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PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF *NELUMBO NUCIFERA* PETALS

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ABSTRACT

For a long period of time, plants have been a valuable source of medicines. Phytochemicals like alkaloids, flavanoids, tannins, phenols are the non-nutritive, chemical compounds that occur naturally on plants and have diverse protective properties. Reactive oxygen species are formed in biological system as part of normal metabolism. To avoid damage caused by these excess ROS, plants have developed elaborate mechanism to manage them at sustainable level. Enzyme play an important role in lowering the ROS level and and avoid oxidative stress. Catalase, play vital role in combating oxidative stress. Measuring these enzymes activities provide an easy and precise way to study and understand an important part of the defense against oxidative stress.

KEYWORDS: Phytochemicals, Reactive oxygen species, oxidative stress, alkaloids, flavanoids, tannins.

INTRODUCTION

Since ancient times, different plants have been used as a source of medicines. A variety of drugs could be obtained from medicinal plants. For thousand years ago, the medical knowledge of the Indian subcontinent was termed as Ayurveda. It remains an important system of medicine and drug therapy in India(Gururajan *et al.*, 2017). About 80% individuals from developing countries rely on plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care (Ellof, 1998). Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicine (Nair *et al.*, 2005).

Mostly, these compounds are secondary metabolites such as alkaloids, flavonoids, steroids, resins, fatty acids, tannins and phenol compounds, etc. The phenolic compounds possess different biological activities, but most of the important one are their antioxidant activities (Raja *et al.*, 2019). Compounds extracted from different parts of the plants can be used in treatment of diarrhea, dysentery, cough, cold, fever, bronchitis, cholera, etc. (Joshi *et al.*, 2011). As phytochemicals often play an important role in plant defense against prey, microorganism, stress as well as interspecies protections(C Mohan *et al.*, 2017) Plant derived products can be exploited with a large number of sustainable advantages like more effectiveness, less side effects, reduced cost, and easy availability (Moorthy *et al.*, 2007).

MATERIALS AND METHODS

Plant Collection

Freshly harvested flowers of *Nelumbo nucifera* were purchased from the Koyambedu flower bazaar of Chennai in Tamil Nadu. Plant was authenticated by Dr. Seetha lakshmi, Life Teck Research Centre, Arumbakkam, Chennai-600 106.

Extraction of Nelumbo Nucifera

The petals alone removed from the flower of the collected *Nelumbo nucifera*. The flower was washed thoroughly with tap water to eliminate earthy matters, free from the debris, shade dried under room temperature for a few weeks and coarsely powdered using a food processor.

Extraction was performed by hot continuous percolation method using Soxhlet apparatus. About 500g of coarsely powdered *Nelumbo nucifera* petals were extracted in 70% ethanol by the continuous hot extraction method at 50˚C was decanted from the Soxhlet apparatus and the filtrate was evaporated for the total elimination of alcohol using a Rota flash Vacuum evaporator. The concentrated liquid extract obtained was then transferred to a China dish and kept in a water bath for 50˚C for dryness. The residual extract was transferred to an airtight container free from contamination until it was used.

Qualitative Phytochemical Analysis

The aqueous extract of petals of *Nelumbo nucifera* were subjected to phytochemical screening to determine the

presence of secondary metabolites such as tannin, saponin, flavonoid, alkaloid, steroid, phenol using standard procedures.

Test For Tannins: Ferric chloride test (Sani *et al.*, 2007)
To 1 ml of the extract, few drops of 5% aqueous ferric chloride solution was added. A bluish black colour which disappeared on addition of few ml of dilute sulphuric acids formed yellowish brown precipitate indicated the presence of tannins.

Test For Saponins: Foam test(Kokate, 1994)
To 1 ml of the extract, added a few drops of sodium bicarbonate solution. Shaken vigorously and kept for 3 minutes. A honey comb like froth was formed, indicated the presence of saponins.

Test For Flavonoids: Alkaline reagent test (Sani *et al.*, 2007)
To a few drops of extract, added few drops of sodium hydroxide solution. Intense yellow colour was formed which turned colourless on addition of few drops of dilute hydrochloric acid, indicated the presence of flavanoids.

Test For Alkaloids: Dragendroff's test (Sani *et al.*, 2007)
To 0.5 ml of the extract, added 2 ml of hydrochloric acid. To this acidic medium, 1 ml of Dragendroff's reagent was added. Formation of red precipitate indicated the presence of alkaloids.

Test For Steroids: Libermann – burchard's test (Kokate, 1994)
To 1 ml of the extract, 2 ml of concentrated sulphuric acid was added followed by the addition of 2 ml of acetic anhydride solution. Green colour developed turned blue, indicated the presence of steroids.

Test For Phenol: Ferric chloride test (Kokate, 1994)
To 1 ml of the extract, added 2 ml of distilled water followed by few drops of 10% aqueous ferric chloride solution. Formation of blue or green or violet colour indicated the presence of phenols

TOTAL ANTIOXIDANT ASSAY
DPPH ASSAY(Cuendet *et al.*, 1997)
Principle
Antioxidants react with DPPH (1, 1, diphenyl – 2 – picrylhydrazine) and convert it to diphenyl–picryl hydrazine. The degree of discoloration from purple to yellow colour was measured at 518 nm, which is the measure of the scavenging potential of antioxidant extract.

Procedure
The DPPH (1,1 diphenyl - 2 - picryl hydrazine) radical scavenging assay was carried out as described by Cuendet, 1997. Briefly, 5.0 ml of DPPH solution (0.004%) in methanol was added to 50 µl of extracts. After 30 minutes of incubation at 37ºC, the absorbance was read against control at 517 nm. Ascorbic acid was used as positive control.

FRAP ASSAY
Ferric Ion Reduction Potential (FRAP) (Benzie and Strain, 1996).
Principle
Ferric to Ferrous ion reduction at low pH causes a coloured ferrous- tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm test reaction mixtures with those containing ferrous ion in known concentrations.

Procedure
1ml of distilled water and 80µl of test sample was pipette into the standard 4ml plastic cuvette. 600µl of incubated FRAP Reagent was added to the cuvette, which was briefly inverted to mix the solutions. The reagent blank was also prepared as described above but 80µl of distilled water was added instead of test sample. Change in absorbance at 593nm (as a result of the reduction of the Fe 3+ -TPTZ complex at low pH) was recorded at exactly at 4 minutes using spectrophotometer. Each test sample dilution was tested in triplicate to allow a mean absorbance to be calculated.

CATALASE ASSAY (Sinha, 1972)
Principle
Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620nm. The catalase preparation was allowed to spit H_2O_2 for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate-acetic acid mixture and the remaining H_2O_2 as chromic acetate was determined colorimetrically.

Procedure
A 20% homogenate of the samples were prepared in plant extract. Phosphate buffer (0.067M, pH 7.0) and the homogenate were employed for the assay. The samples were read against a control without homogenate, but containing the H_2O_2-phosphate buffer. To 0.9m of phosphate buffer, 0.1mL of supernatant and 0.4mL of hydrogen peroxide was added. The reaction was arrested after 15, 30, 45 and 60 seconds by adding 2.0mL if dichromate-acetic acid mixture. The tubes were kept in boiling water bath for 10 min, cooled and the colour developed was read at 620nm. Catalyse activity was expressed as µmol of H_2O_2consumed/min/mg of protein.

NITRIC OXIDE RADICALS ASSAY(Govindarajan *et al.*, 1996)
Principle
Sodium nitroprusside in aqueous solution, at physiological pH spontaneously generates nitric oxide,

which interacts with oxygen to produce nitric ions that is estimated spectrophotometrically at 546 nm.

Procedure

Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with different concentrations of the extract (250-2500 µg/ml) prepared in ethanol and incubated at 25ºC for 30 minutes. A control without the test compound but with an equivalent amount of ethanol was taken. After 30 minutes, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and percentage scavenging was measured with reference to standard.

RESULTS AND DISCUSSION

PHYTOCHEMICALS PRESENT IN *Nelumbo nucifera*

The results of phytochemical analysis of the *Nelumbo nucifera* is given in the Table. The qualitative phytochemical analysis revealed the presence of secondary metabolites (tannin, saponin, flavanoid, alkaloid, steroids and phenol). Presence of phytochemicals was indicated by the +ve sign and absence by the –ve sign.

This may be due to the higher solubility of constituents in the solvents. Alkaloids are the class of heterocyclic indole compounds which are proved to have pharmacological properties such as hypotensive activity (Ali and Ghatak, 1995), anti convulsant activity (Singh and Kapoor, 1980) etc. Thus, presence of alkaloid, flavanoid, tannins and phenolic compounds may be indicative of medicinal value of *Nelumbo nucifera*.

Table 1: Phytochemicals present in *Nelumbo nucifera*.

S. No	Phytochemical constituents	Nelumbo nucifera petal extract
1	Tannin	-ve
2	Saponin	-ve
3	Flavonoid	+ve
4	Alkaloid	+ve
5	Steroid	-ve
6	Phenol	+ve

DPPH ASSAY OF *Nelumbo nucifera*

In the present study the DPPH assay for different concentration were estimated. The percentage inhibition for concentration 200 was 10.69, for 400 it was 41.71, for 600 it was 71.12, for 800 it was 76.47, for 1000 it was 79.14. Among these five concentration 1000 was found to be the most efficient solvent for scavenging activity of ***Nelumbo nucifera*** and had the highest percentage of inhibition.

Table 2: DPPH Assay Of *Nelumbo nucifera*.

S.No	Concentration (µg/ml)	O.D at 517nm	DPPH Activity %
1	200	0.333	10.69
2	400	0.205	41.71
3	600	0.201	71.12
4	800	0.174	76.47
5	1000	0.132	79.14

DPPH is a very stable free radical and it is widely used to evaluate antioxidant activities in relatively short time (Mokbel and Hashinaga, 2006). This assay is based on the reduction of DPPH radicals in methanol which causes an absorbance to drop at 517 nm (Wong *et al.*, 2006). DPPH accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative property (Chang *et al.*, 2002).

From the present result, it may be postulated that extract of ***Nelumbo nucifera*** can reduce the radical to the corresponding hydrazine, when it reacts with hydrogen donor present in the antioxidants (Sanchz-Morero, 2002). When DPPH radical reacts with suitable reducing agents, the electrons become paired off and the solution looses colour stoichiometrically depending on the number of electrons taken up (Blois, 1958).

The antioxidant activity reflected by the DPPH radical scavenging assay was clearly observed in ***Nelumbo nucifera*** extracts in dose dependent manner. This suggests that physico-chemical nature of the individual phenolics in the extracts may be important in contributing the antioxidant property of the plant and is well correlated with the presence of active principles in the extract (Ramkumar *et al.*, 2007) such as flavanoids and tannins.

FRAP ASSAY OF *Nelumbo nucifera*

The percentage inhibition for concentration 200 was 885, for 400 it was 1405, for 600 it was 1480, for 800 it was 2100, for 1000 it was 2110. Among these five concentration 1000 was found to be the most efficient solvent for scavenging activity of ***Nelumbo nucifera*** and had the highest percentage of inhibition.

Table 3: FRAP Assay of *Nelumbo nucifera*.

S.No	Concentration (µg/ml)	O.D Value for Sample at 0 min	O.D Value for Sample at 4 min	FRAP(µM)
1	200	0.430	0.253	885
2	400	0.319	0.038	1405
3	600	0.373	0.077	1480
4	800	0.470	0.050	2100
5	1000	0.753	0.331	2110

FRAP Assay measures the reducing potential of an antioxidants against the oxidative effect of ROS. Electron donating antoxidans can be described as reductants and inactivation of reductants can be described as redox reaction. This assay is based on the ability of antioxidants to react with ferric tripyridyltriazine complex and producing a coloured ferrous tripyridyltriazine(Gordan., 1990) (Duh *et al.*, 1999). The reducing properties associatedwith the presence of compound exerts their action by breaking the free radicals chain through donating a hydrogen atom(Evans *et al.*, 1997)

CATALASE ASSAY OF *Nelumbo nucifera*

The percentage inhibition for concentration 200 was 0.7, for 400 it was 1.11, for 600 it was 2.6, for 800 it was 2.7, for 1000 it was 2.9. Among these five concentration 1000 was found to be the most efficient solvent for scavenging activity of lotus flower and had the highest percentage of inhibition.

Table 4: Catalase Assay *Nelumbo nucifera*.

S.No	Concentration (µg/ml)	O.D	Catalase Activity (Moles of H_2O_2 Consumed /min)
1	200	0.087	0.7
2	400	0.134	1.11
3	600	0.313	2.6
4	800	0.324	2.7
5	1000	0.348	2.9

Catalase are responsible for the removal of H_2O_2 to 2 H_2O. Catalase are localized to peroxisomes. Plant posses multiple CAT encoded specific genes which respond differentially to various stress that are known to generate ROS.(Scandolios., 2002) (Scandolios., 2005).

NITRIC OXIDE ASSAY of *Nelumbo nucifera*

Nitric oxide radical quenching activity of Nelumbo nucifera identified and compared with the standard ascorbic acid. The extract of Nelumbo nucifera displayed the maximum inhibition of 37.30% at a concentration of 1000(µg/ml). In the current study, nitrite was produced by incubation of sodium nitroprusside in standard phosphatesaline buffer at 29˚C. Significant scavenging activity may be due to the antioxidant property of flavonoid, which compete with oxygen to react with nitric oxide, leading to less production of nitric oxide.

Table 5: NITRIC OXIDE ASSAY of *Nelumbo nucifera.*

S.No	Concentration (µg/ml)	O.D	NO2 Inhibition
1	200	0.360	6.73
2	400	0.355	8.03
3	600	0.348	9.84
4	800	0.302	21.76
5	1000	0.242	37.30

Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems as neuronal messenger, vasodilator, anti-microbial agent and as anti-tumour agent (Hangarman *et al.*, 1998). It is also an important chemical mediator generated by endothelial cells, macrophages etc. and is involved in the regulation of various physiological processes (Lata and Ahuja, 2003). Excess concentration of NO is associated with several diseases (Ross, 1993). Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which acts as free radicals (Cortan *et al.*, 1999). In this present study, the extract competes with oxygen to react with nitric oxide and thus inhibits the generation of anions.

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