INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

FREE RADICAL SCAVENGING AND ANTIOXIDANT POTENTIAL OF DIFFERENT EXTRACTS OF *COLOCASIA GIGANTEA* (BLUME) HOOK. F. *IN VITRO*

Nambam Bonika Devi, Ganesh Chandra Jagetia *

Department of Zoology, Mizoram University, Tanhril, Aizawl, Mizoram, India

*Corresponding Author Email: gc.jagetia@gmail.com

Article Received on: 23/08/17 Approved for publication: 20/09/17

DOI: 10.7897/2230-8407.0810184

ABSTRACT

Free radicals have been implicated in various diseases including cancer. The agents that can inactivate the formation of free radicals or scavenge free radicals may be of great potential to reduce the oxidative stress induced health disorders in humans. The leaves and rhizomes of *Colocasia gigantea* form part of human diet as they are consumed by humans as vegetable regularly. The present study was undertaken to study the free radical scavenging activity of *Colocasia gigantea* in vitro. The rhizomes of *Colocasia gigantea* were collected, shade dried, powdered and sequentially extracted in chloroform, ethanol, and water. The antioxidant activity of various extracts was evaluated by their ability to inhibit the generation of DPPH, hydroxyl (˙OH), superoxide ($O_2^{\bullet-}$), $ABTS^{\bullet+}$and nitric oxide (NO˙) radicals in vitro. Total flavonoid and the total phenol contents were also determined to understand their role in free radical scavenging. The chloroform, ethanol, and aqueous extracts of *Colocasia gigantea* showed a concentration dependent inhibition in DPPH, ˙OH, $O_2^{\bullet-}$, $ABTS^{\bullet+}$ and NO˙ radical generation. Different extracts of *Colocasia gigantea* showed the presence of polyphenols. The *C. gigantea* scavenged DPPH, ˙OH, $O_2^{\bullet-}$, $ABTS^{\bullet+}$and NO˙ radicals in a concentration dependent manner and this activity may be due the presence of various polyphenols and flavonoids in its rhizomes.

Keywords: *Colocasia gigantea*, Free radical, polyphenols, flavonoids.

INTRODUCTION

A free radical is an atom or molecule with an unpaired electron in its outer most orbit[1], which is freely available for reaction. Such unpaired electrons make these species very unstable and highly reactive with other molecules and they try to pair their electron(s) and generate a more stable compound. The oxygen derived radicals also known as Reactive Oxygen Species (ROS) are an important class of radicals that are produced in living system for various purposes[2]. The ROS are dangerous species and are highly reactive with the molecules around them[3]. ROS is a collective term, which includes not only the oxygen radicals (Ö and OH) but also some non-radical derivatives of oxygen, including hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3)[4].

The free radicals are usually produced in organisms that utilize oxygen for energy production, especially during oxidative phosphorylation in the mitochondria. Similarly, the reactive nitrogen species (RNS) are equally important in biological systems as they are involved in several cellular processes including cell signaling[5]. Antioxidant means "against oxidation." An antioxidant is any substance that retards or prevents deterioration, damage or destruction by oxidation[6]. Antioxidants includes several organic substances, including vitamin C, E, and A (which is converted from beta-carotene), selenium and a group known as carotenoids[7]. Despite, the fact that organisms have in built safety mechanisms to neutralize free radicals by different antioxidant molecules present in the cell, excess induction of ROS and RNS does occur. This excess generation of ROS and RNS may overwhelm the endogenous defense system and supplementation by exogenous antioxidants may be essential to neutralize the additional induction of ROS and RNS since the increased induction of ROS and RNS leads to several inflammatory diseases, especially autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular, gastrointestinal and neurodegenerative diseases and cancer[8]. The exogenous supply of antioxidants may be required to neutralize the deleterious effect of ROS/RNS and support the endogenous antioxidants system[9]. Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters have been suspected to cause negative health effects, which has led to strong restrictions on their use in humans. This indicates that there is a need of non-toxic naturally occurring antioxidants, which do not trigger adverse effects. Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical-induced tissue injury. Many plant extracts and phytochemicals have shown to possess free radical scavenging activities[10] but generally there is still a need to find more information concerning the antioxidant potential of other plant species.

*Colocasia gigantea (*family: Araceae) also called giant elephant ear or Indian taro is 1.5-3 m tall herb with a whorl of large leaves at its apex. It bears a large, fibrous, and inedible corm. *C. gigantea* grows commonly in India, Thailand and other Southeast Asian countries[11]. The leaf stalks are used as a vegetable in some areas in, India, Southeast Asia and Japan. In India and Bangladesh, the tubers are cooked and used as a vegetable[12]. In Thailand, *C. gigantea* is considered as a minor food crop and is mainly utilized as a stem vegetable. *C. gigantea*'s stalks are often used for making homemade Thai food called "Bon curry". In the Pacific islands, the tubers are cooked and eaten as a starch[11]. In Thai traditional medicine, *C. gigantea* tuber is heated over a fire and is used to reduce

"internal heat" (fever) and also for the treatment of drowsiness. The fresh or dried tubers mixed with honey are used in the treatment of phlegm in northern Thailand. Fresh tubers have been shown to ameliorate stomach problems, combat infection, and accelerate the healing of wounds. Recently the leaf and tuber extracts have been found to be cytotoxic to HeLa cells[13]. The supplementation of antioxidants from dietary source will be of great importance than those given exogenously from other sources. Since not much information is available on *Colocasia gigantea* despite the fact that it is commonly used as a vegetable in India and Southeast Asia and is part of human diet, the present study was undertaken to investigate the antioxidant potential of different extracts of *Colocasia gigantea* in vitro.

MATERIALS AND METHODS

Collection and extraction of plant

The non-infected and matured rhizomes of *Colocasia gigantea* (family- Araceae) were collected from Manipur. The plant was identified by PG Department of Botany, DM College, Imphal-West, Manipur, India and further authenticated by Botanical Survey of India, Shillong, Meghalaya, India. The non-infected and matured rhizomes of *Colocasia gigantea* were collected and cleaned and chopped into small pieces. The rhizome pieces were spread into the stainless steel trays for drying under shade at room temperature in dark in clean and hygienic conditions to avoid entry of insects, animals, fungus, and extraneous terrestrial materials. The exhaust and free air circulation was allowed. The dried rhizomes were powdered in an electrical grinder at room temperature. Usually 100 g of rhizome powder was extracted sequentially in chloroform, ethanol and water in a Soxhlet apparatus, evaporated to dryness under reduced pressure and stored at -80°C until use. The chloroform, ethanol and water extracts of *Colocasia gigantea* will be called as CGC, CGE and CGA henceforth.

Chemicals

All the chemicals used were of analytical grade and Milli Q water was used for the entire analysis. 1,1- dimethylsulfoxide (DMSO), ascorbic acid, nitroblue tetrazolium (NBT), ethylenediaminetetra acetic acid (EDTA), trichloroacetic acid (TCA), sodium nitroprusside, and Griess reagent were procured from Sigma-Aldrich Chemical Co. Bangalore, India. Methanol, ethanol, sodium acetate, ferric chloride, Folin-Ciocalteau reagent, sodium carbonate, sodium hydroxide, sodium chloride, potassium chloride, disodium hydrogen phosphate (anhydrous), potassium dihydrogen phosphate, aluminum chloride, potassium acetate, gallic acid, glacial acetic acid and acetyl acetone were procured from Merck India, Mumbai.

Estimation of free radical scavenging in vitro

The scavenging activity of CGC, CGE and CGA was estimated using the following protocols:

DPPH free radical scavenging assay

The DPPH scavenging activity of CGC, CGE and CGA was carried out according to earlier described method[14] with minor modifications. To 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200µg/ml CGC, CGE or CGA 1 ml of 0.1 mM DPPH in methanol was added. After thorough mixing, the mixture was kept in the dark for 30 min and the absorbance was measured at 523 nm using UV-VIS spectrophotometer (SW 3.5.1.0. Biospectrometer, Eppendorf India Ltd., Chennai). Methanol was utilized for the baseline correction. The results have been compared with that of the control prepared as above without sample. Radical scavenging activity has been expressed as a percentage and calculated using the following formula:

% Scavenging= (Acontrol-Asample)/ Acontrol X 100.

Where Asample is the absorbance of the test sample and Acontrol is the absorbance of the control.

Hydroxyl radical scavenging activity

Scavenging of the hydroxyl ($^{\bullet}OH$) free radical was determined by the earlier described method[15]. Briefly, the reaction mixture contained deoxyribose (2.8 mM), KH_2PO_4-NaOH buffer, pH 7.4 (0.05 M), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM) and 20, 40, 60, 80, 100, 120, 140, 160, 180,200,220 or 240 µg/ml CGC, CGE or CGA in a final volume of 2 ml. The reaction mixture was incubated for 30 min at ambient temperature followed by the addition of 2 ml trichloroacetic acid (2.8% w/v) and thiobarbituric acid. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was read at 532 nm in a UV-VIS spectrophotometer. The results have been expressed as gallic acid equivalent which was used as a standard.

Superoxide anion scavenging activity

Superoxide free radicals formed by alkaline DMSO react with NBT to produce coloured diformazan. Scavenging of the superoxide ($O_2^{\bullet-}$) anion radical was measured using a modified method[16]. Briefly, the reaction mixture contained 0.2 ml NBT (1 mg/ml in DMSO) and 0.6 ml of various concentrations (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µg/ml) of CGC, CGE or CGA , 2 ml of alkaline DMSO (1 ml DMSO containing, 5 m M NaOH in 0.1 ml water). The blank consisted of pure DMSO instead of alkaline DMSO. The absorbance was recorded at 560 nm in a UV/VIS spectrophotometer. The antioxidant capacity of the CGE based on its ability to inhibit formazan formation has been expressed as mg ascorbic acid equivalent /100 g of extract.

ABTS scavenging activity

ABTS scavenging activity of CGC, CGE or CGA was carried out as described earlier[17]. Briefly, 37.5 mg of potassium persulphate was dissolved in 1 ml of distilled water. 44 µl of this solution was added to 9.7 mg of ABTS dissolved in 2.5 ml of distilled water to prepare ABTS solution. The ABTS solution was allowed to stand in the dark at room temperature for 12-16 hours. The working solution consisted 1 ml of ABTS solution, 88 ml of 50% ethanol. 25 µl of different concentrations (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µg/ml) of the different extracts of *C. gigantea* were mixed with 250 µl of the working ABTS solution and allowed to react for 4 minutes. The absorbance was then measured at 734 nm in a UV-VIS spectrophotometer. Gallic acid was used as the standard antioxidant and the activity was expressed as gallic acid equivalent. The percentage scavenging activity was calculated as follows:

Scavenging (%) = Control O.D – Sample O.D/ Control O.D x 100.

Nitric oxide scavenging activity

The nitric oxide scavenging activity was estimated by spectrophotometric method[18]. Briefly, sodium nitroprusside (5 mM) in phosphate buffer saline was mixed with 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 µg/ml CGC, CGE or CGA and incubated at 25°C for 150 min. The samples were mixed with Greiss reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% napthylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with napthylenediamine was read at 546 nm using a UV-VIS spectrophotometer. The inhibition of nitric oxide formation was determined with respect to standard potassium nitrite treated in the same way with Greiss

reagent. The results have been expressed as potassium nitrite equivalent which has been used as a standard.

Determination of Total phenol contents

The total phenol contents were estimated by Folin-Ciocalteau reagent[19], where 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µg/ml CGC, CGE or CGA after dilution or gallic acid (standard phenolic compound) were mixed with Folin-Ciocalteau reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1M). The mixture was allowed to stand for 15 minutes and the total phenol contents were measured at 756 nm with a UV-VIS spectrophotometer. The total phenol contents are expressed in terms of gallic acid equivalent (mg/100 g of extracts).

Total Flavonoids Determination

The total flavonoid contents in CGC, CGE or CGA were estimated using Aluminum chloride method as described earlier[20]. Different concentrations of *C. gigantea* extract(20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µg/ml) were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml distilled water and incubated at room temperature for 30 min. The absorbance was recorded at 415 nm with a UV-VIS spectrophotometer. Quercetin was used as the standard and the results have been expressed as quercetin equivalent.

RESULTS

The results are shown in table 1-4 and figures 1-7.

DPPH radical scavenging activity

Various extracts of *Colocasia gigantea* showed a concentration dependent rise in the scavenging of DPPH radicals as indicated by the discolouration of DPPH which is purple in colour (Table1). Maximum scavenging was observed at a concentration of 140µg/ml for CGC, CGE or CGA that ranged between 50.41±0.30, 51.08±0.68 to 51.2±0.23 mg of ascorbic acid equivalent, respectively (Figure 1).

Hydroxyl Radical Scavenging activity

Different extracts of *C. gigantea* inhibited the generation of hydroxyl radical in a concentration dependent manner and a maximum inhibition in OH generation was observed at 200µg/ml for chloroform, ethanol and aqueous extracts, respectively (Figure 2). When the efficiency of scavenging was determined with respect to gallic acid equivalent the most effective extract was CGA followed by, CGE and CGC, respectively (Table 2).

Superoxide anion scavenging activity

The chloroform, ethanol and aqueous extracts of *C. gigantea* showed a concentration dependent rise in the inhibition of superoxide radical generation up to a concentration of 140µg/ml that declined thereafter (Figure 3). The maximum effect was observed for ethanol extract which scavenged superoxide radical more efficiently than the other two extracts and this was 59.17±0.23 mg ascorbic acid equivalent, whereas it was 51.02±0.07mg and 50.63±0.11 mg ascorbic acid equivalent for chloroform and aqueous extracts, respectively (Table 1).

ABTS scavenging activity

The different extracts of *C. gigantea* showed a concentration dependent rise in the scavenging activity of the ABTS free radicals up to a concentration of 140µg/ml CGE and CGC and declined thereafter, whereas a maximum scavenging effect for aqueous extract was recorded at 180 µg/ml and a decline thereafter (Figure 4 and Table 2).

Nitric oxide scavenging activity

Various extracts of *C. gigantea* showed a concentration dependent increase in the scavenging activity of nitric oxide radicals and a highest scavenging of NO was observed for 140µg/ml for chloroform and ethanol, whereas and for aqueous extracts showed the highest scavenging activity at 120µg/ml (Figure 5). Among all the three extract the best effect was observed for CGA that revealed maximum activity at a lower concentration than the other two extract (Figure 5 and Table 3).

Total phenol contents

The presence of phenolic compounds in the CGC, CGE and CGA was estimated as total phenol contents that increased in a concentration dependent manner up to 200µg/ml for chloroform, ethanol and aqueous extracts, respectively (Figure 6). The CGA showed the least amount of total phenols when compared to CGE and CGC, where the amount was maximum (Table 4).

Total flavonoid contents

The total flavonoid contents in CGC, CGE and CGA increased in a concentration dependent manner up to 200µg/ml equivalent to gallic acid contents (Table 4). The maximum amount of total flavonoid was estimated for CGC followed by CGA, whereas it was least for CGE (Table 4).

Table 1: DPPH and superoxide radical scavenging activities of different extracts of *Colocasia gigantea*.

Conc. (µg/ml)	DPPH (%±SEM)			Superoxide (%±SEM)		
	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform
20	26.75±0.38	36±0.14	32.33±0.24	26.06±0.11	11.16±0.45	25.61±0.13
40	30.83±1.04	41±0.14	34.6± 0.11	28.86±0.08	14.29±0.34	28.13±0.07
60	34.25±0.38	44±0.38	37.13±0.17	29.22±3.24	26.20±0.60	31.26±0.04
80	39.08±0.22	48.5±0.14	39.46±0.24	37.89±0.37	31.46±0.64	33.89±0.04
100	41.25±0.62	48.91±0.60	41.33±0.40	43.76±0.31	38.88±0.87	37.31±0.04
120	43.91±0.36	54.08±0.68	46.6±0.23	49.45±0.30	52.15±0.19	47.86±0.10
140	50.41±0.30	51.33±0.65	51.2±0.23	50.63±0.11	59.17±0.23	51.02±0.07
160	45.91±0.44	49.91±0.22	46.86±0.35	47.53±0.11	56.87±0.19	48.92±0.09
180	44.41±0.22	47.83±0.50	42.8±0.11	45.87±0.08	54.27±0.16	47.83±0.16
200	42.91±0.20	45.91±0.22	40.2±0.07	43.85±0.11	52.33±0.20	46±0.05

Data are Mean± SEM, N=5.

Table 2: Hydroxyl and ABTS scavenging activities of different extracts of *Colocasia gigantea*.

Conc. (µg/ml)	Hydroxyl Radical (%±SEM)			ABTS (%±SEM)		
	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform
20	22.16±0.15	22.87±0.13	24.05±0.15	23.87±0.19	26.12±0.33	24.35±0.62
40	23.76±0.18	23.75±0.13	26.33±0.10	25.35±0.07	32.96±0.12	26.79±0.11
60	26.51±0.28	30.22±0.15	30.22±0.26	28.11±0.05	35.43±0.09	30.70±0.08
80	32.47±0.37	33.90±0.13	34.44±0.18	30.02±0.15	37.85±0.29	38.34±0.05
100	40.32±1.28	36.77±0.07	35.42±0.18	33.42±0.30	41.61±0.22	38.50±0.22
120	38.31±0.23	41.31±0.10	36.89±0.23	36.90±0.05	43.18±0.19	45.70±0.17
140	48.38±0.39	44.75±0.18	39.07±0.10	42.73±0.45	46.88±0.04	48.17±0.13
160	49.86±0.56	46.66±0.10	42.87±0.43	49.34±0.10	50.28±0.52	51.47±0.14
180	57.83±0.24	51.07±0.28	46.25±0.91	53.75±0.07	47.39±0.09	45.98±3.25
200	60.94±0.36	56.39±0.13	51.91±0.10	49.37±0.22	44.64±0.07	42.15±2.90
220	54.31±0.54	56.39±0.13	46.97±0.10	-	-	-
240	48.32±0.34	48.50±0.18	42.30±0.53	-	-	-

Data are Mean± SEM, N=5.

Table 3: Effect of different extracts of *Colocasia gigantean* on nitric oxide scavenging.

Conc. (µg/ml)	Nitric Oxide Radical (%±SEM)		
	Aqueous	Ethanol	Chloroform
20	19.59±0.77	22.09±0.67	25.19±0.45
40	22.80±0.50	26.74±0.67	27.82±0.69
60	34.50±0.77	28.68±1.02	31.49±0.45
80	42.10±0.50	32.94±1.02	38.58±0.45
100	46.49±0.50	39.92±1.02	40.94±0.45
120	54.97±0.77	45.34±0.67	48.03±0.45
140	50.87±0.50	53.10±1.02	54.85±0.69
160	49.12±0.50	47.28±1.02	47.50±0.94
180	42.69±0.77	33.72±0.67	43.56±0.69
200	35.08±1.01	28.68±1.02	30.97±0.69
220	32.49±0.77	26.74±0.67	27.56±0.45
240	30.69±0.50	23.68±1.02	24.85±0.69

Data are Mean± SEM, N=5.

Table 4: Total phenol and total flavonoid contents of different extracts of *Colocasia gigantea.*

Conc. (µg/ml)	Total Phenols (µg/ml ±SEM)			Total Flavonoids (µg/ml ±SEM)		
	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform
20	84.16±2.20	102.50±1.44	114±1.73	271.15±8.96	189.06±20.36	280.60±15.79
40	90.00±1.44	162.5±12.82	184±2.64	353.83±19.74	213.93±4.97	331.35±5.17
60	113.33±7.12	204.16±2.20	258±1.73	385.58±15.13	231.35±4.30	373.15±10.76
80	147.50±1.44	240.00±1.44	264±1.73	447.78±4.30	253.74±4.30	420.91±10.34
100	162.50±1.44	289.16±2.20	310±3.60	502.50±8.96	308.47±6.58	459.72±7.89
120	161.66±4.63	333.33±2.20	365±2.64	537.33±4.30	350.76±8.61	501.51±5.17
140	182.50±1.44	355.00±1.44	424±2.64	567.18±4.30	393.05±8.96	588.08±16.62
160	205.00±1.44	387.50±1.44	488±2.64	634.35±12.92	432.85±4.30	680.62±5.17
180	222.5±1.44	430.83±3.63	527±2.64	684.10±6.58	457.73±6.58	761.22±15.51
200	265±1.44	505.83±2.20	576±1.73	726.39±6.5	849.53±6.58	829.88±7.89

Data are Mean± SEM, N=5

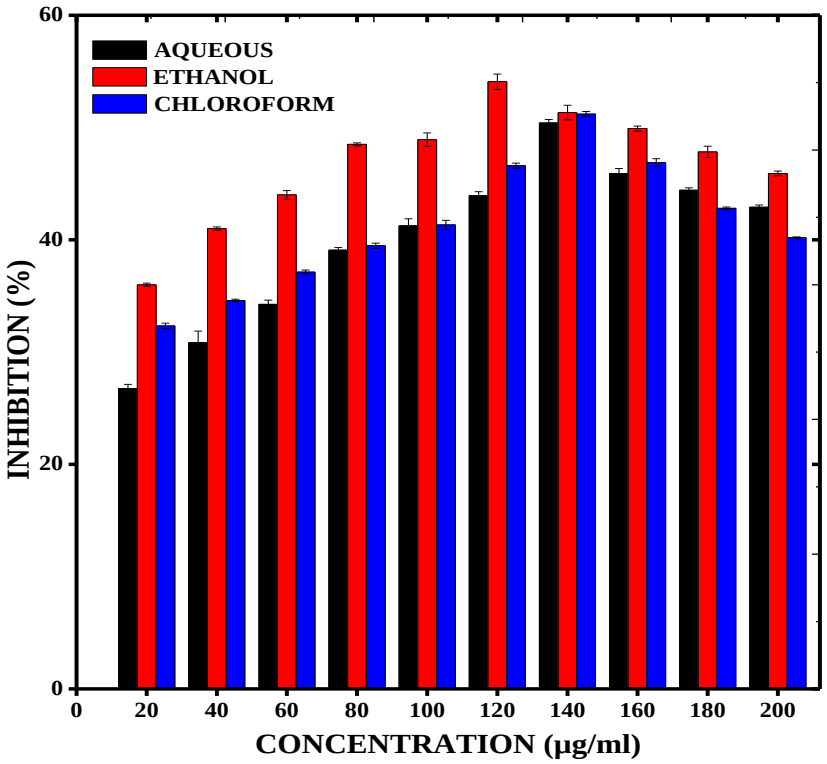

Figure 1: Effect of different extracts of *Colocasia gigantean* on DPPH radical scavenging activity. Data are Mean± SEM, N=5

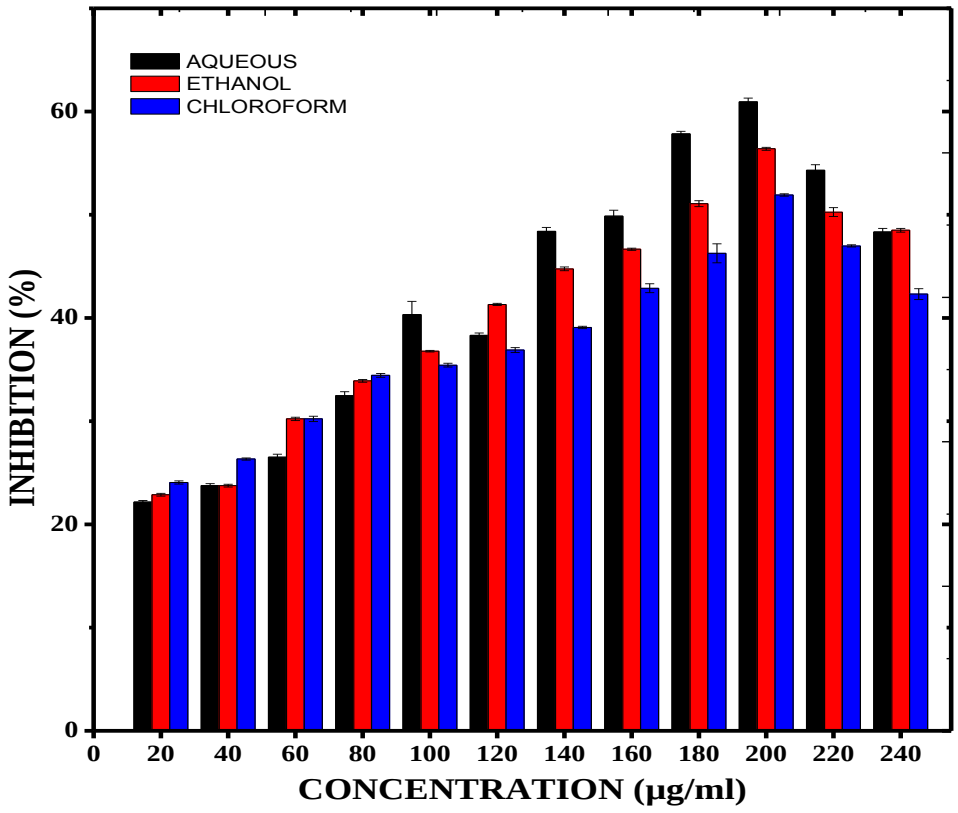

Figure 2: Hydroxyl radical scavenging activity of different extracts of *Colocasia gigantea* expressed as gallic acid equivalent. Data are Mean± SEM, N=5

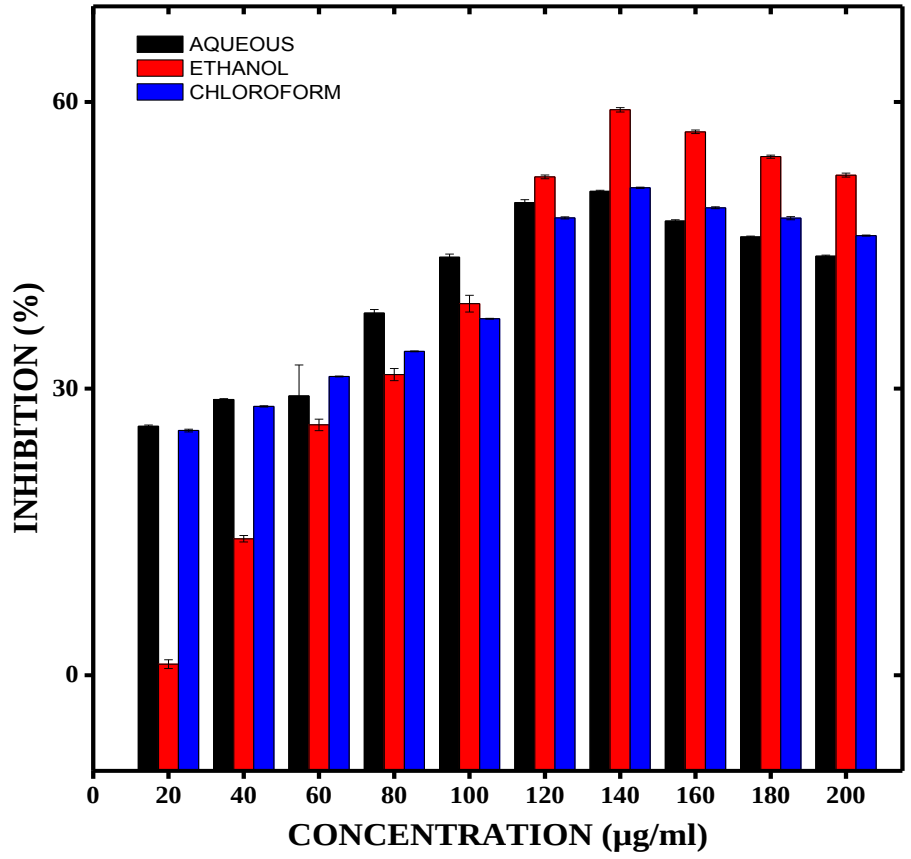

Figure 3: Superoxide radical scavenging activity of different extracts of *Colocasia gigantea*. Data are Mean± SEM, N=5.

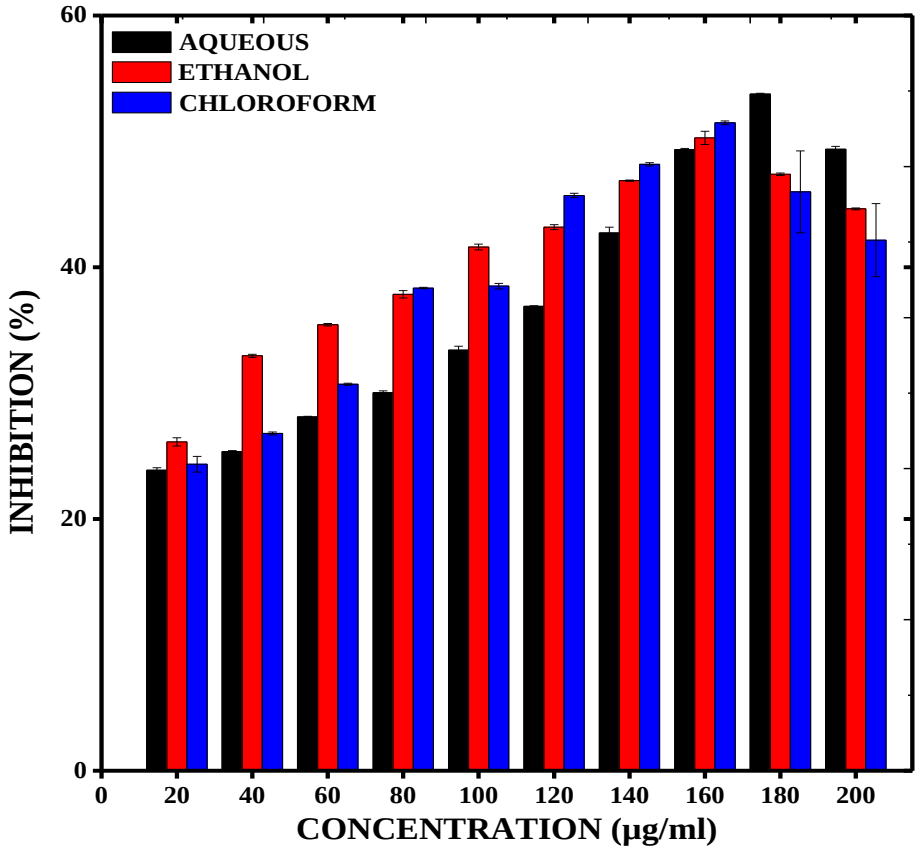

Figure 4: ABTS radical scavenging activity of different extracts of *Colocasia gigantea*. Data are Mean± SEM, N=5.

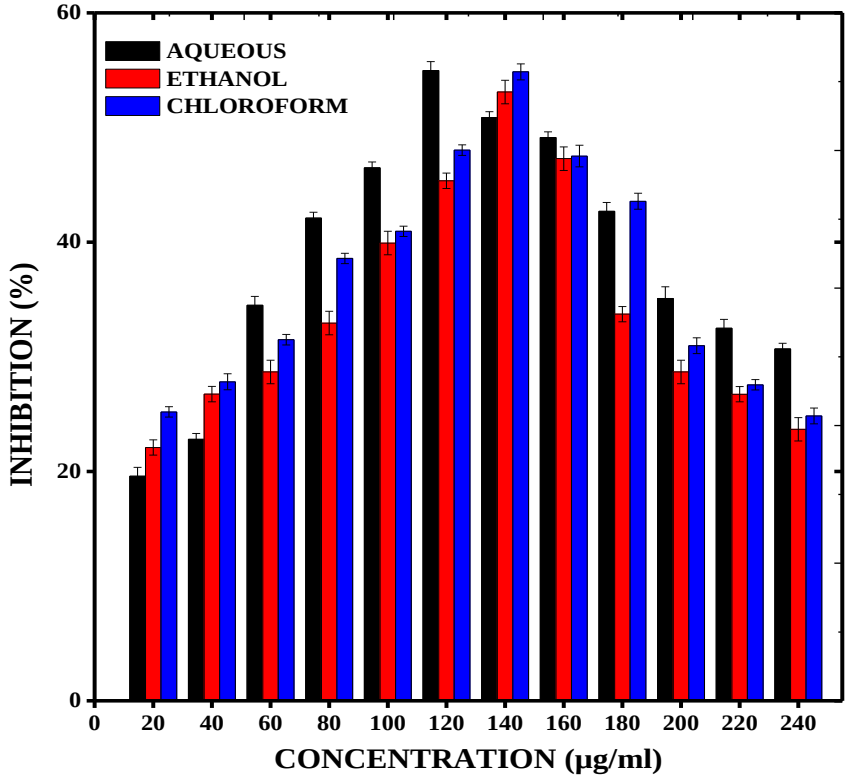

Figure 5: NO radical scavenging activity of different extracts of *Colocasia gigantea* (. Data are Mean± SEM, N=5

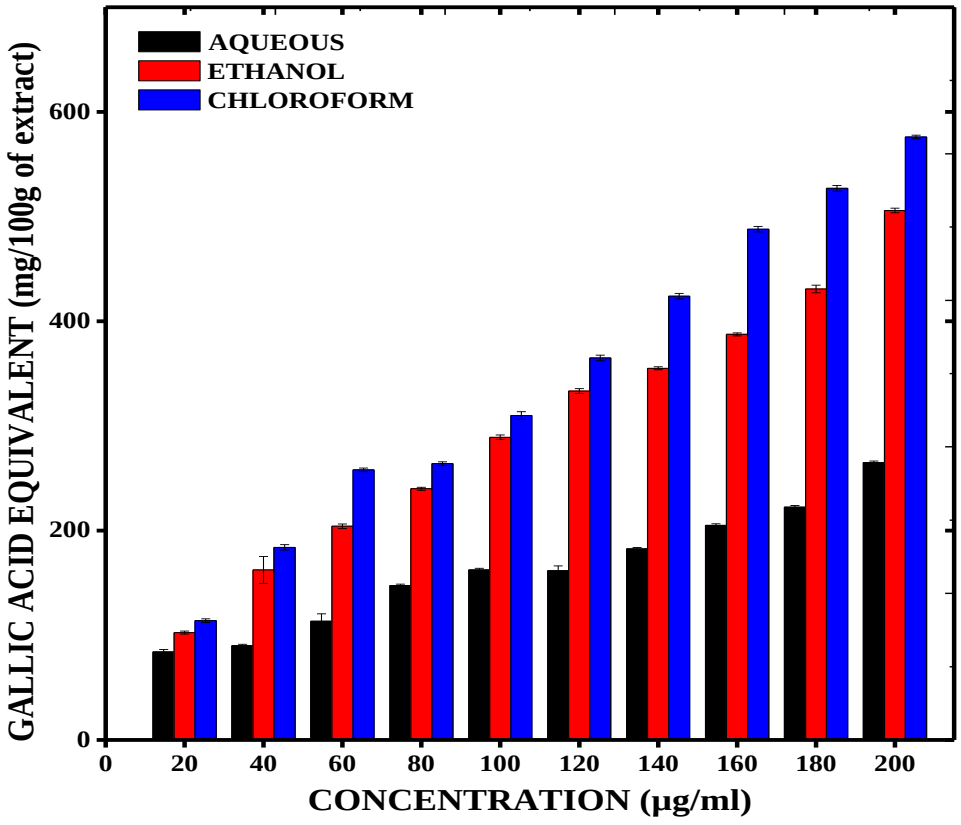

Figure 6: Total phenol contents of different extracts of *Colocasia gigantea* (20-200µg/ml). Data are Mean± SEM, N=5.

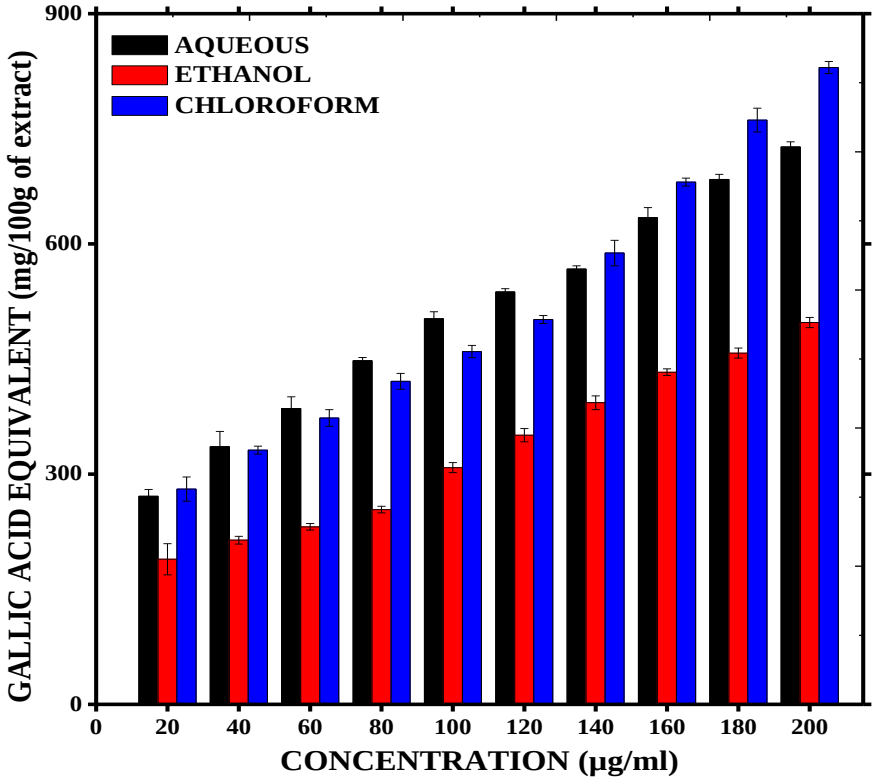

Figure 7: Total flavonoids contents of different extracts of *Colocasia gigantea* (20-200µg/ml). Data are Mean± SEM, N=5.

DISCUSSION

The oxidative stress is the price organisms have to pay for using oxygen as a chemical energy source that is required for various activities. The oxidative stress is induced due to the production of free radicals during various metabolic activities and respiration in particular. The cells are equipped with a repertoire of antioxidant or antioxidant enzymes that usually take care of the normal oxidative stress induced during respiration however in situation of excess oxidative stress it may not be possible for the endogenous antioxidant system to passivate it. Moreover, generation of excess oxidative stress has been indicated as a causative factor of several disorders including, aging, autoimmune, benign oral, cardiovascular, kidney, liver, intestine, and Alzheimer diseases and arthritis, diabetes and cancer [21]. It is also known that external supplementation with antioxidants have been helpful in reducing the risk of oxidative stress. It will be better if the antioxidants come from the dietary sources. The *Colocasia gigantea* or Indian taro is part of human diet and it is consumed frequently during the season when it is available. Therefore, the present study was undertaken to investigate the antioxidant activity of various extracts of *Colocasia gigantea* in vitro.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay is one of the simple and convenient techniques to estimate the antioxidant property of any agent. DPPH is a stable free radical by virtue of the delocalization of the free electron over the molecule, which is violet coloured, and faints into the yellow coloured congener (DPPH-H) once it accepts an electron donated by the antioxidant and subsequently get reduced with a high λ-shift in the visible spectra (from 520 nm to 330 nm). This redox process was first reported by Goldschmidt and Renn (1922). All extracts of *Colocasia gigantea* scavenged DPPH free radical in a concentration dependent manner up to 140µg/ml. Somewhat similar results have been reported for *Agele marmelos*, *Croton caudatus*, *Milletia pachycarpa*, *Schima wallichi*, *Eleagnus caudata*,*Castanopsis indica* and *Dysoxylum gobara*, *Oroxylum indicium*[10,21,22]. The other phytochemicals including naringin, and mangiferin have been reported to scavenge DPPH free radicals in a concentration dependent manner[23].

The hydroxyl free radical is highly reactive species, which reacts in the close vicinity of its formation[24]. During respiration superoxide radical is converted into H_2O_2 which is a highly toxic and oxidizing agent. Despite H_2O_2 is not very reactive, it becomes highly toxic to the cell due to its ability to generate hydroxyl radical in the cells in presence of metals by Haber Weiss and/or Fenton reaction[15]. The hydroxyl radical is capable of inducing detrimental effects on the important macromolecules including proteins and nucleic acids. It reacts with DNA leading to base and sugar damages[24,25]. Hence, neutralization of hydroxyl radical is crucial to protect cells from its deleterious effects. The various extracts of *Colocasia gigantea* inhibited the generation of OH radicals in a concentration dependent manner and hence it may be a useful agent to inactivate this radical in vivo. Many other plant extracts and flavonoids have been found to scavenge hydroxyl free radicals in a concentration dependent manner[21-23,26,27].

The superoxide free radical is an intermediate during cellular respiration which is produced as a result of incomplete metabolism of oxygen[28]. It has been reported to play important role in cell signaling[29]. However, the superoxide anion produces H_2O_2, which in turn generates hydroxyl free radicals in the presence of metals leading to pathologic alteration of several important macromolecules in the cell[30]. Therefore, neutralization of superoxide radical will be able to arrest the chain of ROS generation and protect the cells from the oxidative stress. The various extracts of *C. gigantea* have been found to inhibit the production of superoxide radical in a concentration dependent manner. Other plant extracts and some flavonoids

have been reported to scavenge the superoxide anion free radical earlier[21-23,26,27].

The nitric oxide radical (NO•) is a labile molecule and it is generated in mammalian cells as a byproduct of respiration. It is also used by neutrophils to eliminate invading bacteria. NO• also plays an important role in signal transduction and nerve conduction. However, excess production of NO• is toxic, especially after reaction with oxygen or superoxide anion radicals and the reaction products includes NOx and ONOO- (peroxynitrite). These products are able to inflict severe cellular damage[31]. The various extracts of *C. gigantea* reduced the generation of NO• in a concentration dependent manner. Several plant extracts and plant formulations have also been reported to scavenge NO• in a concentration dependent manner[28]. Similarly, some of the plant flavonoids including naringin and mangiferin have been reported to scavenge nitric oxide free radical in a concentration dependent manner earlier[21-23,26,27,32].

The $ABTS^{\bullet+}$ chromophore was produced through the reaction between ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) and potassium persulfate which is indicated by blue/green colouration. The addition of antioxidants to the pre-formed radical cation reduces the ABTS, indicated by the discoloration of the blue/green colour. The extent of decolorization was expressed as the percentage inhibition of the $ABTS^{\bullet+}$[17]. This trapping of ABTS derived radical cation ($ABTS^{\bullet+}$) by free radical scavengers is a commonly employed method to evaluate the total charge of antioxidants present in complex mixtures[33]. The inhibitory action of $ABTS^{\bullet+}$ indicates the antioxidant potential of any chemical agent. The different extracts of *C. gigantea* did inhibit the generation of $ABTS^{\bullet+}$ radical in a dose dependent manner.

A similar effect has been observed with *Agele marmelos*, *Syzygium cumini* earlier[22, 31]. The exact mechanism of free radical scavenging by *C. gigantea* is not known. However, the free radical scavenging and antioxidant activity of *C .gigantea* may be due to the presence of various phytochemicals like polyphenols and flavonoids, which may be able to donate or accept electron thus neutralizing their oxidative effects. Plants produce phenolic compounds and flavonoids in particular as secondary metabolites that help plants in pollination, to ward off against fungal attacks and also give attractive colours to flowers[34]. These flavonoids have been reported to exert a conducive effect on human health owing to their free radical scavenging ability and antioxidant nature.

CONCLUSION

The present study indicates that all the extracts of *C. gigantea* showed a concentration dependent inhibition of free radicals. These activities of *C. gigantea* may be due to the presence of various phenolic compounds and flavonoids. Therefore, *C. gigantea* could be a potential source of natural antioxidant which may act as therapeutic agent in preventing or slowing down the progression of oxidative stress related degenerative diseases.

ACKNOWLEDGEMENTS

The authors are thankful to the Indian Council of Medical Research, University Grants Commission and Department of Biotechnology, Government of India, New Delhi for providing financial assistance to carry out this study.

REFERENCES

1. Gutteridge JM, Halliwell B. Antioxidants: Molecules, medicines, and myths. Biochem Biophys Res Commun 2010; 393(4):561-4.
2. Lushchak VI. Free radicals, reactive oxygen species, oxidative stress and its classification. Chemico-biological interactions. 2014;224:164-175.
3. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82(1):47-95.
4. Sjodin T, Westing YH, Apple FS. Biochemical mechanisms for oxygen free radical formation during exercise. Sports Med 1990; 10:236-254.
5. Kreuz S, Fischle W. Oxidative stress signaling to chromatin in health and disease. Epigenomics 2016; 8(6):843-62.
6. Halliwell B, Aerchabach R, Lologer J, Aruoma OI. Food Chem. Toxicol 1995; 33(7):601-617.
7. Kaczmarski MJ, Wojicicki L, Samochowiee T. The influence of exogenous antioxidants and physical exercise on some parameters associated with production and removal of free radicals. Pharmazie 1999; 54:303-306.
8. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev 2014; 94(2):329-54.
9. Jagetia GC, Reddy TK. Alleviation of iron induced oxidative stress by the grape fruit flavanone naringin in vitro. Chemico-Biol Inter 2011; 190:121-128.
10. Lalrinzuali, K. Vabeiryureilai, M., and Jagetia G.C. The analysis of antioxidant activity and phenolic contents of selected medicinal plants of Mizoram. Genomics and Applied Biology, 2015, Vol.6, No.11 1-12.
11. Manner HI. Farm and forestry production and marketing profile for giant taro (Alocasia macrorrhiza). In: Elevitch CR, editor. Specialty crops for pacific island agroforestry [Internet]. Holualoa, Hawaii: Permanent Agriculture Resources (PAR) 2011.
12. Kay DE. (revised by Gooding EGB). Crop and product digest No. 2-root crops. 2nd ed. London: Tropical Development and Research Institute 1987.
13. Pornprasertpol A, Sereemaspun A, Sooklert K, Satirapipatkul C, Sukrong S. Anticancer activity of selected Colocasia gigantia fractions. J Med Assoc Thai 2015; 98(1):S98-106.
14. Leong LP and Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem 2002 76:69-75.
15. Halliwell B. Oxidants and human disease: some new concepts. FASEB J 1987; 1(5)358-64.
16. Hyland K, Voisin E, Banou H and Auclair C. Superoxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generating system. Anal. Biochem 1983; 135:280–287.
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical Cation decolorization assay. Free Rad Biol Med 1999; 26(9/10):1231–1237.
18. Marcocci L, Packer L, Droy-Lefaix MT. Antioxidant action of Ginkgo biloba extract EGb 761. Meth. Enzymol 1994; 234: 462-475.
19. Chang C, Yang M, Wen H. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10:178-182.
20. Kesarwala AH, Krishna MC, Mitchel JB. Oxidative stress in oral diseases. Oral Diseases 2016; 22:9–18.
21. LalrinzualiK. Vabeiryureilai M, Jagetia GC, Lalawmpuii PC. Free radical scavenging and antioxidant potential of

different extracts of *Oroxylum indicum in vitro*. Advances in Biomedicine Pharmacy 2:3 (2015) 120-130.
22. Jagetia GC, Venkatesh P, Baliga MS. Evaluation of radioprotective effect of *Aegle marmelos* (L.) Correa in the cultured human peripheral blood lymphocytes exposed to different doses of□□□-radiation: a micronucleus study. Mutagenesis 2003;18 (4): 387-93.
23. Jagetia GC, Shetty PC, Vidyasagar MS. Inhibition of radiation-induced DNA damage by jamun, *Syzygium cumini*, in the cultured splenocytes of mice exposed to different doses of γ-radiation. Integrative Cancer Therapies 2012;11(2):141-153.
24. Pastor N, Weinstein H, Jamison E. A detailed interpretation of OH radical footprints in a TBP-DNA complex reveals the role of dynamics in the mechanism of sequence-specific binding. J Mole Biol. 2000; 304:55–68.
25. Verlackt CCW, Neyts EC, Jacob T, Fantauzzi D, Golkaram M, Shin YK, Duin ACT van and Bogaerts A. Atomic-scale insight into the interactions between hydroxyl radicals and DNA in solution using the ReaxFF reactive force field. New J. Phys 2015; 17.
26. Shantabi L, Jagetia GC, Ali MA, Singh TT, Devi SV. Antioxidant potential of *Croton caudatus* leaf extract in vitro. Trans Med Biotechnol2014, 2 (6),1-15.
27. Jagetia GC, Venkatesha VA. Effect of mangiferin on radiation-induced micronucleus formation in cultured human peripheral blood lymphocytes. Environmental and Molecular Mutagenesis 2005;46, 12-21.
28. Kirkinezosa IG, Morae CT. Reactive oxygen species and mitochondrial diseases. Cell Dev Biol 2001; 12:449–457.
29. Weidinger A, Kozlov AV. Biological activities of reactive oxygen and nitrogen species: oxidative stress *versus* signal transduction. *Biomolecules* 2015; 5(2):472-484.
30. Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol 2003; 15;552(2):335-244.
31. Roberts RA, Laskin DL, Smith CV, Robertson FM, Allen EM, Doorn JA, Slikker W. Nitrative and oxidative stress in toxicology and disease. Toxicol Sci 2009; 112(1):4-16.
32. Jagetia GC, Rao S, Baliga MS, Babu KS. The evaluation of nitric oxide scavenging activity of certain herbal formulations in vitro: a preliminary study. Phytother Res h2004; 18(7):561-565 ().
33. Miller NJ, Rice-Evans CA. Factors influencing the antioxidant activity determined by the ABTS•+ radical cation assay. Free radical research. 1997 1;26(3):195-9.
34. Ferreyra ML, Rius SP, Casati P. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Front Plant Sci 2012;3.

Cite this article as:

Nambam Bonika Devi and Ganesh Chandra Jagetia. Free radical scavenging and antioxidant potential of different extracts of *Colocasia gigantea* (Blume) Hook. F. *In vitro*. Int. Res. J. Pharm. 2017;8(10):72-81 http://dx.doi.org/10.7897/2230-8407.0810184

Source of support: Indian Council of Medical Research, University Grants Commission and Department of Biotechnology, Government of India, New Delhi, Conflict of interest: None Declared