Antioxidant Potential of *Croton Caudatus* Leaf extract I*nvitro*

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Abstract

Free radicals have been implicated in various diseases including cancer and agents that inhibit the formation of freeradicals or scavenge free radicals may be of great utility to reduce the oxidative stress induced by free radicals in humans. The present study was undertaken to study the antioxidant activity of *Croton caudatusin vitro.* The leaves of *Croton caudatus* were collected, shade dried, powdered and sequentially extracted in chloroform, ethanol, and water. The antioxidant activity of various extracts was evaluated bytheir ability to inhibit the generation of DPPH, hydroxyl ($^{\bullet}OH$), superoxide ($O_2^{\bullet-}$) and nitric oxide ($NO^{\bullet}$) and FRAP free radicals *in vitro*. Total flavonoid and the total phenol contents were also determined to understand their potential in free radical scavenging. The chloroform, ethanol, and aqueous extracts of *Croton caudatus* showed a concentration dependent inhibition in DPPH,$^{\bullet}OH$, $O_2^{\bullet-}$, $NO^{\bullet}$and FRAP free radicals. Amaximum inhibition in the DPPH and FRAP free radicals was observed for 2000 µg/ml, whereas the $^{\bullet}$O Hand $O_2^{\bullet-}$radicals were inhibited to a greatest extent at a concentration of 3000µg/ml and 5000µg/ml, respectively. Similarly, a highest inhibition of $NO^{\bullet}$ free radicals was observed at a concentration of 3500 µg/ml for all the extracts evaluated. The highest amount of total phenols and flavonoids were determined for 2000 and 4000µg/ml for all the extracts. Various extracts of *Croton caudatus* inhibited the generation of DPPH, $^{\bullet}OH$, $O_2^{\bullet-}$, $NO^{\bullet}$ and FRAP radicalsin a concentration dependent manner, which may be due to the presence of various polyphenols.

KEYWORDS: *Croton caudatus,* Free radicals, DPPH, Hydroxyl, superoxide, nitric oxide and FRAP radicals, flavonoids.

INTRODUCTION

Antioxidant means "against oxidation" therefore an antioxidant is a substance that retards or prevents deterioration, damage or destruction induced by oxidation(Dekkers*etal*.,1996).Antioxidants include several organic substances, like vitamins C, E, and A, selenium and carotenoids (Dekkers*etal.,*1996;Kaczmarski*et al.,*1999).

A free radical is an atom or a molecule with an unpaired electron in its outer most orbit (Jesberger and Richardson,1991), which is freely available for reaction. The presence of an unpaired electron makes these species very unstable and highly reactive with other molecules (Koppenol,2000) as they try to pair their electron(s) and generate a more stable compound. There are mainly two classes of free radicals generated: one the oxygen derived, whereas the other nitrogen derived. The oxygen derived radicals are also known asReactive Oxygen Species (ROS). They are an important class of radicals that are produced in living system for various purposes (Miller*et al*.,1990).The ROS are dangerous species and are highly reactive with the molecules around them (Sharma and Clark,1998). 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) (Sjodin*etal.*,1990).

The free radicals are necessary evil as they are generated as a by-product of oxidative metabolism in the mitochondria in the organisms living in an aerobic environment. They are also produced by immune cells to fight against infection and during wound healing. However, excess induction of ROS has been implicated in several inflammatory diseases including autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular disorders, neurodegenerative diseases and cancer (Valko*et al.,* 2007). Similarly, the reactive nitrogen species (RNS) are equally important in biological systems as they are involved in several cellular processes including cell signalling(Valko*et al.,* 2007). The human body is well equipped with various antioxidant mechanisms such as antoxidant enzymes and antioxidant nonenzymatic molecules including glutathione(Beckman and Ames, 1998) to neutralise the free radicals produced during various metabolic processes. The excess generation of ROS and RNS can overwhelm the cellular machinery resulting in the failure of endogenous antioxidant system. In such situations, the exogenous supply of antioxidantsmay be required to neutralize the deleterious effect of ROS/RNS and support the endogenous antioxidants system(Jagetia*et al.,* 2003; Jagetia and Reddy, 2011). 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. Hence, strong restrictions have been placed on their application in humans. This indicates that there is a need of non-toxic naturally occurring antioxidants which do not exert negative health effect on humans. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical-induced tissue injury. Despite the fact that many plant extracts and phytochemicals have shown to possess free radical scavenging activities (Larson, 1988;Kolev*aet al.,* 2002) there is still a need to find more information concerning the antioxidant potential of other plant species that remained unexplored.

Croton caudatus Geiseler (family: Euphorbiaceae) is a traditional medicinal plant, which is fairly widespread in South East Asia including India, Sri Lanka,Bhutan,Borneo,Burma, Indo-Myanmar region,Java,Laos,Malaysia,Nepal,Pakistan,Philippines, Singapore, Sumatra, Thailand and Vietnam.*Croton caudatus* had long been known toChin-

Kuki inhabitants of Manipur and Mizoram states of India.It is traditionallyused as a poultice for fever, sprains and treatment of liver diseases in various parts of Asia. Its roots are purgative.The whole plant is used for medicinal purposes and it has been found to be low in toxicity (Lin *etal.*, 2003).*Croton caudatus*is a traditional Dai Nationalistic medicine. The stems and leaves of *Croton caudatus*have been used for the treatment of malaria, ardent fever, convulsions, rheumatic arthritis, and numbness(Jiangsu New Medical College,China, 1975). It is one of the constituents of Qi Wei KeTengZi Wan, which is a well-known formula used by the Dai tribe of China for the treatment of pain and stomach diseases (Anonymous, 2005). The leaves have been applied on festering wounds of injured cattles to ward off against maggots. In Hmar language,it is called "Ranlung damdawi"(Ranlung = Worm,damdawi = medicine) , "Yong Khullokpi and Khagilaikoi"in Manipuri language and "Ganthan lou"Thadou-Kuki dialect.The oral administration of the juice/extract of *Croton caudatus* has been first experimented at Saikot,Churachandpur district of Manipur by Mr. Chawilen on himself therefore it is also known as "Chawilen damdawi" after him. It is traditionally known as "Kam-Sabut". *Croton caudatus* usually grows in peat swamp, deciduous and thick canopy evergreen forests. Sometimes,it also grows near marginal areas along river or stream tracts. Its traditional uses for various medicinal purposes stimulated us to obtain an insight into its antioxidant properties in vitro.

MATERIALS AND METHODS

Preparation of the extracts

Croton caudatus (CC)Geiseler (family: Euphorbiaceae)was identified by Professor Kumar Singh, a well-known taxonomist of Manipur University, Imphal, India and further authenticated by Botanical Survey of India, Shillong.A voucher specimen has been stored with us. The mature leaves of *Croton caudatus* were collected from Saikot, Churachandpur District of Manipur during the dry season. The cleaned and non-infected leaves were spread into the stainless steel trays and allowed to shade dry at room temperature in dark in clean and hygienic conditions to avoid entry of insects, animals, fungus, and extraneous terrestrial material. The exhaust and free air circulation was allowed. The dried leaves were powdered in a grinder at room temperature. A sample of 100g of leaf powder was extracted sequentially with petroleum ether, chloroform, ethanol and water in a Soxhlet apparatus. The extracts(CCE) were then evaporated to dryness under reduced pressure and stored at -80°C until further use.

Chemicals

All the chemicals used were of analytical grade and Milli Q water was used for entire analysis. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), dimethyl sulfoxide (DMSO),ascorbic acid, nitrobluetetrazolium (NBT), ethylene-diaminetetraacetic acid (EDTA), trichloroacetic acid (TCA), sodium nitroprusside, and Griess reagent were procured from Sigma-AldrichCompany Bangalore, India). Methanol, ethanol, sodium acetate, ferric chloride, Folin-Ciocalteu reagent, sodium carbonate, sodium hydroxide, sodium chloride, potassium chloride, disodium hydrogen phosphate (anhydrous), potassium dihydrogen phosphate, aluminium

chloride, potassium acetate, gallic acid, ferrous ammonium sulphate, ammonium acetate, glacial acetic acid and acetyl acetone were procured from Merck India, Mumbai, India).

Estimation of free radical scavenging in vitro

The free radical inhibitory activity of different extracts of *C. Caudatus* was evaluated using the methods given below and the standards were selected based on the species of free radical scavenging activity being studied.

1. DPPH free radical scavenging

The principle for reduction of the DPPH free radical is that the antioxidant reacts with the stable free radical DPPH and converts it into 2,2-diphenyl-1-picrylhydrazine. The DPPH free radical scavenging activity of *C.caudatus* extracts was determined as described earlier(Leong and Shui,2002). Briefly, various concentrations of CCE (15.625, 31.25, 62.5, 125, 250, 500, 1000or 2000 µg/ml) dissolved in 40µl of appropriate solvents were added to 3 ml methanol solution of 0.1mM DPPH and incubated for 30 min at room temperature. An equal amount of ascorbic was used as a standard, whereas an equal amount of methanol served as blank. After 30 min, absorbance was recorded at 515nm in Spectroscan 2600 UV/VIS spectrophotometer (Chemito Technologies Pvt. Ltd.,Mumbai, India). The DPPH free radical scavenging ability of the CCE has been expressed as mg trolox equivalent/100g as well as mg ascorbic acid equivalent/100g of the extracts.

2. Hydroxyl radical scavenging

The hydroxyl radical scavenging activity of CCE was assayed according to the method of Klein *et al.*,(1981). Briefly, 15.625, 31.25, 62.5, 125, 250, 500, 1000,2000, 2500, 3000, 3500or4000 µg/mlCCEin 1 ml was placed into a test tube and evaporated to dryness. One ml of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml 0.018% EDTA, 1 ml DMSO (0.85%, *V/V*, in 0.1 mol L^{-1} phosphate buffer, pH 7.4) and 0.5 ml 0.22% ascorbic acid were added into each tube. The tubes were capped tightly and heated in a water bath at 80-90°C for 15 min. The reaction was terminated by adding 1 ml of ice-cold TCA (17.5% *m/V*). Three ml of Nash reagent (75.0g ammonium acetate, 3 ml glacial acetic acid and 2 ml acetyl acetone were mixed andMilli Q water was added to make up the volume up to 1 L) was added to each tube; and the tubes were left at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured at 412 nm using a UV/VIS spectrophotometer against blank of the reagent. The antioxidant capacity of CCE based on its ability to decrease hydroxyl radical generated in a Fenton reaction system has been expressed as mg gallic acid equivalent /100g of CCE.

3. Superoxide anion scavenging

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 an earlier described method (Hyland *et al*., 1983) with minor modifications. Briefly, the reaction mixture contained 0.2ml NBT (1mg/ml in DMSO) and 0.6ml various concentrations of CCE (15.625, 31.25, 62.5, 125, 250, 500, 1000,2000, 2500, 3000, 3500, 4000,4500or5000), 2ml of alkaline

DMSO (1ml DMSO containing, 5mMNaOHin 0.1ml water). The blank consisted of pure DMSO instead of alkaline DMSO. The absorbance was recorded at 560nm in aUV/VIS spectrophotometer. The antioxidant capacity of the CCE based on its ability to inhibit formazan formation has been expressed as mg ascorbic acid equivalent/100g of CCE.

4. Nitric oxide scavenging

The nitric oxide scavenging activity of CCE was estimated by Griessreaction as described earlier with minor modifications (Marcocci*et al.,* 1994;Sreejayan and Rao, 1997; Jagetia*et al.,* 2003). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess reagent. Scavengers of nitric oxide compete with oxygen leading to a reduced production of nitric oxide(Marcocci*etal.,* 1994). Briefly, the reaction mixture containing 1ml of 5mM sodium nitroprusside was incubated with 500 µl of15.625, 31.25, 62.5, 125, 250, 500, 1000,2000, 2500, 3000or 3500 µg/ml CCE and incubated at 25℃ for 150 min. Oneml sample from the above was removed and diluted with 1 ml of Griess reagent and the absorbance of the chromophore formed during diazotisation of nitrite with sulphanilamide and subsequent coupling with NED was read at 546nm in a UV/VIS spectrophotometer. Anequal ratio of sodium nitroprusside and Griess reagent treated in an identical manner without the CCE served as a standard, whereas PBS was used as blank. The antioxidant activity of CCE based on its ability to decrease nitrous acid has been expressed as mg ascorbic acid equivalent/100g of CCE.

5. Ferric reducing antioxidant potential (FRAP)

The ability of CCE to reduce ferric ions was measured using an earlier described method (Benzie and Strain, 1999) with minor modifications. 50 µlof various concentrations of CCE (15.625, 31.25, 62.5, 125, 250, 500, 1000, or 2000 µg/ml)were added to 3ml of FRAP reagent (10 parts of 300mM acetate buffer, pH 3.6, 1 part of TPTZ solution and 1 part of 20mM $Fecl_3.6H_2O$ solution) and the reaction mixture was incubated at 37℃ for 30 min. The increase in absorbance was measured at 593nm using UV/VIS spectrophotometer. The antioxidant activity of the extracts is based on their ability to reduce ferric ions and it has been expressed as mg Trolox equivalent/100g of CCE as well as mg ascorbic acid equivalent/100 g of CCE.

6. Total phenolic contents

The total phenolic contents of the CCE were determinedas described earlier(Singleton and Rossi,1965).500µl of 15.625, 31.25, 62.5, 125, 250, 500, 1000, or 2000 µg/mlCCE was mixed with 1ml of 1:10 Folin-Ciocalteu reagent and incubated at room temperature for 5min followed by the addition of 900 µl saturated (7.5%) sodium carbonate solution. After 1 h of incubation at room temperature, the absorbance was recorded at 640nmusing a UV/VIS spectrophotometer.Total phenolic contents of the CCE have been expressed as Gallic acid equivalent mg/100g of the CCE.

7. Total Flavonoids Determination

The total flavonoids were determined by colorimetric method (Chang *et al*., 2002). One ml of various extracts of CCE (15.625, 31.25, 62.5, 125, 250, 500, 1000,2000, 2500, 3000, 3500

or4000 µg/ml) was separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water and incubated for 30 minutesat room temperature. The absorbance of the reaction mixture was recorded at 415 nm with a UV/VIS spectrophotometer. The calibration curve was prepared by preparing different concentrations of gallic acid in methanol. The quantity of flavonoids in CCE has been expressed as mg gallic acid or quercetin equivalent/100g of CCE.

RESULTS

The results of free radical scavenging, total phenol or flavonoid contents have been expressed as ascorbic (AA), Trolox (Tx), gallic acid or quercetin equivalent/100 gofCCEas the case may be in Fig. 1 to 7.

The DPPH assay has been extensively used to indicate antioxidant activity of any pharmacological agent. The violet colour of DPPH is converted into the yellow colour after its reduction into DPPH-H, with a high λ-shift in the visible spectra (from 520 nm to 330 nm).The stable free radical DPPH is well known as a good hydrogen abstractor yielding DPPH-H as a by-product(Forester *etal,* 1968). The chloroform, ethanol and aqueous extracts of CC caused a concentration dependent scavenging of DPPH radical and a maximum effect was observed at a concentration of 2000 µg/ml (Fig. 1). However, the greatest scavenging activity was observed for ethanol extract where it was equivalent to 3193±6.25 mg of trolox or ascorbic acid (Fig.1). This was followed by the chloroform extract, where it was 2843±12.5 mg trolox or ascorbic acid equivalent (Fig. 1). The aqueous extract showed less activity which was 2575±6.25 mg trolox or ascorbic acidequivalent, when compared to other two extracts (Fig. 1).

The hydroxyl free radical scavenging activity of CCE showed a concentration dependent rise until a maximum inhibition of OH radicals was observed at a concentration of 3000 µg/ml for chloroform, ethanol and aqueous extracts (Fig. 2). The maximum effect was observed for ethanol extract which scavenged OH radicals more efficiently than the other two extracts and this was 690 mg gallic acid equivalent, whereas it was 579±4.05 mg and 203±0.675 mg equivalent to gallic acid for aqueous and chloroform extracts, respectively (Fig. 2).

The superoxidescavenging activity of CCE increased markedly with the increase in concentration and the maximum effect was observed for 5000 µg/ml for chloroform, ethanol and aqueous extracts (Fig. 3). The highest effect was observed for chloroform extract which scavenged superoxide radicals more efficiently than the other two extracts and this was 645±1.066mgascorbic acid equivalent, whereas it was 448 mg and 440±0.533mg ascorbic acid equivalent for aqueous and ethanol extracts, respectively (Fig. 3).

The nitric oxide radicalscavenging activity of CCE showed a concentration dependent rise until a maximum inhibition of NO radical was observed at a concentration of 3500 µg/mlfor chloroform, ethanol and aqueous extracts (Fig. 4). The maximum effect was observed for aqueous extract which scavenged superoxide radicals more efficiently than the other two extracts and this was 242±2.5 mg ascorbic acid equivalent, whereas it was 196±3.75 mg, and 185±15.0 mg equivalent to ascorbic

acid for ethanol and chloroform extracts, respectively (Fig. 4).

The FRAP radicalscavenging activity of CCE showed a concentration dependent rise until a maximum inhibition of FRAPradical was observed at a concentration of 2000 µg/mlfor chloroform, ethanol and aqueous extracts (Fig.5). The maximum effect was observed for ethanol extract which scavenged FRAP radicals more efficiently than the other two extracts. The FRAP inhibitory activity of ethanol CCE was 3230±27.83mgascorbicacid equivalent and 1588±13.68 mg trolox equivalent,whereas3020±10.0 mgand 995±10.0mgascorbicacid equivalent and1484±4.91mgand489±4.91mgtrolo xequivalent for aqueous and chloroform extracts, respectively (Fig.5).

Total phenol contents of CCE showed a concentration dependent rise up to a concentration of 2000 µg/mlfor chloroform, ethanol and aqueous extracts (Fig. 6). The maximum effect was observed for ethanol extract which showed the highest content (more than the other two extracts) and this was3083±16.66 mg gallic acid equivalent,whereas it was 1860±14.58 mg and 1416±10.41 mg gallicacid equivalent, for chloroform and aqueous extracts, respectively (Fig.6).

Total flavonoid contents of CCE showed a concentration dependent rise and the maximum effect was observed for 3500µg/ml (alcoholic and chloroform extracts) and 4000 µg/ml (aqueous extract) thesecontents were equivalent to 2784mg, 2019 mg and 1847 mg of gallic acid for ethanol, aqueous and chloroform extracts, respectively, where as quercet in equivalent contents were 3171 mg, 2300 mg and 2103mg for ethanol, aqueous and chloroform extracts, respectively (Fig. 7).

DISCUSSION

Free radicals are generated during essential metabolic processes in the cell and their excess generation leads to oxidative stress, which is harbinger of several diseases (Halliwell and Guetteridge, 1999). Although cells are equipped with a repertoire of various cellular defence mechanisms to counter the negativeeffect of free radicals produced in the normal course, the excess generation of free radicals and subsequent increased oxidative stress may overwhelm theintrinsic cellular defence mechanism/sleading to several oxidative stress related disorders, which may be countered by supply of exogenous antioxidants. Several synthetic antioxidants have been used or are in use,however; they exert negative health effects on humans. Therefore, there is always a need to search for new and less toxic or non-toxic molecules/pharmacological agents that can be used for human healthcare. The plants synthesize several biomolecules to protect themselves from insect, pests and other environmental stresses, which may be useful to humans and their biologic origin, make them more biocompatible and acceptable to humans than exotic synthetic molecules(Jagetia*et al.,* 2003a,b; Jagetia, 2007).Therefore, the present study was undertaken to investigate the effect of various *Croton caudatus* extracts on their free radical scavenging ability in vitro.

DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is a simple and convenient method to study the antioxidant property of several agents. DPPH is a stable free radical by virtue of the delocalization of the free electron over the molecule and is violet coloured, which turns into yellow colour after it

accepts an electron from the donor antioxidant and subsequently getsreduced into the congener (DPPH-H), with a high λ-shift in the visible spectra (from 520 nm to 330 nm). This is a redox process and was first mentioned by Goldschmidt and Renn (1922). All extracts of *Croton caudatus* scavenged DPPH free radicals in a concentration dependent manner. Several other plants extracts have beenreported to scavenge DPPH free radicals in a concentration dependent manner(Jagetia and Baliga, 2003;Jagetia*et al*., 2003b, 2012; Wong *et al.,* 2006;Narayanaswamy and Balakrishnan, 2011, Aparadh*et al.,* 2012). Theother agents like naringin, mangiferin have also been reported to scavenge DPPH free radicals in a concentration dependent manner (Jagetia*et al,* 2003a; Jagetia and Venketasha, 2005). The DPPH scavenging effect of *Croton caudatus* extracts may be due to the donation of an electron to DPPH free radical.

The hydroxyl radical is the neutral form of the hydroxide ion and it is highly reactive. It reacts in the close vicinity of its formation(Pastor *et al.,* 2000). Hydroxyl radicals are the major reactive oxygen species that cause lipid oxidation and enormous biological damage to important macromolecules including proteins and nucleic acid, especially the DNA (Aurand et al, 1977). H_2O_2 is generated in the cells as a metabolic by-product. Although it is not very reactive, it becomes highly toxic to the cell due to its ability togenerate hydroxyl radical in the cells in presence of metals by Haber Weiss and/or Fenton reaction (Halliwell, 2006; Valko*et al.,* 2007). Thus, removal of hydroxyl radical is very important to protect cells from its deleterious effects. *Croton caudatus* extracts inhibited the generation of hydroxyl free radicals in a concentration dependent manner and it may be a useful agent to neutralize this radical in vivo. Many other plant extracts and flavonoids have been found to scavenge hydroxyl free radicals in a concentration dependent manner (Jagetia*et al.,* 2003a,b;2012;Jagetia and Venketasha, 2005;).

It is well known that superoxide anions damage biomolecules directly or indirectly byforming H_2O_2, $^{\bullet}OH$, peroxy nitrite or singlet oxygen during aging and other pathological eventssuch as ischemic reperfusion injury. Superoxide has also been observed to directly initiatelipid peroxidation (Yen and Duh, 1994) and subsequently damage DNA. The superoxide anion radical scavenging activity of *Croton caudatus* extractsassayed by alkaline DMSO method showed a concentration dependent inhibition of superoxide anion radical formation. The other plant extracts and certain plant falvonoids including naringin and mangiferin have been found to scavenge superoxide free radicals in a concentration dependent manner (Jagetia*et al*., 2003a,b, 2012; Jagetia and Venketasha, 2005).

The nitric oxide radical ($NO^{\bullet}$) is a labile molecule, which is generated in mammalian cells. Apart from its role in physiological processes, $NO^{\bullet}$ is also toxic, especially after reaction with oxygen or superoxide anion radicals. The reaction products formed include NOx and $ONOO^-$ (peroxynitrite), which are able to inflict severe cellular damage (Beckman *et al.,* 1990,Radi*et al.,* 1991, Lipton *et al*., 1993 andStamler*et al.,* 1994). Thenitric oxide radicals decreased with the increasing concentration of *Croton caudatus*extracts and a maximum inhibition of $NO^{\bullet}$ generation was observed at 1 mg/ml indicating that

Croton caudatus extracts effectively scavenged NO• at low concentration. Several plants and plant formulations have been reported to scavenge NO• in a concentration dependent manner(Jagetia and Baliga, 2003;Jagetia*et al*., 2003b,2012). Similarly, some of the plant flavnaoids including naring in and mangiferin have been reported to scavenge nitric oxide free radicals in a concentration dependent manner earlier (Jagetia and Baliga, 2003; Jagetia*et al*., 2003a; Jagetia and Venketasha, 2005).

FRAP assay is a simple technique to evaluate the antioxidant activity of any agent. CCE extract inhibited the generation of FRAP radical in a concentration dependent manner. Several plant extracts have been reported to exhibit antioxidant activity by scavenging of FRAP radical in vitro(Wong *et al.,* 2006;Aparadh*et al.,* 2012). The phytochemical analysis has shown thepresence of phenols and flavonoids and the free radical scavenging and antioxidant activities of CCE may be due to the presence of various phenols and flavonoids. 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 (Middleton and Chithan, 1993; Harborne and Baxter, 1999; Harborne and Williams 2000), which have been found to exert beneficial effect on human health.

CONCLUSIONS

The importance of free radical generated oxidative stress cannot be underestimated as free radicals are essential in many cellular pathways and at the same time excess generation leads to oxidative stress and several free radical related diseases. CCE has scavenged DPPH, hydroxyl, superoxide, FRAP and nitric oxide free radicals in a concentration dependent manner indicating its potential as an antioxidant agent. The antioxidant activity of CCE may be attributed to the presence of various phenolic compounds and other phytochemicals. The *Croton caudatus* leaves could be a potential source of natural antioxidants that may act as therapeutic agent in preventing or slowing the progression of oxidative stress related degenerative diseases.

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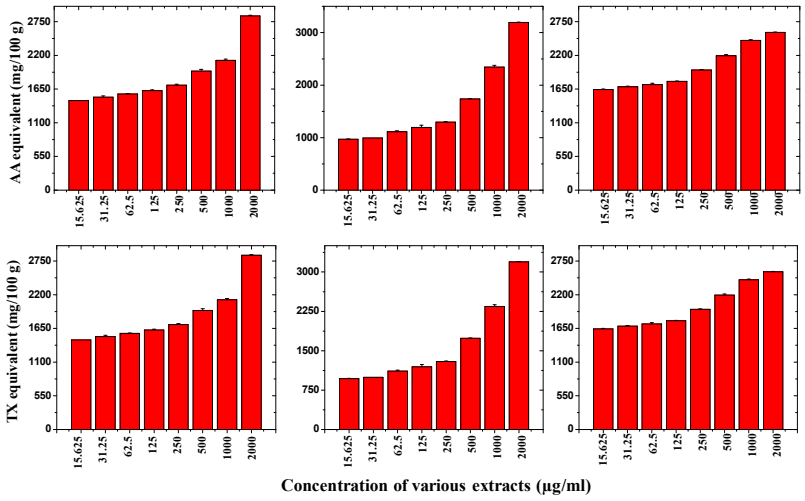

Fig. 1: Inhibition of DPPH radical formation by various concentrations of *Croton caudatus in vitro*. Left panel: chloroform ; Middle panel: ethanol and Right panel: aqueous extracts. AA: Ascorbic acid; TX: Trolox .

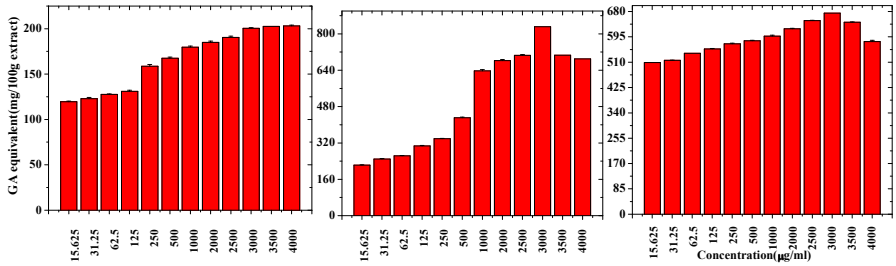

Fig. 2: Inhibition of hydroxyl radical formation by various extracts of *Croton caudatus in vitro*. Left : chloroform; Middle: ethanol and Right: aqueous extracts. GA : Gallic acid.

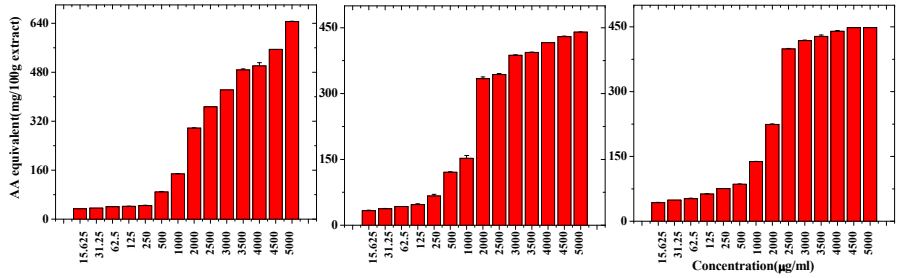

Fig. 3: Inhibition of super oxide radical formation by various extracts of *Croton caudatus in vitro*. Left: chloroform; Middle : ethanol and Right: aqueous extracts. AA : Ascorbic acid

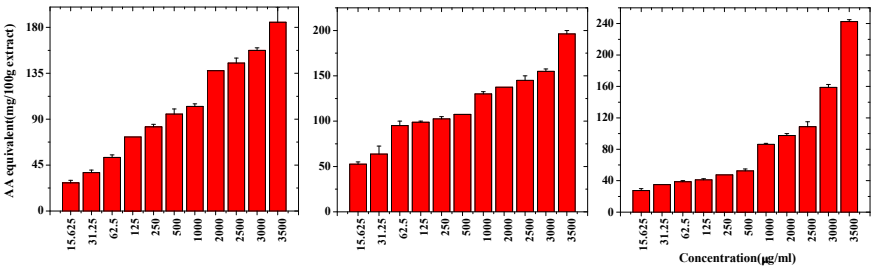

Fig. 4: Inhibition of Nitric oxide radical formation by various extracts of *Croton caudatus in vitro*. Left : chloroform; Middle : ethanol and Right: aqueous extracts. AA : Ascorbic acid.

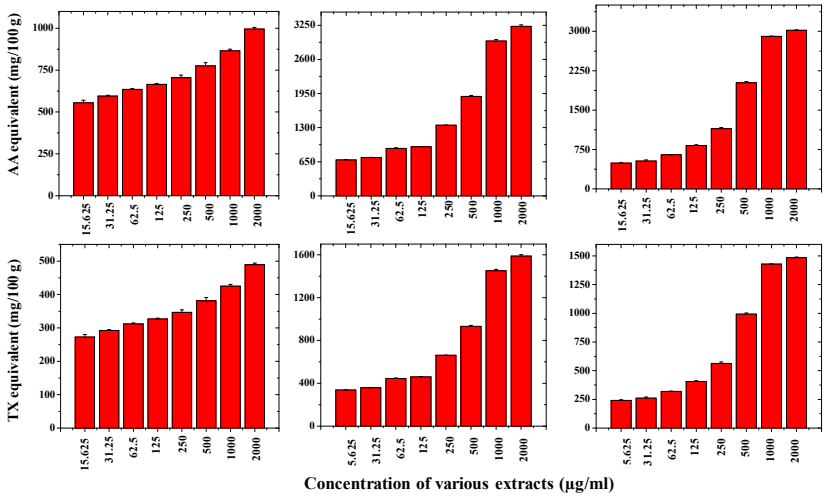

Fig. 5: Inhibition of FRAP radical formation by various concentrations of *Croton caudatus in vitro*. Left panel: chloroform ; Middle panel: ethanol and Right panel: aqueous extracts. AA: Ascorbic acid and TX: Trolox.

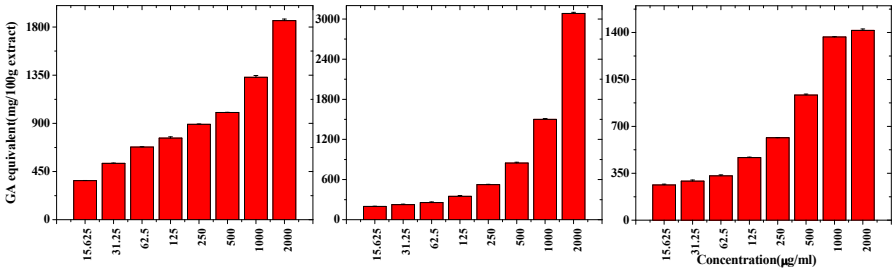

Fig. 6 : Total phenol contents in various extracts of *Croton caudatus in vitro*. Left : chloroform; Middle: ethanol and Right: aqueous extracts. GA : Gallic acid.

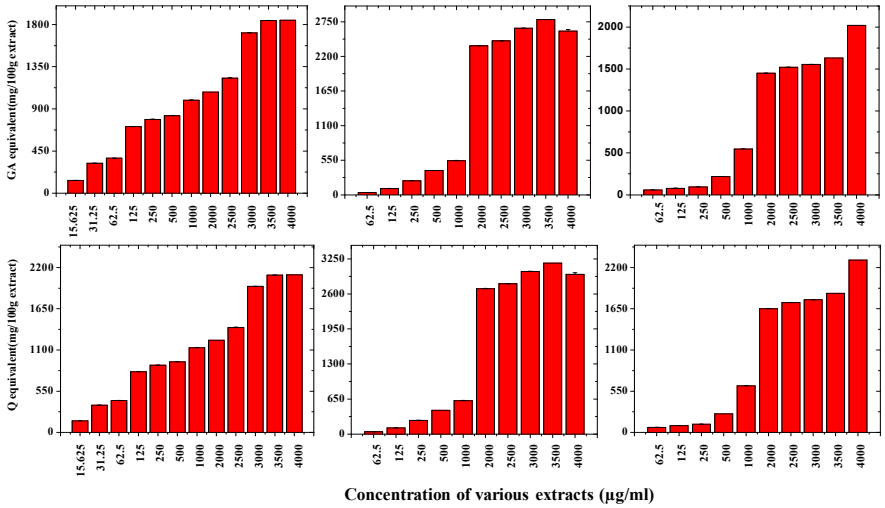

Fig. 7 : Total flavonoid contents in various extracts of *Croton caudatus in vitro*. Left panel: chloroform; Middle panel: ethanol and Right panel: aqueous extracts. GA: Gallic acid ; Q: Quercetin.