ORIGINAL RESEARCH

ANTIOXIDANT ACTIVITIES OF CRUDE EXTRACTS OF *PULSATILLA NIGRICANS* STOCK

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ABSTRACT:

The present study investigates the *in-vitro* radical scavenging activity of different extracts obtained from the dry arial parts of *Pulsatilla Nigricans* by 1,1-diphenyl-2-picryl hydrazyl (DPPH) and nitric oxide assay. The methanolic extract of *Pulsatilla Nigricans* showed signifcant DPPH radical scavenging activity at IC_{50} of 25.67 µg/ml and low radical scavenging activity at 747.15 µg/ml with methanolic extract in nitric oxide assay.

KEYWORDS: *Pulsatilla Nigricans*, Ranunculaceae, Antioxidant activity

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1. INTRODUCTION

Free radicals and other oxygen derived species are constantly generated *in vivo*, both by accidents of chemistry and for specific metabolic purposes. The reactivity of different free radical varies, but some can cause severe damage to biological molecules especially to DNA, lipids and proteins. Antioxidant system of the body is generally able to combat the oxidative stress produced after normal physiological processes, but they are not completely effective. The increased production of free radicals leads to various diseases such as cancer, cardiovascular diseases, diabetes mellitus, liver disorders, brain dysfunction and inflammation hence, externally given antioxidants may be particularly important in a diminishing cumulative oxidative damage. Therefore the uses of antioxidants, both natural and synthetic are gaining wide importance prevent chronic disease.[1]

Pulsatilla Nigricans belongs to buttercup family, Ranunculaceae and grows in Turkey, Russia, Germany,France, Denmark, Sweden, England and Asia. .*Pulsatilla nigricans* Linn. (Ranunculaceae) has been traditionally used in the treatment of nervousness, restlessness, ovaritis, ovaralgia, pain associated with debility and due to acute inflammation, uterine affections, acute meningitis, and astaeniafuge. Despite a long tradition of use, no work has been carried out to justify its traditional claims, specially,NS depressant properties and pain associated with debility due to acute inflammation [2,3].

2. MATERIALS AND METHODS

2.1. General experimental procedures

Spectroscopic grade solvents were used for all spectroscopic studies without further purification. UV/visible absorption spectra were recorded on a Shimadzu TCC240A spectrometer. ELISA reader in Bio-Rad Laboratories Inc, California, USA, Model 550 with the filter of 490 nm[4].

2.2. Materials

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDD), Sodium nitroprusside and sulphanilic acid were purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai) India. All other chemicals and solvents [ethanol, methanol, chloroform, petroleum ether (60-80^{o}C), ethyl acetate, dimethyl sulphoxide (DMSO)] used in this study were of analytical grade and obtained from Merck Specialties Pvt. Ltd. (Mumbai) India. Microtiter plates were purchased from Tarsons India Pvt. Ltd., (Kolkata), India.

2.3. Collection of plant material

The arial parts of Plant of *P. nigricans* was procured from K. R. Indo German American Trading Company, Kurukshetra, India. Dr J. M. Majumder, East India Horticulture and Biotech Centre, West Bengal, India has authenticated the plant materials. *Pulsatilla Nigrican stock* is also present in Phanerogams herbarium in hungeryand specimen no Cat. P00040545 available in Central National Herbarium Botanical Survey of India, Calcutta.

2.4. Preparation of the extracts

The arial parts of *P. nigricans* were dried under shade, ground to half dust (each 1 kg) and soaked in petroleum ether (60-80°C) for 72 h at room temperature with occasional shaking. The extract was filtered and the filtrate evaporated to dryness under reduced pressure. The residue was soaked with the fresh solvent. The entire procedure repeated twice more to get maximum extract of constituents. The residue was extracted in the same way with chloroform, ethyl acetate and methanol. The extracts were collected and the solvents were evaporated using rotary evaporator at 40ºC to get 41g (4.1 %), 83g (8.3%), 77g (7.7%) and 72g (7.2 %) of crude extracts from respective solvent extracts. All the crude extracts were stored at 4ºC before performing biological activities[4,5,6]

2.5. In vitro antioxidant assays

2.5.1. Diphenyl picryl hydrazyl method (DPPH) free radical-scavenging activity

Free radical scavenging activity was measured by DPPH assay, which was adopted from Wang *et al*. (1998) and modified as reported by Badami *et al*. (2003). A 10 µL aliquot of the extract (21 mgmL^{-1} to 21µgmL^{-1}) was added to 200 µL of DPPH in methanol solution (100 µM) in a 96-well microtitre plate. After incubation at 37°C for 20 min., the absorbance of each solution determined at 490 nm using ELISA reader (Bio-Rad Laboratories Inc,

California, USA, Model 550). The corresponding blank reading was also taken and the remaing DPPH was calculated. IC_{50} is the concentration of sample required to scavenge 50% DPPH free radical and was obtained by plotting the percentage of free radicals scavenged versus the putative antioxidant concentration. All determinations were performed thrice in duplicate sets, and the average of the values is reported [8,9] .

2.5.2. Nitric oxide radical inhibition assay

Free radical scavenging activity was measured by nitric oxide assay, which was adopted from Garrat. (1964). The reaction mixture (6 mL) containing sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (1 mL) and extract or standard solutions (1 mL) were incubated at 25ºC for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrite was added and mixed with 1mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1mL of napththylethelene diammine dihydrochloride (NEDD) was added, mixed and allowed to stand for 30 min at 25°C. A pink colored chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions using spectrophotometer[10,11,12,13].

3. STATISTICAL ANALYSIS

All data were expressed as mean ± SEM. The statistical analysis of all the observations was carried out using one-way ANOVA followed by the multiple comparison test of Tukey–Kramer, where necessary. $P < 0.05$ was considered as significant compared with the control.

4. RESULTS AND DISCUSSION

Pulsatilla Nigricans known as traditional medicinal plants; grows in Turkey, Russia, Germany,France, Denmark, Sweden, England and Asia. [4-6]. They are used used in the treatment of nervousness, restlessness, ovaritis, ovaralgia, pain associated with debility and due to acute inflammation, uterine affections, acute meningitis, and astaeniafuge. The arial parts of plant *Pulsatilla Nigricans*is was dried under shade, ground to half dust and repeatedly extracted with petroleum ether, chloroform, ethyl acetate and methanol. .

The overproduction of radical reactive species leads to lipidic peroxidation, harming the membranes of biological systems and causing serious pathologies such as cancer, senescence, and inflammation. The research into natural products as health protecting factors against oxidative damage is an interesting field. Diverse studies have shown that natural products have a large range of biological activities .Natural products from plant are have potent antioxidant activity . (Badami, Gupta, Suresh, 2003; Hallwell, Gutteritge, Aruoma, 1987). So the present work was study of DPPH and nitric oxide radical scavenging activities of various extracts of *P.Nigricanesis*[14].

Table 1: Antioxidant activity of crude extracts from *Pulsatilla Nigricans*

Plants	Extracts	IC $_{50}$ in µg/ml	
		DPPH	Nitric oxide
Pulsatilla Nigricans	ME	25.67 ±1.41	747.15 ± 1.15
	ET	62.07 ± 3.55	876.3.38± 4.15
	CH	349.99 ± 13	1533.99 ± 3.38
	PE	252.50 ±4.60	1897.55 ± 2.64
Ascorbic Acid (Standard)	-	22.4 ±. 1.15	144.15 ± 1.88

Notes: [a] ME: Methanol Extract; ET: Ethyl acetate Extract; PE: Petroleum ether Extract; CH: Chloroform Extract. Values represent means ± SEM of three different experiments

Among all the extracts (Table 1), the methanolic extract of *Pulsatilla Nigricans*is showed DPPH radical scavenging activity with IC_{50} 25.67µgmL^{-1} as potent as the standard ascorbic acid (IC_{50} 22.7 µgmL^{-1}). The potent DPPH radical scavenging activity of methanolic extracts of the plants might be explained due to the rich contents of active metabolites s as compared to ethyl acetate, chloroform and petroleum ether extracts. As anticipated, the extracts of *Pulsatilla Nigricans*is showed less nitric oxide radical scavenging activity as compared to the standard ascorbic acid (Table 1). In conclusion, the present investigation revealed that the methanolic extracts of aerial parts *Pulsatilla Nigricans*is possess significant antioxidant activity at 25.67 µg/ml which is comparable with thw standard (Ascorbic acid)at 22.4 µg/ml Further work is in progresses to identify the bioactive compounds which are responsible for antioxidant, antimicrobial and cytotoxic activities.

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