International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Influence of Thermal Treatments on Polyphenolic Contents and Antioxidant Properties of Underutilized Edible Flowers of *Nelumbo nucifera* and *Nymphaea alba*

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ABSTRACT

The present study was proposed to investigate the antioxidant and radical scavenging property of aqueous acetone extract of raw flower petals and raw and processed (boiling and blanching) core parts from *Nelumbo nucifera* and *Nymphaea alba*. Total phenolics and tannin content of raw and processed petals and core extracts were ranged from 173.13 - 312.81mg/g extract and 149.50- 302.43mg/g extract respectively. In general, the processed core part extracts registered greater polyphenolic contents than the raw extracts. Interestingly, among the various processing methods, boiled core sample of *N. alba* registered higher DPPH (535121.6 mmol TE/g extract) and ABTS (302642.21 mmol TE/g extract), superoxide (74.92%), hydroxyl (73.4%) radical scavenging activity, metal chelating property (0.43 mg EDTA/g extract), FRAP (6156 mmol Fe (II)/g extract). These results revealed that processing methods significantly increased the content and potential of antioxidant components of *N. nucifera* and *N. alba*.

Keywords: *Nelumbo nucifera*, *Nymphaea alba*, underutilized edible flowers, polyphenols, antioxidant activity.

1. INTRODUCTION

Edible plants which are rich in phenolics and flavonoids have been reported to potential free radical scavengers, those are found in all parts of plants such as leaves, fruits, seeds, roots and bark. However, flower is an important part of plant which contains a great variety of natural antioxidants such as many of the phenolic compounds. In most of the countries it is believed that consumption of flower vegetables can cure illness and diseases. Also natural antioxidants particularly from plant sources are widely used in food industry as food additives instead of synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole were restricted in recent years, since they were found to be toxic. [1]

Lotus (*Nelumbo nucifera*) is a perennial aquatic plant of economic important grown in wetlands or ponds which are widely distributed in temperate and tropical regions. [2] In China and Japan the lotus leaves are used to treat summer heat syndrome. [3] It is one of the most popular edible aquatic plants, especially its seed, rhizome and leaf which have been used as food products like dessert, porridge and soup. [4] The dietary fiber rich mature leaves are consumed as a functional food and it is also used for preparation of antioxidant beverages and tea bags in Asia. [5] Further, the roasted seeds contain appreciable quantities of bioactive constituents like saponins, phenolics and

carbohydrates and it also found to be a good source of coffee substitute. Many pharmacological and physiological properties such as antioxidant, anti-inflammatory, antidiabetic, anticancer, antiobesity, hepatoprotection and atherosclerosis of whole plant of the *N. nucifera* were also studied. [4,6-9] The essential oil from lotus flower extract contains rich amount of palmitic acid methyl ester which regulate the melanin content [10] which are useful in the treatment of cardiovascular disease and chronic liver damage. [11]

Water lily (*Nymphaea alba*) which is belonging to the family Nymphaeceae is also an aquatic flowering plant grown in large ponds and lakes which are distributed globally. The flowers are white and they have many small stamens inside contains alkaloids which act as sedative agent. Many important bioactive compounds have been obtained from *Nymphaea* spp, and all parts of the plants are used in medicine and pharmacy, especially in the treatment of insomnia, anxiety, cancer, viral and free radical mediated diseases. [12] They are rich in tannic acid, gallic acid, alkaloids, sterols, flavonoids, glycosides, hydrolysable tannins and high molecular weight polyphenolic compounds. Due to the presence of apigen, ellagic acid and quercetin in *N. alba* plants having the prophylactic effects which act against renal carcinogenesis, oxidative stress and hyperproliferative activity. [13] It is used in Ayurvedic medicine for dyspepsia, enteritis, diarrhea, urinary problems, fever and heart palpitation. The flowers of *N. stellata* are used for the hyperglycaemic, hyperlipidaemic complaints, atherosclerosis and cardiovascular diseases. [14] Further, the rhizome of the plant is found to have the anti-diarrheal, anti-inflammatory [15] and analgesic potential. [16] On the other hand, the root and rhizome are used to cure gastrointestinal, genital and bronchial complaints. [17]

There is a strong need for exploration of underutilized/ indigenous vegetable sources which containing effective dietary antioxidant potential along with adequate nutritional compositions to prevent the various disorders implicated by the free radicals and promote health attributes. Thermal processing is applied for the purpose of food preservation, increase the edibility, digestibility and bioavailability of nutrients and phytochemicals in foods. It is also important to investigate whether processing increases or decreases the antioxidant potential of flowers. Therefore, this present study proposed to investigate the effect of heat processing on free radical scavenging capacity and antioxidant potential using aqueous acetone extract of raw core and petals and processed core of underutilized flower samples from *N. nucifera* and *N. alba*.

2. MATERIALS AND METHODS

2.1 Plant samples and preparation of plant extracts

N. alba and *N. nucifera* are water plants and flowers were collected from Alappuzha, Kerala, India. The respective petals of the flowers were separated and allowed to dry under room temperature whereas the remaining core samples were divided into three parts. One part (250 g) was cut into small pieces and dried at 45° C. The second part of the respective core sample was boiled in water at 100° C for 15 min in the ratio of 1:10 (w/v). The third part of the respective core sample was bleached in boiled water (at 100° C) for 10 min in the ratio of 1:10 (w/v). After boiling and bleaching, the remaining water was discarded and the respective processed cores were cut into small pieces and dried at 45°C. All the raw and processed samples were ground to fine powder and stored in separate screw capped bottles at room temperature for further analysis. Both raw and processed flower samples were subjected to extraction. After defatting by petroleum ether, the raw and processed flower samples (15 g) were extracted by stirring with 100 ml 70:30 of aqueous acetone at 25^{o} C for 48 h and filtering

through Whatman No. 4 filter paper. The residues were re extracted with an additional 75 ml of aqueous acetone, as described above, for 3 h. The solvent of the combined extract was evaporated using reduced pressure in a rotary vacuum-evaporator and the remaining water was removed by lyophilisation. The freeze dried extract was used for analysis.

2.2. Chemicals

Butylated hydroxyanisole (BHA), 2,2′–diphenyl-1-picrylhydrazyl (DPPH˙), potassium persulfate, 2,2 azinobis (3-ethylbenzo-thiozoline-6-sulfonic acid) disodium salt (ABTS), 6-hydroxy -2,5,7,8-tetra-methylchroman 2- carboxylic acid (Trolox), β-carotene and linoleic acid were obtained from Hi Media, Merck and Sigma. All chemicals were of analytical grade. All analysis was performed with UV-visible spectrophotometer (Cyberlab-UV 100, USA).

2.3 Determination of total phenolics and tannin contents

The total phenolics and tannins were measured as gallic acid equivalents. [18] Aliquots of the samples to the final volume of 1 mL, was added with 0.5 mL of Folin–Ciocalteu reagent (1N) and 2.5 mL of sodium carbonate solution (20%). Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm with UV-visible spectrophotometer (Cyberlab-UV100, USA) against the reagent blank. TPC of samples were expressed as mg gallic acid equivalents (GAE)/L. Using the same samples and method, the tannins were estimated after treatment with polyvinylpolypyrrolidone (PVPP).

2.4 Estimation of total flavonoids

Total flavonoid content was measured according to the method of Zhishen et al., [19] outlined by Siddhuraju and Becker. [20] Flower samples was added with 0.3 mL of 5% sodium nitrite and well mixed. After 5 min of incubation, 0.3 mL of 10% aluminum chloride solution was added. Then, after 6 min, 2 mL of 1 M sodium hydroxide was added to the mixture and made up the volume to 10 mL with water. The absorbance was measured at 510 nm with UV–visible spectrophotometer. Total flavonoids were measured from rutin (20–100 µg) standard curve and expressed as mg rutin equivalents/L.

2.5 Free radical scavenging activity on 2, 2-diphenyl-1-picrylhydrazyl (DPPH˙)

The antioxidant activity of samples and standards (BHA, rutin and tannic acid) was measured in terms of hydrogen donating ability using a stable, commercially available organic and nitrogen centered DPPH radical by the method of Brand-Williams et al. [21] with slight modifications. Flower samples were mixed with 3.9 mL of methanol containing DPPH˙ (0.025 g/L) and incubated in dark for 30 min. The absorbance was measured at 515 nm with UV–visible spectrophotometer. The trolox standards were prepared in the range of 0–2.5 mM. The concentration of DPPH was calculated from trolox standard curve and expressed as mmol trolox equivalents/L.

2.6. Antioxidant activity by the ABTS˙+ assay

The ABTS˙+ radical cation decolorization assay was performed to evaluate the radical scavenging ability of flower samples by the method of Re et al. [22] with slight modification made by Siddhuraju and Becker. [20] ABTS radical cation (ABTS˙+) was generated by adding 2.45 mM potassium persulfate to 7 mM ABTS and incubated in dark at room temperature for 12–16 h. This stock solution of ABTS˙+ was diluted with ethanol to give an absorbance of 0.70 (± 0.02) at 734 nm, which act as a positive control. Ten microliters of flower samples was mixed with 1.0 mL of diluted $ABTS^{\cdot+}$ solution and incubated at 30 °C for 30 min. The absorbance value was measured at 734 nm with UV–visible spectrophotometer. Trolox standard was also prepared (in ethanol: 0–1.5 mM) to get the concentration response curve. The unit of trolox equivalent antioxidant activity (TEA) was defined as the concentration of Trolox having the

equivalent antioxidant activity expressed as mmol/L. The TEA of BHA, rutin and tannic acid was also measured by $ABTS^{\cdot+}$ method for comparison.

2.7. Ferric reducing antioxidant power assay (FRAP)

FRAP assay can be used to evaluate the electron donating ability of antioxidants according to the method of Pulido et al. [23] An aliquot of 30 µL flower samples was mixed with 90 µL of water and 900 µL of FRAP reagent (2.5 mL of 20 mmol/L of TPTZ in 40 mM of HCl, 2.5 mL of 20 mmol/L of ferric chloride, 25 mL of 0.3 mol/L of acetate buffer (pH 3.6)) and incubated at 37 °C for 30 min. After incubation, the absorbance values were recorded at 593 nm with UV–visible spectrophotometer. Known ferrous sulphate concentrations ranging from 400 to 2000 µmol were used to generate the calibration curve. From the curve, the ferrous ions reduced by the flower sample were calculated using regression equation. The antioxidant activity was expressed as amount of samples required to reduce 1 mmol of ferrous ions. The antioxidant activity of samples was compared with the following standards: BHA, rutin and tannic acid.

2.8. Metal chelating activity

The chelating activities of samples, standards like BHA and α-tocopherol were estimated by the method of Dinis et al. [24] An aliquot of 0.1 mL flower samples, 0.6 mL of distilled water and 0.1 mL of ferrous chloride (2 mmol/L) were well mixed and incubated for 30 s. Then, 0.2 mL of ferrozine (5 mmol/L) was added to the above mixture and incubated for 10 min at room temperature and the absorbance was recorded at 562 nm with UV–visible spectrophotometer. EDTA (0-2 µg) was used as standard for the preparation of calibration curve. Metal chelating ability of antioxidant was expressed as mg EDTA/L.

2.9. Nitric oxide scavenging activity assay

Nitric oxide generated from sodium nitroprusside (SNP) was measured by the Griess reaction. Nitric oxide interacts with oxygen to produce nitrite ions that can be observed by Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. [25] Various concentrations (500µg) of sample extracts and sodium nitroprusside (SNP, 5 mM final concentration) in phosphate buffer saline, pH 7.4, in a final volume of 1 ml were incubated at 25° C for 150 min. A control experiment without samples but with equivalent amount of vehicles was conducted in an identical manner of the sample. After incubation, the reaction mixtures were mixed with Griess reagent (1% sulfanilamide and 0.1 % naphthylethylene diamine dihydrochloride in 5 % H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 540 nm. The total antioxidant activity of ASC (500 µg) and QUE (500 µg) were also measured by nitric oxide scavenging method for comparison. The % Nitric oxide scavenging activity was calculated by the following equation;

$NOSA = (A_c\text{-}A_s)/A_c \times 100$

Where A_c- Absorbance of control; A_s- Absorbance of samples.

2.9. Superoxide anion radical scavenging assay

The superoxide anion radical ($O2^{\cdot-}$) scavenging capacity of standards (BHA, catechin, trolox and rutin) and flower samples were determined by the method of Martinez et al. [26] for the determination of superoxide dismutase with some modifications made by Dasgupta and De [27] in the riboflavin-light-nitroblue tetrazolium system. Each 3 mL of reaction mixture consists of 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM EDTA, 75 µM NBT and 1 mL of samples/standard was kept for 10 min of illumination under 20 W fluorescent lamps. The production of blue formazan was monitored and recorded at 560 nm with UV-visible spectrophotometer. The degree of

superoxide anion radical scavenging activity (SRSA) was calculated as follows,

% SRSA = $(A_c-A_s)/A_c \times 100$

Where A_c- Absorbance of control; A_s- Absorbance of samples. The scavenging activity was compared with the positive standards (150 µg) BHA, rutin and trolox.

2.10. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging ability of samples and standard (catechin) was measured according to the method of Klein et al. [28] Flower samples and standards were mixed with 1 mL of iron-EDTA solution (0.13% ferrous ammonium sulfate in 0.26% EDTA), 0.5 mL of 0.018% EDTA and 1 mL of DMSO solution (0.85% in phosphate buffered saline 0.1 M, pH 7.4). The reaction was terminated by the addition of 1 mL of ice cold TCA (17.5 w/v). Then, 3 mL of Nash reagent (7.5 g ammonium acetate, 0.3 mL of glacial acetic acid, 0.2 mL of acetyl acetone and distilled water – 100 mL) was added to the above mixture and incubated at room temperature for 15 min and the absorbance values were recorded at 412 nm with UV–visible spectrophotometer. Sample control was also run with the substitution of phosphate buffer instead of ascorbic acid. The % hydroxyl radical scavenging activity (HRSA) was calculated using the following formula,

% HRSA=1– (Difference in absorbance of sample/ Difference in absorbance in blank) x 100.

The activity was compared with the positive standard catechin (250 µg).

2.11. β-carotene/linoleic acid bleaching activity

The antioxidant activity of flower samples and standards (BHA, rutin and trolox) was analyzed according to the method of Taga et al. [29] with slight modifications. Two milligrams of β-carotene were dissolved in 1 mL of chloroform containing 40 mg of linoleic acid and 400 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45 °C for 4 min and 100 mL of distilled water was added slowly to the semisolid residue with vigorous agitation to form an emulsion. A 5 mL aliquot of the emulsion was added to a tube containing standards (50 µg) and samples (50 µl), and the absorbance was measured at 470 nm with UV–visible spectrophotometer, immediately, against a blank, consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the absorbance measurements were conducted at 120 min. All determinations were carried out in triplicates. The antioxidant activity (AA) of the samples was evaluated in terms of bleaching of β-carotene using the following formula: AA= [1- (A0 - At) / (A′0 - A′t)] × 100, where A0 and A′0 are the absorbance of values measured at zero time of the incubation for test sample and control, respectively and At and A′t are the absorbances measured in the test sample and control, respectively, after incubation for 120 min.

2.12 Statistical analysis

Values are expressed as mean of triplicate determinations ± standard deviation. The data were subjected to one way analysis of variance and the significant difference between means was determined by Duncan's multiple test ($P< 0.05$) using Statistical Package for the social Sciences Version 13.0. (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1 Total phenolics and tannins

The total phenolics and tannins of raw and processed *N. nucifera* and *N. alba* are presented in Table 1. Plant phenolics are the largest class of plant secondary metabolites, which serve in plant defense mechanisms to counteract the reactive oxygen species and prevent molecular damage and damage caused by microbes, insects and herbivores. [30] Polyphenols are the important phytochemicals those are effective hydrogen donors which having excellent antioxidant potential and play an essential role as free radical scavengers needs to the maintenance of redox homeostasis responsible for various degenerative diseases. So, it is sensible to

estimate the phenolics and tannins in selected plant extracts. *N. alba* extracts expressed highest phenolics and tannin contents than *N. nucifera* extracts (Table 1). Phenolics and tannins were resistant to thermal processing and phenol content of raw samples were 170.92-294.27 mg TAE/g extract and processing samples were 173.13-312.81 mg TAE/g extract respectively. The amount of tannins in both raw and processed flower samples of lotus and lily was within the range of 149.50-302.43 mg TAE/g extract. All processed samples were observed to contain higher amount of phenolics and tannins than their respective raw samples. The results on the increase of TPC by processing are in good agreement with those reported by Adefegha and Oboh [31] that cooking increases the TPC in vegetables (*Talinium triangulare* and *Ocimum gratissimum*). These significant increases of TPC during cooking could be attributed to the breakdown of tough cell walls and more release of extractable phenolic compounds. [32] In plants phenolic acids are accumulate in vacuoles, during thermal processing the bound phenolic acids are released from cellular constituents and which are trapped in the fiber of green leafy vegetables [33] and also it could be due to Millard reaction (non-enzymatic browning). Similarly, the increase in total phenolic content on hydrothermal treatment of tomato was reported by Dewanto et al. [34]

Table 1: Extract yield, Total phenolics, tannins and flavonoids of raw and processed core and raw petal parts of *N. alba* and *N. nucifera.*

Sample	Recovery (%)	Total Phenolics*	Tannins*	Flavonoids**
A1	19.00	294.27^{b}±0.18	286.15^{b}±0.20	67.84^{c}±0.83
A2	13.83	312.81^{a}±1.33	302.43^{a}±1.78	70.11^{b}±0.85
A3	15.99	220.40^{c}±1.23	212.53^{c}±1.03	129.82^{a}±0.81
N1	17.22	202.79^{b}±1.30	190.21^{b}±0.96	109.56^{b}±0.80
N2	12.22	230.01^{a}±1.68	224.77^{a}±1.59	120.23^{b}±1.08
N3	2.22	173.13^{c}±1.81	163.48^{c}±1.55	109.61^{a}±0.86
A4	17.33	265.27±0.65	256.00±0.66	83.85±0.82
N4	14.11	170.92±0.68	149.50±0.91	109.74±0.65

Values are mean of triplicate determinations ± standard deviations. Mean values followed by different superscript letters in the same column are significantly (p<0.05) different.

*-mg Tannic acid equivalents/g extract; **-mg Rutin equivalents/g extract.

A1- *Nymphea alba* core raw acetone extracts; A2- *Nymphea alba* core boiled acetone extracts; A3- *Nymphea alba* core bleached acetone extracts; N1- *Nelumbo nucifera* core raw acetone extracts; N2- *Nelumbo nucifera* core boiled acetone extracts; N3- *Nelumbo nucifera* core bleached acetone extracts; A4- *Nymphea alba* petal raw acetone extracts; N4- *Nelumbo nucifera* petal raw acetone extracts.

3.2. Total flavonoid content

The presence of flavonoids might be responsible for the antioxidant activity of the plant that constitutes a large group of naturally occurring plant phenolics compounds. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation and their consumption through food will reduces the risk of cardiovascular disease and certain types of cancer. Through the *In vitro* and *In vivo* experiments, flavonoids have been shown antiallergic, anti inflammatory, anti-viral and antioxidant activities. [35] Levels of flavonoids content are shown in (Table 1). As like phenolics and tannins all the processed samples showed higher flavonoid contents in the range of 70.11-129.82 mg rutin/g extract than the raw samples (67.84-109.74 mg rutin/g extract) respectively. The increase might be due to the release of bound flavonoid compounds during the thermal treatment. The findings of the present study are in good agreement with the previous report of Adefegha and

Oboh [31] in cooked leaf of *T. triangulare*. Therefore the presence of high amount of phenolics and flavonoids in the sample extracts might be responsible for their high antioxidative activity.

3.3 Free radical scavenging activity on DPPH and ABTS$^{\cdot+}$ assay

It is one of the most extensively used antioxidant assay for plant samples. This assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable radical. In its radical form, DPPH$^{\cdot}$ absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, the absorbance disappears. Sample extracts react very quickly with DPPH$^{\cdot}$, reducing a number of DPPH$^{\cdot}$ molecules equal to their number of available hydroxyl groups. Radical scavenging activities of the samples were shown in (Table 2). All samples showed lower scavenging activity than standards because the interference of many compounds in the crude extract. The range of DPPH radical scavenging activity was 306581.50-535121.6 mmol TE/ g extract. This is higher than the values of previous reports on edible flowers, *Brassica rapa*, [36] *Dillenia pentagyna.* [37] The result revealed that DPPH radical scavenging ability significantly increased with processing than the raw samples. Boiling and blanching of flower samples would attribute to the suppression of oxidation by antioxidants due to the thermal inactivation of oxidative enzymes and also the release of potent antioxidants from the cell wall of plant materials during thermal process. This increase in radical scavenging potential might be attributed to the increase in the total phenol and total flavonoid contents of the flower extracts. Potential DPPH radical scavenging activities revealed by acetone extract of *N. alba* and *N. nuciferra* flower samples might confirm its hydrogen donating capacity and also its proposed ability to protect the consumer's health from various free-radical mediated diseases.

The free radical scavenging activity of the flower extract was further studied using a moderately stable nitrogen centered radical species, ABTS$^{\cdot+}$. This is a radical generated from potassium persulphate and ABTS in a dark for 16 hrs and forms blue-green chromophore. The increase in concentration of extracts decreased the absorbance. The order of the samples were A3> A2> A4> A1> N2> N1> N3> N4 and ranges from 130597.87-302642.21 mmol trolox/g extract respectively. The results also revealed that processing caused a significant increase in ABTS$^{\cdot+}$ scavenging activity of flower extracts. Processed samples have high phenolic content and it might be responsible to scavenge ABTS radical efficiently than raw sample. Huang et al. [4] reported that in the ABTS assay, the IC_{50} value of LLE was 2.195 ±0.17 µg/ml trolox. The present study report is good agreement with earlier reports of the cooking on antioxidant properties of green leafy vegetables, [31] maize, [32] carrots [38] and tomatoes. [34]

Table 2: DPPH$^{\cdot}$ radical, ABTS$^{\cdot+}$ cation, FRAP and metal chelating scavenging activity of raw and processed core and raw petal parts of *N. alba* and *N. nucifera*.

Samples	DPPH$^{\cdot}$ radical scavenging activity[1]	ABTS$^{\cdot+}$ radical scavenging activity[2]	FRAP[3]	Metal Chelating activity[4]
A1	362043.79^{a} ±373.83	202507.51^{b} ±6787.99	2829.69^{a} ±66.79	0.36^{a} ±0.05
A2	535121.61^{c} ±879.95	274873.07^{a} ±1228.26	6156.56^{b} ±85.07	0.43^{a} ±0.06
A3	420900.24^{b} ±546.63	302642.21±6512.11	5658.40^{c} ±90.95	0.41^{a} ±0.06
N1	379635.03^{b} ±514.84	154139.47^{a} ±15498.77	4053.87^{b} ±13.87	0.26^{a} ±0.06
N2	381496.35^{a} ±1332.49	168479.95^{a} ±10427.48	5855.81^{a} ±24.23	0.25^{a} ±0.01
N3	379635.03^{b} ±514.84	150865.20^{a} ±8112.93	3777.84^{c} ±20.41	0.33^{a} ±0.06
A4	360863.74 ±439.97	258708.94^{a} ±9823.08	5815.37 ±15.29	0.25 ±0.06
N4	321313.86 ±921.12	130597.87±15232.64	5091.36 ±31.96	0.28 ±0.01
BHA	814172.7±187.06	655137.00±61415.86	350760.44 ±72476.7	10.49 ±0.06
Rutin	748175.2±598.32	433569.06±23178.33	174032.83 ±26869.47	-
TAN	848540±547.45	751735.57±62890.85	562955.03 ±42130.92	-

Values are mean of triplicate determination ± standard deviation. Mean values followed by different superscript letters in the column are significantly (p<0.05) different.

[1]-g extract/g DPPH; [2]- mmol Trolox equivalent antioxidant activity (mmol equivalent trolox performed by using ABTS radical cation); [3]- mM Fe (II) equivalents/g extract; [4]- mg EDTA equivalents/g extract.

A1- *Nymphea alba* core raw acetone extracts; A2- *Nymphea alba* core boiled acetone extracts; A3- *Nymphea alba* core bleached acetone extracts; N1- *Nelumbo nucifera* core raw acetone extracts; N2- *Nelumbo nucifera* core boiled acetone extracts; N3- *Nelumbo nucifera* core bleached acetone extracts; A4- *Nymphea alba* petal raw acetone extracts; N4- *Nelumbo nucifera* petal raw acetone extracts; BHA- Butylated hydroxyanisole. TAN- Tannic acid.

3.4 FRAP assay

FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-Tri(2-Pyridyl)- S-Triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The absorbance decrease is proportional to the antioxidant content. [39] The reducing activities of raw and processed *N. nucifera* and *N. alba* samples are presented in Table 2. The reducing ability of both samples is as follows: A2> N2> A4> A3> N4> N1> N3> and range from 2829.69-6156.56 mmol Fe (II)/g extract. The results revealed that processed extracts had higher reducing power than raw extracts. Presence of polyphenolic constituents of the sample extracts appears to function as good electron and hydrogen atom donors and enable to terminate radical chain reaction by converting the free radicals and reactive oxygen species to more stable products. [40] The result of the reducing power agreed with the $ABTS^{+\cdot}$ and DPPH free radical scavenging abilities which have been discussed earlier confirm that processing could increase antioxidant activity, as a result of increase in total phenolics and flavonoid content during the processing as shown in (Table 1). The presence of Phenolic compounds in the sample has redox properties which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

3.5 Metal chelating activity

Metal chelating is one of the important antioxidant mechanisms which retard metal-catalyzed oxidation. In food systems, ferrous ions are the most effective pro-oxidants. Ferrozine can quantitatively chelate with Fe^{2+} and form a complex with a red color. This reaction is limited in the presence of other chelating agents and results in a decrease of the red color of the ferrozine-Fe^{2+} complexes. Measurement of the color reduction estimates the chelating activity to compete with ferrozine for the ferrous ions. [41] The chelating activity of samples were shown in (Table 2), they were ranging from 0.2500-0.4300 mg EDTA equivalents/g extract. Yen et al. [42] reported the strongest chelating effect of boiling water extract of lotus seed (57.4%). Wu et al. [43] reported that the lotus extracts having remarkable metal chelating ability. Such kind of lower metal chelating ability does not made interfere into essential mineral absorption phenomena.

3.6 Nitric oxide Radical Scavenging Activity

Nitric oxides generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent. Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical, which plays many roles as an effective molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities. [44] The order of nitric oxide radical scavenging activity of the samples (at 250 µg) were N2> N3> A2> A3> A4> A1> N4> N1 and the ranges of 12.17-32.28 % respectively (Fig 1). When compared to standards, all samples registered lower scavenging activity towards nitric oxide radicals. Processing of core part

of both flowers extracts increases their nitric oxide radical scavenging activity than the respective raw samples. Compared with the result of Madhusudanan et al. [45] the extract might contain bioactive compounds capable of inhibiting nitric oxide radicals and offers scientific evidence for the use of the *N. alba* flower extract in the indigenous system of medicine for the treatment of various diseases.

3.7 Superoxide Anion Radical Scavenging Activity

Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress and ultimately lead to the genesis of several chronic diseases in human beings. [46] The reduction of the yellow dye (NBT) to produce the blue formazan and it measured with spectrophotometrically at 560 nm. Raw and processed samples of *N. alba* and *N. nucifera* have potent antioxidant capacity were shown in (Fig 1) The decrease of absorbance at 560 nm indicated the high antioxidant power. At a concentration of 250 µg the extracts exhibited 51.15%-76.72% of radical scavenging activity and the order of activity was RUT(76.72) > A2(74.92%) > N3(72.98) > A3(71.09%) > N2(70.15%) > BHA(69.28) > N1(68.96) > A4(68.11%) > N4(61.19%) > A1(51.15%). The present results showed higher SO scavenging activity than the lotus germ oil. [47] In the present investigation, processed samples exhibited higher scavenging activity than the raw samples and showing higher values than the standard BHA. All the samples revealed the excellent scavenging activity against superoxide radicals and thus the regular consumption of above said vegetables may play a preventive role against dangerous superoxide radical, which is produced continuously during metabolism.

3.8 Hydroxyl radical scavenging activity

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. It has been implicated as major active oxygen centered radical formed from the reaction of various hydroperoxides with transition metal ions, which is capable of damaging almost every molecule found in living system causing lipid peroxidation and biological damage. [48] Scavenging hydroxyl radicals is an important antioxidant activity for protecting living cells, since the hydroxyl radicals can easily pass through the cell membrane at specific sites, reacting with most biomolecules and causing tissue damage and cell death. The electron or proton donation capacities of raw and processed samples of acetone extract of both flowers were further confirmed by the Fenton reaction system. The scavenging abilities of raw and processed extract on hydroxyl radical inhibition are shown in (Fig 1). At a concentration of 250 µg, all the extracts showed good hydroxyl radical scavenging activities. The range of hydroxyl radical scavenging activity was 35.13-73.38% and the order of activity was N2> N3> A2> A3> N4> A4> A1> N1. Processing increases the scavenging activity towards hydroxyl radical. The present results showed higher HO radical scavenging activity than the lotus liquor, [49] lotus germ oil, [47] and ten different vegetative parts of lotus. [43]

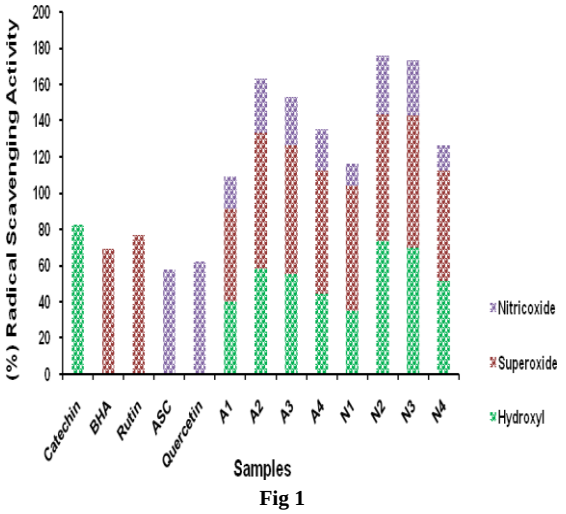

Fig 1

Hydroxyl, superoxide and nitric oxide radical scavenging activity of acetone extract of raw and processed core part and raw petals of *N. alba* and *N. nucifera*. Values are means of triplicate determinations ± standard deviation. Values are mean of triplicate determinations ± standard deviations. Mean values followed by different superscript letters in the same column are significantly (p<0.05) different. BHA- Butylated hydroxyl anisole; ASC- Ascorbic acid; A1- *N. alba* core raw acetone extracts; A2- *N. alba* core boiled acetone extracts; A3- *N.alba* core bleached acetone extracts; N1- *N. nucifera* core raw acetone extracts; N2- *N. nucifera* core boiled acetone extracts; N3- *N. nucifera core* bleached acetone extracts; A4- *N. alba* petal raw acetone extracts; N4- *N. nucifera* petal raw acetone extracts.

3.9 *β- Carotene linoleic acid emulsion assay*

In oil-water emulsion based system, linoleic acid in the reaction mixture forms hydroperoxide at 50 °C, thereby it attacks the β- carotene chromophore resulting in a bleaching effect. [50] This test measures the sample's ability to prevent the bleaching of β- carotene by scavenging hydroperoxides formed in it. The percentage of β- carotene bleaching inhibition by raw and processed flower and core extracts decreased with time duration. Based on a 120 min reaction time (Fig 2), all the samples had lower antioxidant activity (37.18-47.15%) when compared to BHA (67.70%) and trolox (49.56%). In term of β- carotene bleaching effect, those samples exhibited the following order: BHA> trolox> N3> N4> N2> A2> N1> A4> A3> A1. Among all the samples, the processed samples increased the peroxidation inhibition activity than the raw samples and this antioxidant capacity might be due to the presence of high levels of phenolics compounds. However, this is found to be the first report on lipid peroxidation inhibition of *N. nucifera* and *N. alba* flower samples. Thus, consumption of such edible flowers can prevent lipid peroxidation mediated diseases like coronary heart disease, atherosclerosis, cancer and the aging process.

During thermal processing, the phenolics, tannins and flavonoids contents of all the samples from *N. nucifera* and *N. alba* were increased higher amount. The changes in phenolics, tannins and flavonoid content by hydrothermal processing as indicated by the present investigation could be attributed to the complete breakdown or modification of cellular components by thermal treatment and also it might be increase the release of metabolites from the matrix which become more accessible during the extraction. [51] Overall, the thermal processing modifies the phenolic structure and improved antioxidant potential. Other factors for increased antioxidant potential by heat treatment could be due to the formation of Millard reaction. [52] Also it has been reported heat treatment could deactivate endogenous oxidative enzymes that are responsible for the destruction of antioxidants. [32] In the present study, heating plays a significant role in enhancing the free radical scavenging capacity and antioxidant potential of *N. nucifera* and *N. alba*. In some previous studies heating vegetables has been shown to increase total antioxidant activity with the increase in TPC and TFC. [31-34] These reports give a strong support to the findings of the present study that thermal processing could also enhance the antioxidant activity of *N. nucifera* and *N. alba*.

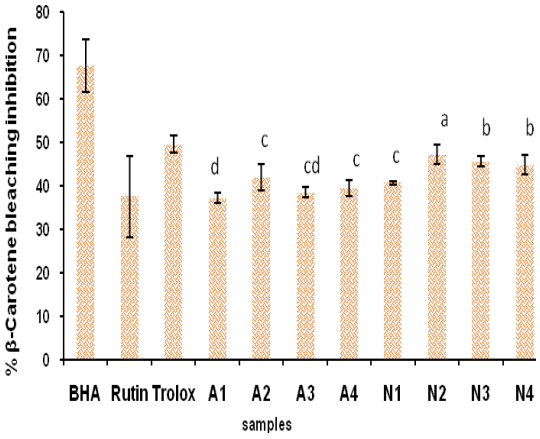

Fig 2

β- Carotene bleaching inhibition activity of acetone extract of raw and processed core part and raw petals of *N. alba* and *N. nucifera*. Values are means of triplicate determinations ± standard deviation. Values are mean of triplicate determinations ± standard deviations. Mean values followed by different superscript letters in the same column are significantly (p<0.05) different. BHA, Butylated hydroxyl anisole; A1- *N. alba* core raw acetone extracts; A2- *N. alba* core boiled acetone extracts; A3- *N. alba* core bleached acetone extracts; N1- *N. nucifera* core raw acetone extracts; N2- *N. nucifera core* boiled acetone extracts; N3- *N. nucifera core* bleached acetone extracts; A4- *N. alba* petal raw acetone extracts; N4- *N. nucifera* petal raw acetone extracts.

4. CONCLUSION

In the present study, the antioxidant activities of acetone extract of raw (core and petal part) and processed (core part) edible flower of *N. nucifera* and *N. alba* were evaluated. Edible portion of both flower samples possessed higher dietary phenolic and flavonoid content and exhibited good antioxidant activity against free radicals and reactive oxygen species. Thermal processing enhanced the antioxidant activity might be due to the release of bound phenolics along with other bioactive compounds from the cell wall and subsequent formation of synergetic activity among them. Based on above said preliminary investigation, the wider consumption of such processed underutilized edible flowers of *N. nucifera* and *N. alba* may be further encouraged for supplying the nutrient components along with a natural antioxidants to promote health aspects and alleviate the free radical mediated diseases. On the other hand, the isolation and characterization of bioactive components from flower vegetables would certainly help to ascertain the individual potency of the compounds to be used in the food and pharmaceutical industry.

ACKNOWLEDGMENT

The authors wish to thank University Grants Commission (UGC), New Delhi, India (F.No. 34-259\2008) for financial assistance.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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How to cite this article: Abirami A, Nagarani G, Saipriya V et al. Influence of thermal treatments on polyphenolic contents and antioxidant properties of underutilized edible flowers of *nelumbo nucifera* and *nymphaea alba*. Int J Health Sci Res. 2017; 7(10):210-223.

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