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Research Article

FREE RADICAL SCAVENGING ACTIVITY OF THALAMUS OF N*YMPHACEA STELLATA* WILLD

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ABSTRACT

The in vitro antioxidant activity of extracts of *Nymphacea stellata* thalamus has been investigated by 2,2-diphenyl-2-picryl hydrazyl radical (DPPH) free radical and nitric oxide (NO) scavenging activity. The ethyl acetate, ethanol, and aqueous extracts extract of *Nymphacea stellata* thalamus showed significant antioxidant activity by inhibiting DPPH and nitric oxide radical when compared with compared with standard BHT and Ascorbic acid. Free radical scavenging activity might be due to the presence of phenolic and anthocyanins.

Keywords: *Nymphacea stellata,* D.P.P.H., B.H.T., Antioxidant

INTRODUCTION

Oxidation was an essential to many living organism for their normal physiological function. However, oxygen- centred free radicals and other reactive oxygen species (ROS), which were continuously, produced *in vivo*, result in cell death and tissue damage[1]. ROS such as superoxide anions (O_2^-), hydroxyl radical (.OH) and nitric oxide (NO) inactivate enzyme and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation[2]. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Meanwhile it was found that there was a correlation between antioxidant capacity and phenolics content. The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides and exert beneficial pharmacological effects on neurological disorders on the basis of *in vitro* observations[3,4]. Plant material containing phenolic constituents are increasingly of interest as they retard oxidative degradation of lipids[5]. The importance of the antioxidant constituents of plant material is the maintained of health and protection from coronary heart disease, diabetes and cancer[6].*Nymphacea stellata* willd. (Family- Nymphaeaceae) an aquatic herb has been used in the Indian system of medicine. Ethnoboatnical information reveals that the powdered rhizome is given in dyspepsia, diarrhoea and piles[7]. They are also used for blennorrhagia and diseases of the urinary tract. A decoction of flowers is considered narcotic and antiaphrodisiac[8]. In the present study it was aimed to evaluate antioxidant activity of ethyl acetate and ethanol extract of *Nymphacea stellata* willd thalamus.

MATERIALS AND METHODS

The thalamus of *Nymphacea stellata* willd were collected from the pond and ditches near the bank of Subarnarekha riverside in the district of Mayurbhanj, Orissa and identified by Botanical survey of India, Howrah. These were dried in shade and crushed to moderately coarse powder and stored in air tight container. The powder was successively extracted with organic solvent like petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distil water**.** The extracts were reduced under vacuum with rotary evaporator to obtain a molten mass. The percentage yield of ethyl acetate and ethanol extract is 1.59 and 9.75% respectively.

Preliminary phytochemical screening reveals the presence of flavonoid and free phenolic compounds in ethyl acetate and ethanol extract. The present evaluation of antioxidant activity was done by two invitro processes like nitric oxide scavenging method and DPPH radical scavenging activity. Ascorbic acid and Butylated hydroxyl toluene are taken as standard drugs.

Nitric Oxide Scavenging Activity

Nitric oxide scavenging Activity was measured by using a spectrophotometrically[9]. Sodium nitroprusside (5mM) in phosphate buffer saline was mixed with different concentration of aqueous extract (20 to 300 µg/ml) dissolved in methanol and incubated at 25^{0}C for 30 minutes. A control without test compound but with equivalent amount of methanol was taken. After 30 mins, 1.5ml of the incubated solution was removed and diluted with 1.5ml of griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethelene diamine dihydrochloride). The absorbance (optical density in colorimeter) of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine was measured at 546 nm.

The % inhibition was calculated by the formula (Control – test)/control × 100.

Ascorbic acid (Vitamin-C) is used as standard drug in concentration 100µg/ml & 200µg/ml. Test drug aqueous extract is tested in concentrations of 20, 50, 100, 200, 250, & 300µg/ml.

DPPH radical scavenging activity

DPPH scavenging Activity was measured by using a spectrophotometrically[10]. Free radical scavenging activity is determined using 2,2-diphenyl-2-picryl hydrazyl radical (DPPH), which is a stable free radical having a purple colour. When free radical scavengers are added, DPPH is reduced and its colour is changed to yellow, based on the efficacy of antioxidants.

DPPH solution in methanol in concentration 0.2mM was prepared and 1ml of this solution was added to 3ml of control (without the test compound but with an equivalent amount of methanol) and different concentration (5-100µgm/ml) of ethyl acetate, ethanol, aqueous extract and standard drug (25-100µgm/ml) butylated hydroxyl toluene (BHT). 30minutes latter the decrease in absorbance of the test and standard drugs (due to quenching of DPPH radical) was measured at 517nm and the percentage inhibition was calculated by using the formula.

% inhibition = (control- test)/ control × 100

RESULTS AND DISCUSSION

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, and inflammation and neurodegenerative diseases. Many flavonoids may help to provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense

system of the human body. Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several disease[11, 12]. Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signalling, 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 including neuronal messenger, vasodilation and antimicrobial and antitumor activities [13].

Active oxygen species and free radicals are involved in a variety of pathological events. In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as NO_2, N_2O_4, N_3O_4, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular components. In the present study the nitrite produced by the Incubation of solution of sodium nitroprusside in phosphate buffer saline at 25^0C for 2 hrs resulted in linear time dependent nitrite ion production, which was reduced by the ethyl acetate, ethanol and aqueous extract of *Nymphacea stellata.*

This may be due to the antioxidant principles in the extract which complete with oxygen to react with nitric oxide, thereby inhibiting the generation of nitrite. However, the ethyl acetate, ethanol and aqueous extract of *Nymphacea stellata* showed significant response in quenching nitric oxide radicals with an IC_{50} 95, 64, 96 µg/ml compare to ascorbic acid as standard having value of IC_{50} 40 µg/ml.

Table 1: Tests are done in triplicate and average value is written in the table.

Concentration (µg/ml)	% Inhibition in standard	% Inhibition in Ethyl acetate extract.	% Inhibition in ethanol extract.	% Inhibition in aqueous extract
20	40	25	36	30
50	60	40	48	39
100	85	52	60	55
200	90	60	66	70
300	92	78	80	85

*$p_<$ 0.05 when compared to control. Values are expressed as mean ± SEM.

The optical density of the control was found to be 0.124

The free radical scavenging activity was evaluated by various in vitro assays.

Table 2: DPPH free radical scavenging activity

Drugs	Concentration (µg/ml)	DPPH radical inhibition (%)
ETHYL ACETATE EXTRACT	5	21.5±0.16
	10	50±0.82
	25	78.25±1.12
	50	87.48±0.56
	100	90.24±0.38
ETHANOL EXTRACT	5	26.4±0.24
	10	41.26±0.12
	25	77.25±0.42
	50	90.14±0.16
	100	93.24±0.16
AQUEOUS EXTRACT	5	20.2±0.12
	10	29.8±0.14
	25	45.4±0.75
	50	74.5±0.16
	100	88.7±0.16
BUTYLATED HYDROXY TOLUENE	25	86.73±0.39
	50	88.47±0.15
	100	91.45±0.17

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. It involves reaction of specific antioxidant with a stable free radical 2, 2-diphenyl-1-picrylhydrazyl DPPH. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in the scavenging of the radical by hydrogen donation. Figure 1illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of extracts of *Nymphacea stellata* where as BHT was use as standards. The scavenging effect of ethyl acetate, ethanol and aqueous extract of Nymphacea stellata on the DPPH radical was 90.24%, 93.24 and 88.7 at a concentration of 100 µg/ml. These results indicated that extract has a noticeable effect on scavenging the free radicals. The results obtained from DPPH radical scavenging assay also reveals that the ethyl acetate, ethanol and aqueous extract gave an IC50 value of 13.63,16.66, 34.841µg/ml when compared with BHT. Free radical is chemical entities that can exist separately with one or more unpaired electrons. The generation of free radical can bring about thousands of reactions and thus can cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radical. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals.

The 2,2-diphenyl-2-picryl hydrazyl (DPPH) radical are widely used as the model system to investigate scavenging activities of several natural compounds such as phenolic and anthocyanins or crude

mixture such as aqueous extract of plants. DPPH radical is scavenged by anti oxidants through donation of proton forming the reduced DPPH. The colour changes from purple to yellow after reduction, which can be quantified by decrease of absorbance at wave length 517nm. Radicals scavenging activity increased with increasing percentage of free radical inhibition.

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517nm which is induced by antioxidants. The significant decrease in the concentration of DPPH radical is due to the scavenging activity of aqueous extract of the plant.

CONCLUSION

Nymphacea stellata has the potential to be rich source of phenolic and anthocyanins. Consideration of the antioxidant properties of ethyl acetate, ethanol, and aqueous extracts of *Nymphacea stellata* reported here and the potential disease preventive properties, suggests that it is appropriate for further work with this, to be directed at exploration of its chemo preventive properties.

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