were collected from Western-ghats during flowering season, shade dried, powder prepared and used for further study. In vitro antioxidant activity for DPPH, Nitric oxide, Hydroxyl radicals and inhibitory activity for Hydrogen peroxide was planned as per standard protocol. **Results:** The antioxidant activity property of *H. longicorniculata* J. Graham tuber extract exhibited the IC_{50} value for DPPH >1000, for NO and OH >5000 and inhibitory activity for H_2O_2 68.6189 respectively. Thus tubers of test drug proved to be potent H_2O_2 inhibitor.

Keywords: *Habenaria longicorniculata*J.Graham, In vitro antioxidant, H_2O_2

Article Info: Received 28 April 2019; Review Completed 29 May 2019; Accepted 01 June 2019; Available online 15 June 2019

Cite this article as:

BN Satish, Mallya SV, *In-vitro* Antioxidant activity assay of *Habenaria longicorniculata* J. Graham wild medicinal tubers, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):342-344 **http://dx.doi.org/10.22270/jddt.v9i3-s.2851**

***Address for Correspondence:**

Mallya Suma V, Associate Professor, Department of Dravyagauna, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, 574118,

INTRODUCTION

*Habenaria longicorniculata*J.Graham are wild medicinal orchids found flowering during rainy season[1]. The underground tubers are used in traditional system of medicine in cancer treatment as rejuvenator. These are said to be used in place of *ashtavarga dravya*, the eight immuno-modulators which are endangered now[2]. The tubers are said to be rejuvenators, and rich with nutritional matter and trace elements. The drug with rejuvenating properties are said to be *Rasayana* , that which prevent ageing, re-establish youth, prevent diseases, and strengthen life as per *Ayurveda* pharmacology and its has been observed that many *Rasayana* drugs possess antioxidant property[3].

Antioxidants may be defined as any substance, when present at low concentrations compared with that of an oxidizable substrate, that significantly delays or prevents oxidations of that substrate[4]. Hydroxyl and oxygen are the highly reactive atoms that are capable of becoming part of potentially damaging molecules commonly called “free radicals[5].”Free radicals are capable of attacking the healthy cells of the body, causing them to loose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction[6]. It has been observed that population is crazy behind natural antioxidants, which prevent above mentioned diseases, with many healthy additives to life[7].

Hence with all these background a plan has been made to record antioxidant profile of these wild tubers.

MATERIALS AND METHODS

Tubers of *Habenaria longicorniculata*J.Graham were collected from Western-ghats during flowering season. Samples were cleaned properly from extraneous matter, authenticated, tubers separated from whole plant, shade dried, powder prepared and used for further study.

Antioxidnat activity:

In vitro antioxidant activity for DPPH, Nitric oxide, Hydroxyl radicals and inhibitory activity for Hydrogen peroxide was planned as per standard protocol. The antioxidant activity of tuber extract for DPPH at 1000 to 6.25µg/ml, Nitric oxide and Hydroxyl radical at 5000 to 312.5µg/ml and inhibitory activity for H_2O_2 at 1000 to 62.5µg/ml will be performed[8].

Antioxidant activity for DPPH assay

In the test and test blank 0.01ml of different concentration of tuber extract or standards were taken, in control and control blank 0.01ml of DMSO was taken in place of test sample. To the test and control 0.2 ml of DPPH was added whereas to the test blank and control blank 0.2ml of methanol was added in place of DPPH. All the tubes were incubated at 37^{0}C for 30 minutes. After incubation 0.1ml of reaction mixture was pipetted out to microtitre plate. Absorbance was measured in ELISA reader at 490 nm and values were recorded. Same procedure was repeated for standard by replacing test sample with standard. Test and control were performed in triplicate and test blank and control blank were conducted in singlet[9].

Antioxidant activity for Nitric oxide radical

In the test 0.1ml of tuber extract was taken and to this 0.4ml of 10mM Sodium nitroprusside, 0.1ml of PBS was added. For control and control blank 0.1ml of DMSO was taken in the place of test. For test blank and control blank 0.4ml of distilled water was added in place of Sodium nitroprusside. The reaction mixture was incubated at 25^{0}C for 150 minutes. After incubation 50µl of reaction mixture was transferred to microtiter plate and to this reaction mixture 0.1ml of Sulphanilic acid was added and kept for 5 minutes. Then 0.1ml of NEDD was added to all and allows it to stand for 30 minutes in diffused light. A pink colored chromophore was formed according to the concentration of the test samples. Same procedure was repeated for standard by replacing the test sample with standard. Test and control were performed in triplicate and test blank and control blank were conducted in singlet. An antioxidant activity was determined by measuring the pink colored chromophore released from NEDD at 540nm in ELISA reader[10].

Antioxidant activity for Hydroxyl radical

In test and test blank 0.1ml of tuber extract was taken and to the control and control blank 0.1ml of DMSO was taken in the place of test. For test and control 0.1ml of Iron-EDTA, 0.05ml of EDTA and 0.05ml of 0.22% of Ascorbic acid was added. To the test blank and control blank in place of all reagents distilled water was added. To all the eppendorf's tube 0.1ml of 0.85% DMSO was added. The reaction mixture was incubated at 80^0-90^{0}C for 15 minutes. After incubation, 0.3ml of Nash reagent was added to test and control. In order to stop the reaction 0.1ml of 17.5% TCA was added to the test and control. To the test blank and control blank 0.4ml of distilled water was added and kept at room temperature for 15 minutes.100µl of reaction mixture was transferred to microtiter plate and the reaction mixture was turned to yellow color according to the concentration of the test samples. Same procedure was repeated for standard by replacing test sample with standard. Test and control were performed in triplicate and test blank and control blank were conducted in singlet. An antioxidant activity was determined by measuring at 405nm in ELISA reader[11].

Inhibitory activity for Hydrogen peroxide

In test and test blank 0.5ml of tuber extract was taken and to the control and control blank 0.5ml of distilled water was taken in the place of test. For test and control 0.6ml of $H_2O_2$was added. To the test blank and control blank in place of H_2O_2, phosphate buffer was added. The reaction mixture was incubated at 27^{0}C for 15 minutes. After incubation, the absorbance was measured at 260nm using spectrophotometer and record the values. Same procedure was repeated for standard by replacing test sample with standard[12].

RESULTS

Antioxidant activity of ethanolic extract of test drug by in vitro DPPH Assay did not show antioxidant potential and it has the scavenging capacity of >1000. (Table 1)

Antioxidant activity of H. longicorniculata J. Graham extracts by in vitro Nitric oxide Assay did not show antioxidant potential and it has the scavenging capacity of >5000 µg/ml. (Table 2)

Antioxidant activity of test tuber extracts by in vitro Hydroxyl radicals Assay did not show antioxidant potential and it has the scavenging capacity of >5000 µg/ml. (Table 3)

Antioxidant activity of tuber extracts by in-vitro Hydrogen peroxide assay showed antioxidant potential and it has the scavenging capacity of 68.6189µg/ml. (Table 4 & Figure 1)

Table 1: The antioxidant activity of *H. longicorniculata* J. Graham extract for DPPH radical

S. No	Test Substance	IC_{50} (µg/ml)
1	Tuber powder	>1000

Table 2: The antioxidant activity of *H. longicorniculata* J. Graham extract for Nitric oxide radical

S. No	Test Substance	IC_{50} (µg/ml)
1	Tuber powder	>5000

Table 3: The antioxidant activity of *H. longicorniculata* J. Graham extract for Hydroxyl radical

S. No	Test Substance	IC_{50} (µg/ml)
1	Tuber powder	>5000

Table 4: The inhibitory activity of *H. longicorniculata* J. Graham extract for H_2O_2

S. No	Test Substance	IC_{50} (µg/ml)
1	Tuber powder	68.6189

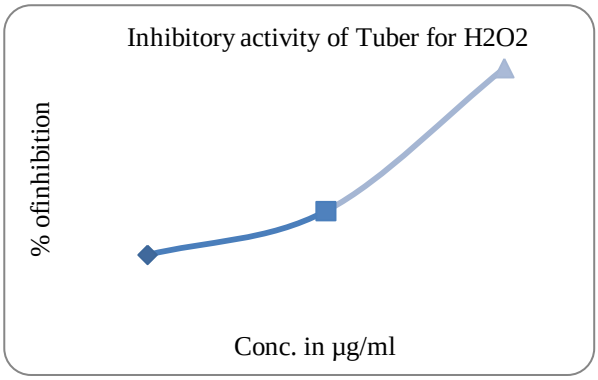

Figure 1: Graphical representation of H_2O_2inhibitory activity of *H. longicorniculata* J. Graham extract

DISCUSSION AND CONCLUSION

Habenaria longicorniculata J. Graham from family *Orchidaceae*, are tuberous herbs, endemic to peninsular India. The underground tubers are widely used by traditional physicians in the treatment of malignancy as a

rejuvenator and these also found to be admixed with *Ashtavarga dravyas* in *Ayurveda*[1]. Natural antioxidants derived from herbs can prevent oxidative stress. Free radicals are an atom or molecule that bears an unpaired electron and is extremely reactive, capable of engaging in rapid change reaction that destabilize other molecules and generate many more free radicals. Free oxygen radicals play a cardinal role in the etiology of several diseases like cancer, arthritis, atherosclerosis etc. The oxidative damage to DNA may play a vital role in aging[13]. Free radicals are generated in the human body when oxidation occurs during aerobic respiration.

In-vitro Antioxidant activity of ethanolic extract of test drug was performed through DPPH, Nitric oxide, Hydroxyl radicals and Inhibitory activity for Hydrogen peroxide. The antioxidant activity for DPPH at 1000 to 6.25µg/ml, Nitric oxide and Hydroxyl radical at 5000 to 312.5µg/ml and inhibitory activity for H_2O_2 at 1000 to 62.5µg/ml was performed. The antioxidant activity property of *H. longicorniculata* J. Graham tuber extract exhibited the IC_{50} value for DPPH >1000, for NO and OH >5000 and inhibitory activity for H_2O_2 68.6189 respectively. Thus tubers of test drug proved to be potent H_2O_2 inhibitor.

REFERENCES

1. Satish BN, Mallya Suma V, Prabhu S; Authentication parameters of *Habenaria longicorniculata* J. Graham; medicinal orchid used in Ayurveda; Arya vaidyan, 2018; XXXI(3):31-40
2. Dangat et al., Inorganic status of tubers of Habenaria longicorniculata J. GRAHAM; Asian J pharm Clin Res, 2014; 7(2):172-173.
3. BN Satish, Mallya Suma V., Differentiation and authentication of medicinal orchids of Habenaria species using RAPD- a molecular profile; WJPR, 2018; 7(17):886-92
4. Sharma RK, Arora R, Herbal drugs A twenty first Century Perspective, Jaypee Brothers Medical Publishers(P)Ltd. Ist edition 2006, pp, 426-30
5. Mallya Suma V., Potent antioxidant from a traditional herbal heritage; Journal of Biological &Scientific opinion; volume. 2015; 3(2):57-60
6. Vaijanathappa J, Badami S, Bhojaraj S, In Vitro antioxidant activity of enicostemmaaxillare. J. Health Science. 2008; 54:524 – 528.
7. Repetto M.G., Llesuy S.F., Antioxidant properties of natural compounds used in popular medicine for gastric ulcers; Braz J Med Biol Res, May 2002; 35(5):523-534
8. Farrukh AQIL, Iqbal AHMAD, Zafar MEHMOOD, Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants; Turk J Biol 30 (2006) 177-183
9. Nabavi S.M., Ebrahimzadeh M.A., Nabavi, S.F. and Jafari, M. *Free radical scavenging activity and antioxidant capacity of EryngiumcaucasicumTrautv and Froripiasubpinnata.* Pharmacologyonline. 2008b; 3:9 – 25
10. Krishnayya D, Srabatly R, Nityanadam R, A review of the antioxidant potential of medicinal plant species; Food and bioproducts processing; 2001; 89(3):217-233
11. Vijayarajan M. and Rajasekara Pandian, M. Antioxidant and free radical scavenging activity of *MoringaConcanensis*root bark extract, International journal of Zoology and Applied Biosciences, 2016; 1:46-56.
12. Ali M, Seyed Mohammad E, Nabavi, Seyed Fazel Nabavi., FatemehBahramian and Ahmad, Reza, Bekhradnia. *Antioxidant and Freeradical scavenging activity of H.Officinalis L. VAR. Angustifolius, V. Odorata, B. Hyrcana and C.Speciosum. Pak.J.Pharm. Sci.,* 2010; *23(1):29 – 34*
13. Badakhshan Mahdi-Pour etal., Antioxidant activity of methanol extracts of different parts of Lantana camara; APJTB, 2012; 2(12):960-65