Indian Journal of Natural Products and Resources
Vol. 10(1), March 2019, pp 59-67

Nutritional composition and antioxidative stress properties in boiled tuberous rhizome of *Neel Kamal* (*Nymphaea nouchali* Burm. f.)

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Received 13 November 2017; Revised 15 March 2019

Nymphaea nouchali (Burm. f.) is known as *Neel Kamal, Utpal*, *Kumud* and *Indeevar* in India. Its tuberous rhizome is used for the preparation of various vegetable items, curries and pickles, and is also consumed boiled or roasted. It finds application in folk medicine for the treatment of a number of disease conditions. In this research macro- and micronutrients profiling along with phytochemical analysis of the boiled tuberous rhizome of *N.nouchali* was carried out. Furthermore, antioxidant activities and antioxidative stress potentials of aqueous methanol extract of boiled tuber were also evaluated. Boiled tuber presents a rich source of carbohydrate, protein, fat, fibres, essential amino acids and fatty acids. Simultaneously, vitamins and minerals were also present in ample amounts. The aqueous methanol extract of boiled tuber contains potent antioxidant phytochemicals. *In vitro* analysis on HEK-293, CHO and NIH 3T3 cell lines reveal that it effectively quashes H_2O_2 induced oxidative stress and protects DNA against free radical-induced damage. This research demonstrates that *N. nouchali* tuber can become an economical dietary adjunct and functional food full of macro- and micronutrient that can help fight against oxidative stress originating due to modern lifestyle induced metabolic disorders. This is the first report presenting nutritional composition and antioxidative stress properties in the boiled tuber of *N. nouchali*.

Keywords: Antioxidant, Antioxidative stress property, DNA damage, *Neel kamal*, Nutritional composition, *Nymphaea nouchali*.

IPC code; Int. cl. (2015.01)- A61K 36/00, A61P 39/00

Introduction

The grace and beauty of water lilies (*Nymphaea*) had impressed mankind for eons for its flower and tuberous rhizome. From ancient time, the tuberous rhizome of *Nymphaea* species has been the staple food for many savage races and aesthetic food of civilized people[1,2]. Underprivileged people used tuberous rhizome of *Nymphaea nouchali* Burm. f. (Syn. *Nymphaea stellata* Willd., Family *Nymphaeaceae*) as food and medicine[1,3-5].

In India, *N. nouchali* is known as *Neel Kamal, Utpal, Kumud* and *Indeevar*. Henceforth in this article, the tuberous rhizome will be referred to as tuber. In many parts of the world, it is eaten raw, roasted or boiled. In Bangladesh and some Indian states also, different types of vegetable, curries and pickles are prepared with its tuber. In India, wild *N. nouchali* is used as food[4,6] and is considered a rich source of starch, protein and fibres[7]. In Sri Lanka, it is cultivated in rice-fields during monsoon period to gather edible tubers[8]. A toxic alkaloid nupharin is reported to occur in the raw tuber of *Nymphaea* species[2]. It is reported that the toxic effect of this chemical gets neutralized when the tuber is cooked[8]. In Assam and West Bengal states of India, the tuber of *N. nouchali* is called *Sheluk*. Villagers of these states collect tuber for their own utilization and boil in water adding a pinch of salt whereas flower is sold in the market for monetary gains[6].

Tubers of *N. nouchali* are astringent and tonic in nature and have been used in diarrhoea, colic, dysentery, dyspepsia, and also used as emollient, diuretic, for back and stomach ache in South-East Asia[8]. Raw tubers have been accorded best medicine for dysentery[4] and possess cooling properties[2]. In Sri-Lankan folk medicine, the tuber is used for cystitis, nephritis, enteritis, fever and in insomnia conditions[9]. A novel Ca^{2+}-dependent

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lectin with antiproliferative activity has been identified from *N. nouchali* tuber[10]. The tuber of *N. nouchali* along with refined jaggery and roots of *Lawsonia inermis* ground with rice-washed water is prescribed for diabetic people in India[4]. Antihyperglycemic and antihyperlipidemic properties in *N. nouchali* tubers have also been reported recently[11].

This research analyzed nutritional contents and phytochemical constituents in the boiled tuber of *N. nouchali*. Furthermore, in order to validate its healthful effect, the antioxidant activities and antioxidative stress properties of its aqueous methanol extract were also carried out.

Materials and Methods

Sample collection and preparation

Solid tubers of *N. nouchali* were collected from a pond situated near Chebrulu village of East Godavari district in Andhra Pradesh state of India in the month of March 2017. The pond is situated in the forest area. The pond was devoid of any effluents drainage and pollution activities. Its taxonomic identification was carried out by Professor Dr. Ajmeera Ragan (Department of Botany, Kakatiya University-Warangal, Telangana). The voucher specimen with accession number KUW-1924 was deposited in Kakatiya University herbarium, Warangal.

Tubers were washed properly with clean water to remove muds. Whole tubers with scales were water-boiled in cooking-pot covered with a lid on light flame for 10-15 minutes. Tubers were removed from hot water and cooled. Scales covering tubers were removed. Peeled tubers were chopped into pieces and shade dried. Shade-dried tuber pieces were powdered in food grade mixer-grinder and stored away from direct sunlight at room temperature in air-tight lid bottle.

Nutritional analysis

Nutritional analysis of boiled tuber powder was carried out at MFPI-Quality Control Laboratory of PJTS Agricultural University, Hyderabad (India).

Proximate analysis

Analysis of carbohydrate[12], protein[13], fat[13], fibre[13], ash[14] and moisture[14] contents and acidity value[15] was determined following standard methodologies.

Sugars

Total as well as reducing sugars present in *N.nouchali* boiled tuber powder was analyzed following Somogyi method[16].

Vitamins

Vitamins such as riboflavin, thiamine and niacin were quantified by high performance liquid chromatography method (HPLC) [17]. Ascorbic content was titrated following method as described by Ranganna[18].

Amino acids

Analysis of amino acids was carried out by (HPLC) applying photodiode array (DAD) and fluorescence detection simultaneously (DAD: 338 10 nm; Ref 390 20 nm, Fluorescence: *Ex* 340 nm, *Em* 450 nm)[19].

Fatty acids

Gas chromatographic analysis of fatty acid methyl esters (FAME) was performed and relative retention time of FAME peaks was compared with standard samples of fatty acid for their identification.

Minerals

Iron, zinc and calcium content in the boiled powder of *N. nouchali* tuber were measured applying inductively coupled plasma optical emission spectrometry (ICP-OES) technique[20].

Phytochemicals

Aqueous methanol (1:1) extract of powder was prepared as described earlier[11] for evaluation of biological activities.

Total polyphenol

Total polyphenol content in aqueous methanol extract was measured using Foli-Ciocalteu reagent[11]. Absorbance at 765 nm was recorded spectrophotometrically and results were expressed as gallic acid equivalence.

Total Flavonoid

An equal volume of 2% aluminium chloride was mixed with aqueous methanol extract of tuber to quantify flavonoids content[11]. Absorbance was recorded at 430 nm spectrophotometrically and results were expressed in terms of rutin equivalence.

Phytate

Phytate was extracted from powder with trichloroacetic acid and further precipitated with ferric salt. The iron content of precipitate was determined colourimetrically and phytate content was determined as described by Wheeler & Ferrel[21].

Oxalate

Total oxalate was extracted by hydrochloric acid (0.25 N, HCl). Titration method using permanganate was applied for the determination of oxalate[22].

Total carotenoids

The spectrophotometric technique described by Zakaria *et al.*[23] was adopted for the determination of total carotenoids in tuber powder.

Lycopene

Lycopene from tuber powder was repeatedly extracted with acetone followed by petroleum ether. Lycopene colour was measured at 503 nm spectrophotometrically[18].

Antioxidant activity

[2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation ($ABTS^{·+}$) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging potentials as a measure of antioxidant activity was determined in the aqueous methanol extract of tuber[11]. Trolox served as the standard antioxidant reference compound.

Antioxidative stress potential in different cell lines

HEK 293 (Human embryonic kidney cells), NIH 3T3 (Mouse embryonic fibroblast) and CHO (Chinese hamster ovary) cells were grown in tissue culture flask in DMEM (Dulbecco modified eagle medium, Sigma) supplemented with 10% fetal bovine serum and 1× antibiotic solution (Sigma) in a CO_2 incubator at 37 ˚C (5% CO_2 and 90% relative humidity).

Hydrogen peroxide (H_2O_2) induced oxidative stress and effect of aqueous methanol extract on HEK-293, NIH-3T3 and CHO cells

The effect of aqueous methanol extract on H_2O_2 induced oxidative stress on cells was determined by MTT assay[24]. HEK-293, NIH 3T3 and CHO cells (1×10^6) were seeded in 96 well plates for 24 h. After 24 hours of incubation, cells were treated with different concentrations of extract for 48 h in the presence and absence of 10 µM H_2O_2. After 48 hours of incubation, 10 µL MTT (3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide-Sigma, 5mg/mL) was added to each well and plates were further incubated for 4 hours at 37 °C in dark. Culture medium from each well was carefully removed out and 100 µL of di-methyl sulfoxide (DMSO) was added. Reduced MTT by metabolically viable cell was determined by measuring absorbance at 570 nm spectrophotometrically.

Antioxidative stress potential (AoxP) of test extract was calculated as follows:

$$100-[\{(A_c-A_t)/Ac\} \text{ x}100]$$

where 'A_c' represents absorbance of cells without H_2O_2 treatment and 'A_t' represent absorbance of cells treated with H_2O_2 in the presence or absence of extract.

Fluorescence-activated cell sorting (FACS) analysis of H_2O_2 induced oxidative stress in HEK-293 cells and effect of aqueous methanol extract

Quantification of antioxidative stress potential of the extract was evaluated by flow cytometric analysis in HEK-293 cells. HEK-293 cells were treated sham as above and incubated for 48 h in presence or absence of 10 µM H_2O_2. In order to detect H_2O_2 mediated reactive oxygen species (ROS), cells were treated with fluorogenic dye 2', 7' –dichlorofluorescin diacetate (DCFDA, 2 µM) at 37 ˚C for 30 minutes and flow cytometric analysis was carried out[25]. Numerical values generated in triplicate for DCF-positive cells were used to quantify antioxidative stress potential of the extract.

Free radical-induced DNA damage and effect of aqueous methanol extract

The method described by Chang *et al.*[26] was adopted with suitable modifications to assess free radicals induced damage to pUC18 DNA. The reaction was carried out in *tris*-buffer (pH 7.4) at 37˚C. $FeCl_3$ and H_2O_2 were used to generate hydroxyl radicals ($^{·}OH$). In an Eppendorf tube, for control test, pUC18 DNA (2 µg) was incubated with 5 µL *tris*-buffer. In another set, pUC18 DNA (2µg) along with 5 µL of *tris*-buffer was enacted with $FeCl_3$ (5 µL) and 10 µL of 30% H_2O_2. The next set was prepared with pUC18 DNA (2 µg), 5 µL of *tris*- buffer and 5-10 µL of extract (5 mg/mL prepared in *tris*- buffer) and incubated for 10 minutes at room temperature. $FeCl_3$ (5 µL) and 10 µL of 30% H_2O_2 were added to induce a free radical reaction. The volume of the reaction mixture was equalized with the addition of *tris*-buffer. Tubes were incubated at 37 ºC for 15 minutes. To the reaction mixture, 3 µL of 6x gel loading dye was added. Electrophoresis was performed on 0.8% agarose gel containing 3 µL Ethidium Bromide (10 mg/mL), at 85 V for 35 minutes. Gels were viewed under transilluminating UV light and photographed (Bio-Rad ChemiDoc™ XRS+ with Image Lab™ Software).

Statistical analysis

The results were analyzed using one way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test. The criterion for statistical significance was set at $p < 0.05$. Statistical analysis was performed by using GraphPad PRISM Version 5.01 (GraphPad Software Inc. California, USA). Results were expressed as mean±SEM, n=3.

Results and Discussion

Nutritional composition

Table 1 presents profiles of proximate compositions, sugars, vitamins, minerals and phytochemicals analyzed in the boiled tuber of *N. nouchali*. It is evident from the table that apart

from macronutrients such as carbohydrate, protein, fat and sugars, *N. nouchali* tuber is also a rich source of micronutrients such as ascorbic acid, niacin, riboflavin, thiamin, calcium, iron and zinc. Among non-nutrient factors, polyphenols, flavonoids and carotenoids were amply detected. There are few reports examining the proximate compositions in the dry tuber of *Nymphaea lotus* (L.)[27]. The nutrient and non-nutrient content in the tuber of *Nymphaea petersiana* are also available in literature[28]. The protein and fat contents in *N. nouchali* tuber are very much similar to that present in *Nymphaea lotus* however, the moisture and ash contents were two to three time higher in *Nymphaea lotus*[26]. Analysis of certain nutrient and non-nutrients present in *N. nouchali* of our study are in proximity of the reported literature[27]. The oxalate content in the boiled tuber of *N. nouchali* was estimated to be 25 mg/100 g (Table 1). It is pertinent to mention here that the oxalate-rich foods present a risk of calcium-oxalate crystals formation and result in the development of kidney stones. At the same time, calcium-rich foods are shown to prevent the development of hyperoxaluria and reduce crystallization of calcium oxalate[29]. Therefore, the formation of calcium-oxalate crystals due to the consumption of *N. nouchali* tuber may be averted due to the presence of about six times more calcium content in boiled tuber as observed in our analysis (Table 1).

Table: 1 Nutritional profile of boiled tuber powder of *N. nouchali*

Proximate	Carbohydrate	76.5%
	Crude Protein	10.76 %
	Crude Fat	2.40 %
	Crude Fibers	0.64 %
	Ash	3.0 %
	Moisture	9.07 %
Sugars	Total Sugars	6.06 %
	Reducing Sugars	1.26 %
	Acidity	1.18 %
Vitamins	Ascorbic Acid	3.12 mg/100g
	Riboflavin	1.11 mg/100g
	Thiamine	0.05 mg/100g
	Niacin	1.45 mg/100g
Minerals	Iron	1.98 mg/100g
	Zinc	1.33 mg/100g
	calcium	148.55 mg/100g
Phytochemicals	Total Polyphenols	0.12 mg/mL Gallic acid equivalent
	Total Flavonoids	50.63 mg/mL Rutin equivalent
	Total carotenoids	115.22 µg/100g
	Anthocyanin	0.16%
	Lycopene	0.007 µg/100g
	Phytate	149.86 mg/100g
	Oxalate	24.97 mg/100g

Plant polyphenols[30] and carotenoids[31] constitute an important class of dietary antioxidants. These phytochemicals have emerged as new generation therapeutics for treatment and management of a number of modern lifestyle induced diseases[32]. Supplementation of dietary antioxidants in moderation has been shown to reduce markers of oxidative stress in geriatric population[31]. Result presented in table 1shows that boiled tuber of *N. nouchali* contains in ample amount these phytochemicals.

Plant foods providing about 12% of the calorific value from protein are categorized as a good source of protein[33] and nutritional value of protein is determined by the kind and quantity of amino acids present in proteinaceous diet[28]. The amino acids composition of boiled *N. nouchali* tuber is presented in Fig. 1. Among nine essential amino acids required to be taken through diet, eight (isoleusine, histidine, leucine, methionine, lysine, phenylalanine, threonine and valine) were identified in the boiled tuber. The essential amino acid tryptophan could not be identified in our analysis (Fig. 1). The co-essential amino acid arginine, cysteine, glycine, proline, and tyrosine except for glutamine were also detected in our analysis. The sulfur amino acids methionine and cystine, and aromatic amino acids phenylalanine and tyrosine were also present in *N. nouchali* tuber (Fig. 1). Presence of a majority of essential and co-essential amino acids in the boiled tuber of *N. nouchali* makes it an important source of amino acids for; they are primarily responsible for maintenance and activity of muscle protein anabolism[34].

Availability of essential fatty acids such as linoleic acid (LA, n-6 fatty acid) and linolenic acid (LNA, n-3 fatty acid) through diet is important for the maintenance of health and management of chronic diseases[35]. Fatty acids profiling of boiled tuber of *N. nouchali* is presented in Fig. 2. Presence of dietary n-3 and n-6 fatty acids balance is an important criterion to maintain the health of cardiovascular system[36]. The ratio of n-6/n-3 of ~6:1 and LA (~6%) and LNA (0.75%) represent adequate dietary value[36]. The fatty acid compositions presented in Fig. 2 are in close proximity to this suggestion. Therefore, this food material may become an ideal source of balanced dietary essential fatty acids.

Oleic acid (n-9 fatty acid) has attracted the attention of researchers in the recent past due to its multiple therapeutic applications. Our analysis finds

that oleic acid is also an important constituent in *N. nouchali* tuber (Fig. 2). Dietary Oleic acid offers protection against free radicals induced damage to the cell membrane and resists oxidative stressors better than n-3 or n-6 fatty acids[37]. Diet high in oleic acid is reported beneficial for hypertensive patients and in improving high density lipoprotein cholesterol[38]. Recently, dietary oleic acid has been shown to reduce levels of inflammation arising due to obesity[39]. Furthermore, the failing heart is reported to restore normal functions when perfused with oleic acid[40]. Therefore, *N. nouchali* tuber may become a richer source of dietary oleic acid for the benefit of patients suffering from disorders involving inflammation as a precursor of disease conditions.

Antioxidative stress capacity

Evolutionarily, aerobic organisms have been strained with oxidative stress in order to become bioenergetically more competent and efficient[41]. Since oxidative stress is the result of normal metabolic activities, aerobic organisms are equipped with an inbuilt antioxidant defence mechanism. However, under consistent and over strained circumstances, this defence mechanism fails to protect bodily damages induced due to a variety of free radicals arising under oxidative stress conditions. Therefore, easing out of oxidative stress now-a-days has become a core subject to maintain better health. A diet that encompasses antioxidative stress potentials, therefore, offers a better choice for

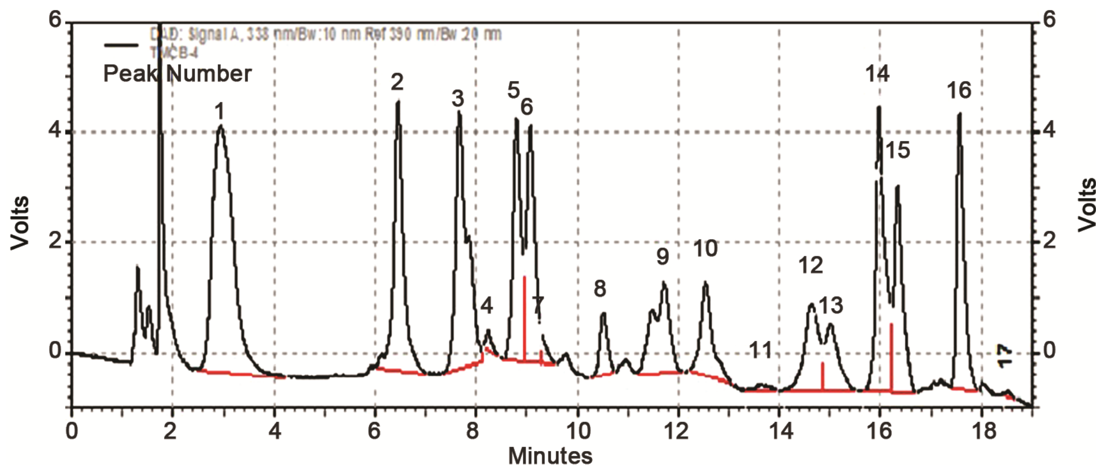

Fig. 1 — HPLC chromatogram showing peaks of identified amino acids in boiled tuber powder of *N. nouchali* . Detection: DAD: Signal A, 338 nm/Bw: 10 nm Ref 390nm Bw: 20 nm Peak denotation: 1. Aspartate; 2. Glutamate; 3. Serine; 4. Histidine; 5. Glycine; 6. Threonine; 7. Arginine; 8. Alanine; 9. Tyrosine; 10. Cystine; 11. Valine; 12. Methionine; 13. Phenylalanine; 14. Isoleucine; 15. Leucine; 16. Lysine; and 17. Proline.

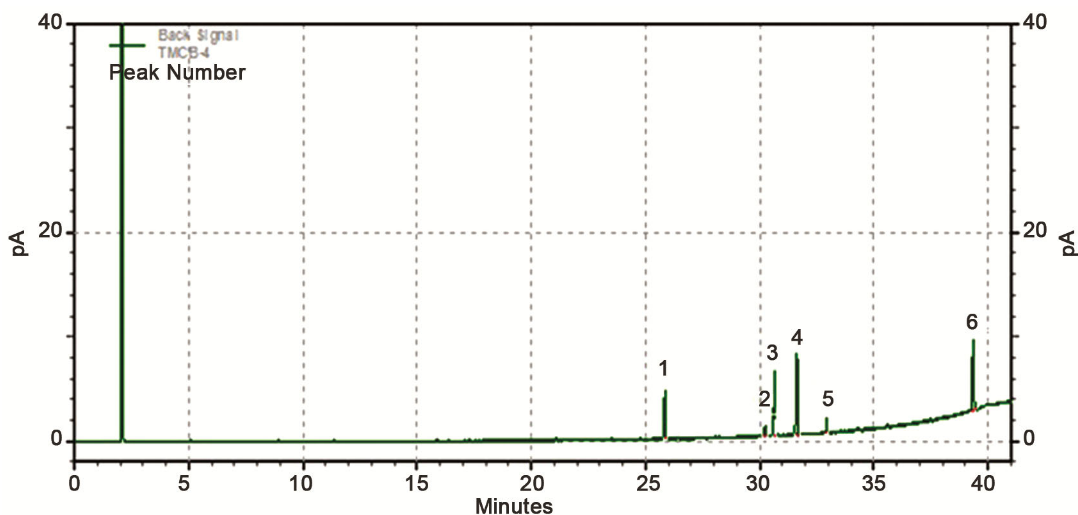

Fig. 2 — Gas Chromatographic analysis of fatty acid methyl esters (FAME) of boiled tuber powder of *N. nouchali*. Peak denotations: 1. Palmitic acid; 2. Stearic acid; 3. Oleic acid; 4.Linolenic acid; 5. A-Linolenic acid; and 6. Cis-13, 16-Docosadienoic acid.

health maintenance and fight against oxidative stress arising due to a disturbance in metabolic activities.

$ABTS^{\cdot+}$ radical cation and DPPH free radical were effectively scavenged by aqueous methanol extract of *N. nouchali* tuber (SC_{50} value 0.24µg for $ABTS^{\cdot+}$, SC_{50} value 0.29 µg for DPPH). These results show that it has the capacity to fight against oxidative stress in demanding situations. To test this ability of N. nouchali extract, we challenged three normal cell lines to H_2O_2 in order to induce oxidative stress. The percentage of cell viability was measured as an antioxidative stress capacity of extract. Results are presented in Fig. 3. H_2O_2 differentially induced oxidative stress in different cell lines. Development of oxidative stress due to H_2O_2 was recorded more in NIH 3T3 (43.31%, viable cells) followed by HEK-293(66.9%, viable cells) and CHO (78.99%, viable cells) cell lines (Fig. 3). These results demonstrated that preconditioning of cells with *N. nouchali* tuber extract significantly prevented the development of oxidative stress induced by H_2O_2. It is important to mention here that the degree of protection offered by different concentrations of extract varied in different cell lines. Although, extract significantly ($p < 0.01$) reduced H_2O_2 induced oxidative stress in HEK-293 cells, increasing extract concentration did not offer additional benefits (Fig. 3a). In CHO cell lines, 0.1µg concentration could not offer significant protection against H_2O_2 induced oxidative stress however, significant ($p < 0.05$) protection was evident when concentration was increased (Fig. 3b). Contrarily, however, tuber extract offered significant ($p < 0.001$) protection to NIH 3T3 cells against H_2O_2 induced oxidative stress, with increasing concentration activity decreased significantly ($p < 0.01$) when compared with a lower dose (Fig. 3c). These observations caution that too much of the antioxidant supplementation may not be always beneficial. Clinical studies with antioxidant concentrated food supplements have recently raised such issues and warranted against the use of such supplements[42].

Antioxidative stress potential of *N. nouchali* boiled tuber extract was further validated applying fluorescence-activated cell sorting (FACS) analysis. DCFDA fluorescence probe was used as an indicator of ROS generation and oxidative stress. Representative FACS diagram and histogram of triplicate analysis is presented in Fig. 4. DCFDA passively diffuses into the cells and gets oxidized by ROS into the fluorescent product known as 2', 7'-dichlorofluorescin (DCF). Right panel shifts (blue) in Fig. 4 depicts DCF-positive cells representing H_2O_2 induced oxidative stress. It was found that H_2O_2 treatment generated

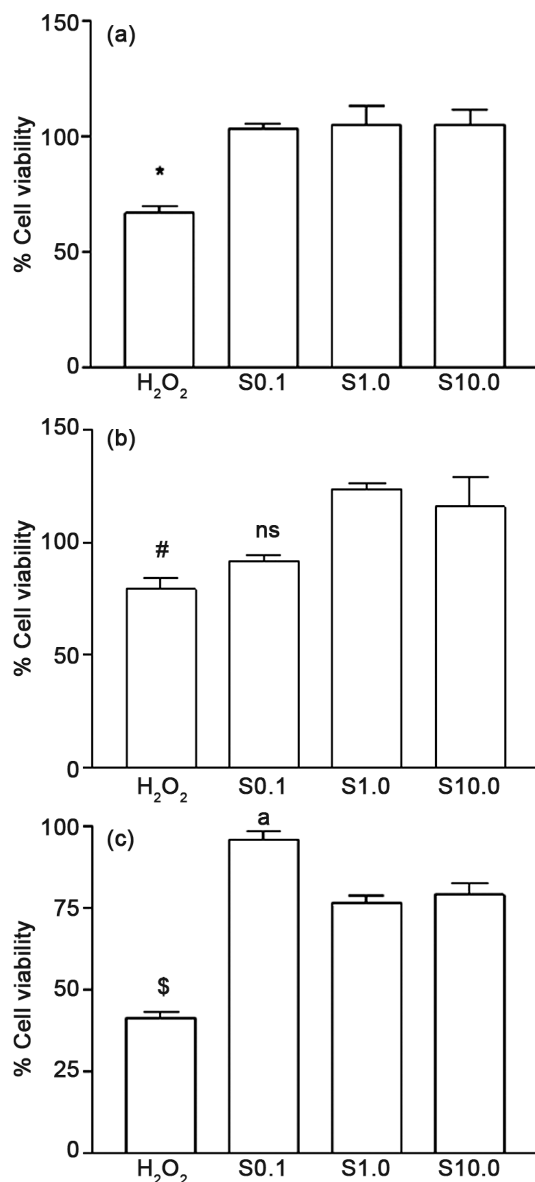

Fig. 3 — Induction of oxidative stress by H_2O_2 in three different types of normal cell lines and the effect of AqMeOH extract of the boiled tuber of *N. nouchali.* (a) HEK-293 cells, (b) CHO cells, and (c) NIH 3T3 cells. S0.1=0.1 µg of tuber extract, S 1.0=1.0 µg of tuber extract and S10.0= 10 µg of tuber extract. Data represent mean±SEM, n=3. One way ANOVA followed by Tukey's Multiple comparison test was applied to assess the degree of significance within the groups. *$p < 0.01$ when compared with S0.1, S1.0 and S10.0. $^{\#}p < 0.05$ when compared with S1.0 and S10.0. S0.1, S1.0 and S10.0 not significant when compared among themselves. P=ns when compared with H_2O_2 and S0.1. $^{\$}p < 0.001$ when compared with S0.1 S1.0 and S10.0. $^{a}p < 0.01$ when compared with S1.0 and S10.0. p=ns (not significant) when compared with S1.0 and S10.0.

significantly (p <0.0001) higher level of oxidants when compared with non- H_2O_2 treated (control) HEK-293 cells. However, preconditioning of cells with *N. nouchali* boiled tuber extract significantly (p <0.0001) mitigated H_2O_2 induced ROS generation in HEK-293 cells (Fig. 4).

Free radical induced damage to DNA has been linked to the etiology of numerous disease conditions[43]. Protective agents against oxidative DNA damage have been identified in dietary materials[44]. We induced hydroxyl radical mediated damage to pUC18 DNA[25] and found that *N. nouchali* tuber extract was effective in preventing hydroxyl radicals induced damage to DNA (Fig. 5).

Diet and beverages in the modern industrialized world have become highly processed calorie-rich and energy-dense however, they lack micronutrients, vitamins, minerals and essential dietary co-factors in sufficiency[45]. Micronutrients rescind diet results in the development of metabolic disorders and consequent oxidative stress. Conversely, whole grain based food accompanied with micronutrients and phytochemicals offer better management of metabolic disorders and counter oxidative stress developed beyond optimal physiological control[46-48]. The amino acids aspartate, glutamate, tyrosine, cysteine, and homocysteine present in *N. nouchali* tuber are not only building blocks of proteins but also serve the purpose of alleviating oxidative stress[49,50]. Similarly, fatty acids such as oleic acid[37] and linoleic acid[51] resist oxidative stress induced damage to biomolecules. These fatty acids are an important constituent of

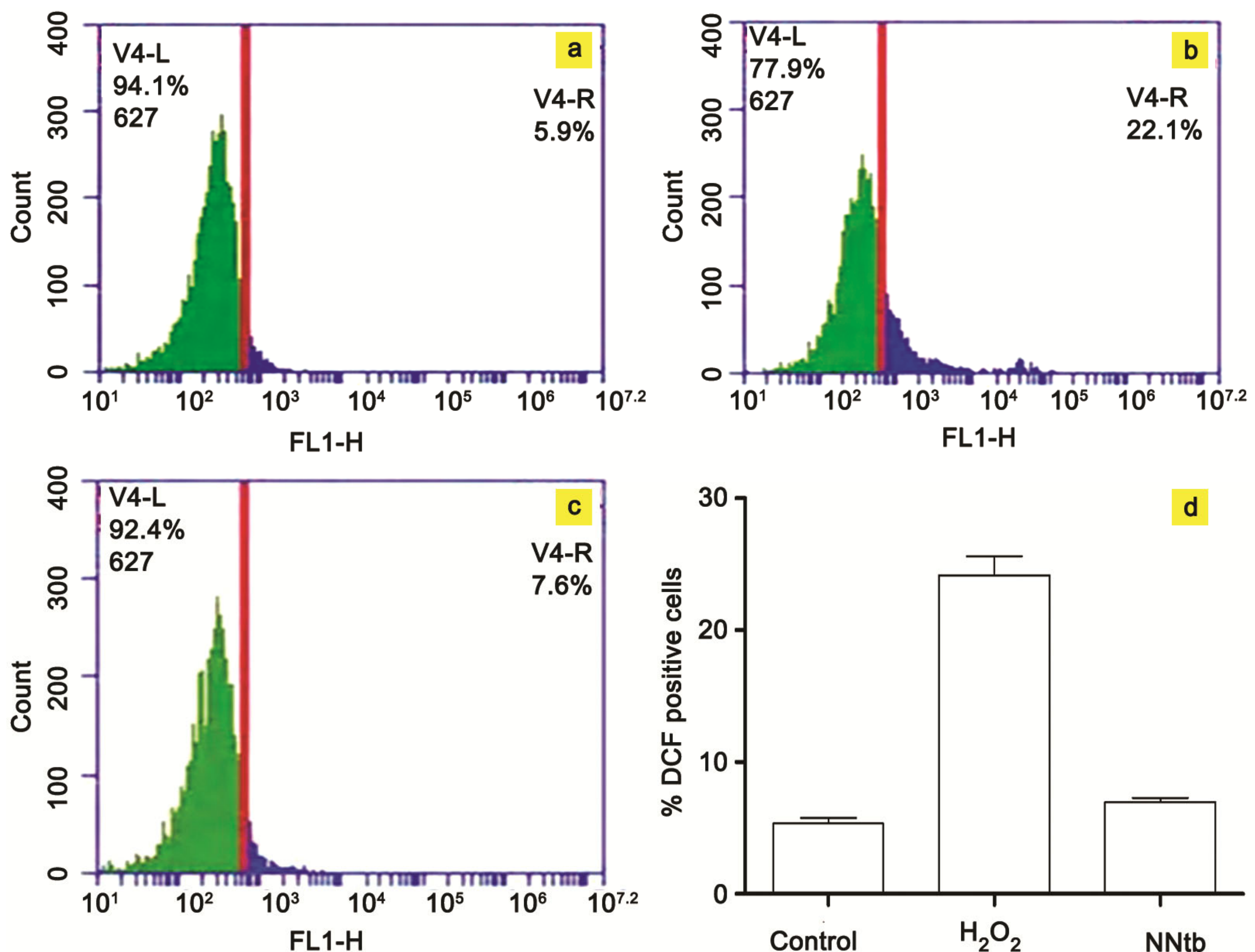

Fig. 4 — Representative diagram of flow cytometric analysis of HEK-293 cells. (a) HEK-293 control cells; (b) HEK-293 cells+H_2O_2 (10 µM); and (c) HEK-293 cells + NNTb (1 µg) +H_2O_2 (10 µM). (d) The histogram presents DCF-positive HEK-293 cells representing the induction of oxidative stress induced due to H_2O_2. Data represent mean±SEM, n=3. One way ANOVA followed by Tukey's Multiple comparison test was applied to assess the degree of significance within the groups. *p <0.0001 when compared with control cells as well as *N. nouchali* boiled tuber (NNTb).

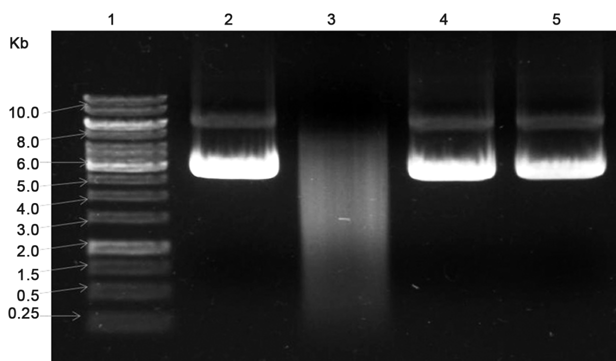

Fig. 5 — Protective effect of free radical induced plasmid DNA damage by AqMeOH extract of the boiled tuber of *N. nouchali*. Lane 1= Marker plasmid DNA, Lane 2= DNA without treatment, Lane 3= DNA+free radicals, Lane 4= DNA incubated with 25 µg extract followed by induction of free radicals reaction, Lane 5= DNA incubated with 50 µg extract followed by induction of free radicals reaction.

N. nouchali tuber. Polyphenols[30], carotenoids[31] and lycopene[52] are well researched antioxidant phytochemicals. They are amply present in *N. nouchali* tuber. The observed antioxidative stress potentials of tuber extract in our study on different cell lines might be originating due to the presence of these multiple antioxidant property bearing micronutrients and phytochemicals. Apart from antioxidative properties, *N. nouchali* tuber extract has also been reported recently to bear antihyperglycemic and antihyperlipidic properties[11]. Taken together, this research demonstrates that tuber of *N. nouchali* can become an economical dietary adjunct of choice to fight metabolic disorders and consequent oxidative stress.

Conclusion

In conclusion, our analysis finds that *N. nouchali* tuber not only possesses dietary macronutrients in sufficient quantity but also it is a rich source of micronutrients and phytochemicals that demonstrate multiple therapeutic and beneficial health properties. Therefore, promotion and inclusion of its tuberous rhizome in modern dietary milieu may become advantageous in fighting diseases of metabolic disturbances. To the best of our knowledge, this is the first comprehensive report analyzing the nutritional composition and antioxidative stress potentials in the boiled tuber of *N. nouchali*.

Acknowledgement

The research was financially supported in part by CSIR Net-Work Program under NaPAHA project (CSC-0130). Ajay Anand thanks UGC-New Delhi for JRF. CSIR-IICT manuscript communication number: IICT/Pubs./2019/099

Conflict of interest

Authors declare no conflict of interest.

References

1 Conard H S, The waterlilies a monograph of the genus *Nymphaea*, *Carnegie Institution of Washington*, 1905, **4**.

2 Irvine F R and Trickett R S, Water lilies as food, *Kew Bull*, 1953, **8**(3), 363-370.

3 Seshavatharam V, Traditional uses and problem of noxious growth, Ecology and Management of Aqautic Vegetation in the Indian Subcontinent, *Springer*, 1990, 201-218.

4 Panda A and Misra M K, Ethnomedicinal survey of some wetland plants of South Orissa and their conservation, *Indian J Tradit Know*, 2011, **10**(2), 296–303.

5 Lim T K, Edible medicinal and non medicinal plants: *Flowers,* 2014*,* **8**, 519-520.

6 Roy D K, Talukdar A D, Choudhry M D and Sinha B K, Less known use of *Nymphaea species* (Nymphaeaceae) as the traditional food item (Vhet-Laddu) in Northeast India, *Int J Food Agr Vet Sci*, 2013, **3**, 82-87.

7 Pareek A and Kumar A, Nutraceutical value of aquatic plants*, J Acad*, 2014, **1**, 10-21.

8 Schmelzer G H, Plant resources of south-east asia*,* Medicinal and poisonous plants 2, *Backhuys publisher Leiden, The Netherlands*, 2001, 12(2).

9 Jayaweera D M A, Medicinal Plants Used in Ceylon, *The National Science Foundation*, Colombo, 1982, 50-55.

10 Kabir S R, Zubair M A, Nurujjaman M, Haque M A, Hasan I, *et al.*, Purification and characterization of a Ca2+-dependent novel lectin from *Nymphaea nouchali* tuber with antiproliferative activities, *Bioscience Reports,* 2011, **31**, 465-475.

11 Priyanka U, Anand A, Koppala B, Zehra A and Tiwari A K, Presence of postprandial antidysmetabolic and antioxidative stress properties in aqueous methanol extract of seeds and tuber of aquatic food plant *Nymphaea nouchali* (Burm.f.), *Cogent Food Agric*, 2016, **2**(1), 1-9.

12 IS 1656:2007, Scheme of testing and inspection for certification of milk-cereal based complementary foods.

13 Horwitz W and Latimer G W, Official methods of analysis of AOAC International, *Gaithersburg MD AOAC International*, 2007 (Online).

14 IS: 1155, Indian standard specification for wheat atta, *Handbook of food analysis*, 1968.

15 IS 13844, Fruit and vegetable products-determination of titratable acidity (first revision), 2003 ISO 750 1998.

16 Somogyi M, Notes on sugar determination, *J Biol Chem*, 1952, **195**, 19-23.

17 Anyakora C, Afolami I, Ehianeta T and Onwumere F, HPLC analysis of nicotinamide, pyridoxine, riboflavin and thiamin in some selected food products in Nigeria, *Afr J Pharm Pharmacol*, 2008, **2**(2), 29-36.

18 Ranganna S, Hand book of analysis and quality control for fruits and vegetable products, *Tata McGraw hill pub book Co, New Delhi*, 2001, 594-625.

19 Steed R, Analysis of amino acids by HPLC, *Agilent Technologies Inc*, 2010.

20 ICP-OES, Determination of metals by ICP-MS and ICP-OES (Optical emission spectrometry), *United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science*, CLG-TM3.05.
21 Wheeler E L and Ferrel R E, A method for phytic acid determination in wheat and wheat fractions, *Cereal Chem*, 1971, **48**(3), 312-320.
22 Sanchez-Alonso F and Lachica M, Oxalate salts in the leaves of plum (*Prunus salicina* L.) and cherry (*P. avium* L.), *New Phytologist*, 1988, **108**(4), 505-508.
23 Zakaria M, Simpson K, Brown P R and Krstulovic A, Use of reversed-phase high-performance liquid chromatographic analysis for the determination of provitamin A carotenes in tomatoes, *J Chromatogr A*, 1979, **176**(1), 109-117.
24 Botta M, Armaroli S, Castagnolo D, Fontana G, Pera P, *et al.*, Synthesis and biological evaluation of new taxoids derived from 2-deacetoxytaxinine, *Bioorg Med Chem Lett*, 2007, **17**(6), 1579-1583.
25 Ding G, Liu F, Yang T, Jiang Y, Fu H, *et al.*, A novel kind of nitrogen heterocycle compound induces apoptosis of human chronic myelogenous leukemia K562 cells, *Bioorg Med Chem Lett*, 2006, **14**(11), 3766-3774.
26 Chang M, Mohammed B,Charles B and Brown G W, A genome-wide screen for methyl methanesulfonate-sensitive mutants reveals genes required for S phase progression in the presence of DNA damage, *Proc Natl Acad Sci,* 2002, 99, 16934-16939.
27 Mohammed H A, Uka U N and Yauri Y A B, Evaluation of nutritional composition of water lily (*Nymphaea lotus* linn.) from Tatabu flood plain, North-central, Nigeria, *J Fish Aqua Sci*, 2013, **8**(1), 261-264.
28 Chawanje C M, Barbeau W E and Grun I, Nutrient and antinutrient content of an underexploited malawian water tuber *Nymphaea petersiana* (Nyika), *Ecol Food Nutr*, 2001, **40**(4), 347-366.
29 Hess B, Jost C, Zipperle L, Takkinen R and Jaeger P, High-calcium intake abolishes hyperoxaluria and reduces urinary crystallization during a 20-fold normal oxalate load in humans, *Nephro Dial Transpl*, 1998, **13**(9), 2241-2247.
30 Pandey K B and Rizvi S I, Plant polyphenols as dietary antioxidants in human health and disease, *Oxid Med cell Longev*, 2009, **2**(5), 270-278.
31 Nelson J L, Bernstein P S, Schmidt M C, Tress M S V and Askew E W, Dietary modification and moderate antioxidant supplementation differentially affect serum carotenoids, antioxidant levels and markers of oxidative stress in older humans, *J Nut*, 2003, **133**(10), 3117-3123.
32 Tiwari A K, Antioxidants: New-generation therapeutic base for treatment of polygenic disorders, *Curr Sci*, 2004, **86**, 1092-1102.
33 Aberoumand A, A comparative study of nutrients and mineral molar ratios of some plant food with recommended dietary allowances, *Adv J Food Sci Tech*, 2010, **2**(2),104-108.
34 Volpi E, Kobayashi H, Moore S M, Mittendorfer B and Wolfe R R, Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults, *Am J Clin Nutr*, 2003, **78**(2), 250-258.
35 Simopoulos A P, Essential fatty acids in health and chronic disease, *Am J Clin Nut*, 1999, **70**(3), 560s-569s.
36 Wijendran V and Hayes K C, Dietry n-6 and n-3 fatty acid balance and cardiovascular health, *Annu Rev Nutr*, 2004, **24**, 597-615.
37 Haug A, Høstmark A T and Harstad O M, Bovine milk in human nutrition – a review, *Lipids Health Dis*, 2007, **6**(1), 25.
38 Ruiz-Gutierrez V, Muriana F J G, Guerrero A, Cert A M and Villar J, Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources, *J Hypertension*, 1996, **14**(12), 1483-1490.
39 Vassiliou E K, Gonzalez A, Garcia C, Tadros J H, Chakraborty G, *et al.*, Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF-α both *in vitro* and *in vivo* systems, *Lipids Health Dis*, 2009, **8**(1), 25.
40 Lahey R, Wang X, Carley A N and Lewandowski E D, Dietary fat supply to failing hearts determine dynamic lipid signalling for nuclear receptor activation and oxidation of stored triglyceride, *Circulation,* 2014, **130**(20), 1790–1799.
41 Perez-Mutute P, Zulet M A and Martinez J A, Reactive species and diabetes: counteracting oxidative stress to improve health,*Curr Opinion Pharmacol*, 2009, **9**(6), 771-779.
42 Di Pierro F, Antioxidants and cancer: a debate on prevention, progression, hormesis, and cruciferous vegetables, *Nutrafoods*, 2015, **14**(4), 175-179.
43 Dizdaroglu M and Jaruga P, Mechanisms of free radical-induced damage to DNA, *Free Radic Res*, 2012, **46**(4), 382-419.
44 Siswoyo T R, Ardyati T and Hosokawa K, Fermentation-induced changes in antioxidant activities and oxidative DNA damage protection of melinjo (*Gnetum gnemon*) flour, *J Food Biochem*, 2017, **41**(4), e12382.
45 Gibbons A, The evolution of diet, *National Geographic Magazine*, 2014, **2**, 81–101.
46 Esmaillzadhe A, Mirmiran P and Azizi F, Whole-grain consumption and the metabolic syndrome: a favorable association in Tehranian adults, *Eur J Clin Nut*, 2005, **59**(3), 353-362.
47 Katcher I H, Legro R S, Kunselman A R, Gillies P J, Demers L M, *et al.*, The effect of a whole grain-enriched hypocaloric diet on cardiovascular disease risk factors in men and women with metabolic syndrome, *Am J Clin Nutr*, 2008, **87**(1), 79-90.
48 Tiwari A K, Praveen K M, Anand D K, Agawane S B, Madhusudana K, *et al*., Ayurvedic diedary formulations and postprandial glycemia in rats, *Int Food Res J*, 2012, **19**(2), 765-773.
49 Meucci E and Mele M C, Amino acids and plasma antioxidatnt capacity, *Amino Acids*, 1997, **12**(3-4), 373-377.
50 Duan J, Yin J, Ren W, Liu T, Cui Z, Huang X, *et al.*, Dietary supplementation with L-glutamate and L-aspartate alleviates oxidative stress in weaned piglets challenged with hydrogen peroxide, *Amino Acids*, 2016, **48**(1), 53-64.
51 Beeharry N, Jillian E L, Hernandez A R, Chambers J A and Fucassi F, *et al.*, Linoleic acid and antioxidants protect against DNA damage and apoptosis induced by palmitic acid, *Mutat Res Fund Mol M*, 2003, **530**(1-2), 27-33.
52 Muller L, Caris-Veyrat C, Lowe G and Bohm V, Lycopene and its antioxidant role in the prevention of cardiovascular diseases-A critical review, *Critl Rev Food Sci Nut*, 2016, **56**(11), 1868-1879.