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In Vitro Antioxidant Activity Of *Plumbago Zeylanica*

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ABSTRACT

This research was taken up to investigate the antioxidant activity of roots of *Plumbago zeylanica*. The *in vitro* antioxidant activity of ethanolic extract of roots of *Plumbago zeylanica* was investigated by DPPH free radical scavenging, nitric oxide scavenging and Superoxide scavenging methods at dose of 100–1000 μg mL^{-1}. The ethanol extract showed good antioxidant activity in these above methods. The maximum activity was found in DPPH free radical scavenging model. The antioxidant activity was dose dependent.

INTRODUCTION

Antioxidants are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexers of prooxidant metals, reducing agents and quenchers of singlet oxygen formation[1]. Antioxidants are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years[2]. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties. In this research *Plumbago zeylanica* was taken up for the purpose.

MATERIAL AND METHODS

Plant material and extraction

The roots of *Plumbago zeylanica* (*plumbaginaceae*) were collected from the Mandsaur, district of Madhaya Pradesh (India) during September. The specimen voucher (BRNCP/P 001) is deposited at the dept of Pharmacognosy, B R Nahata College of Pharmacy, CRC, Mandsaur for future reference. Roots of *P. zeylanica* were air-dried and powdered coarsely. The powder obtained (250 g) was defatted with petroleum ether and extracted with 90% upto complete extraction, filtered, concentrated under reduced pressure.

DPPH radical scavenging activity

DPPH scavenging activity was measured by the spectrophotometric method[3]. To a methanolic solution of DPPH (100 μmol, 2.95 mL), 0.05 mL of the extract dissolved in methanol was added at different concentrations (100-1000 μg mL^{-1}). An equal volume of methanol served as control. Absorbance was recorded at 517 nm at regular intervals of 30 s for 5 min.

Nitric oxide scavenging

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide[4-5]. Which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric oxide[5].

Nitric oxide scavenging activity was measured spectrophotometrically[6]. Sodium nitroprusside (5 mmol) in phosphate buffered saline was mixed with different concentrations of the extract (100-1000 μg mL^{-1}) dissolved in methanol and incubated at 25°C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 mL of the incubation solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1%naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm.

Superoxide scavenging

Superoxide scavenging was carried out using the alkaline dimethyl sulphoxide (DMSO) method[7]. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 μL) was added to 2.8 mL of an aqueous solution containing nitroblue tetrazolium (56 μmol), EDTA (10 μmol) and potassium phosphate buffer (10 mmol, pH 7.4). Sample extract (1 mL) of various concentrations (100–1000 μg mL^{-1}) was added and the absorbance was recorded at 560 nm against the control in which pure DMSO had been added instead of alkaline DMSO.

RESULTS AND DISCUSSION

Free radicals are highly reactive species produced in the body during normal metabolic functions or introduced from the environment. These are atoms or groups of atoms that have at least one unpaired electron, which makes them highly reactive. Oxygen, although essential to life, is the source of the potentially damaging free radicals. Antioxidants counteract these cellular by-products, called free radicals, and bind them before they can cause damage. In fact, free radicals are believed to play a role in more than sixty different health conditions, including the aging process, cancer and atherosclerosis[8]. Antioxidants work in several ways: they may stop the free radical from forming in the first place, or interrupt an oxidizing chain reaction to minimize the damage caused by free radicals.

There was 94.1% decrease of the DPPH radical at a 1 mg mL^{-1} extract concentration and it was found to be dose dependent (TABLE 1). DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm; reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. DPPH radicals

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TABLE 1: Free radical scavenging activity of *Plumbago zeylanica*

Extract concentration (μg mL^{-1})	Inhibition (%)		
	DPPH	Super oxide	Nitric oxide
100	15.2±1.1	11.5±2.3	3.7±0.8
200	25.1±2.3	23.1±1.2	11.9±1.3
300	34.7±2.1	29.2±2.6	17.2±2.3
400	46.2±3.6	34.1±2.2	21.2±2.1
500	58.3±1.9	38.3±1.9	29.1±1.9
600	70.1±3.4	44.1±3.1	39.9±3.2
700	84.1±3.7	52.2±3.4	48.5±3.4
800	88.2±3.2	59.1±3.3	53.2±3.3
900	91.2±3.7	63.8±3.1	56.2±3.1
1000	94.1±3.3	66.1±3.4	59.4±3.1
Ascorbic acid (100 mol L^{-1})	92.9 ± 3.6	88.1 ± 2.3	84.6 ± 4.2

react with suitable reducing agents and then electrons become paired off and the solution loses colour stoichometrically with the number of electrons taken up[9]. Such reactivity has been widely used to test the ability of compounds/plant extracts to act as free radical scavengers.

There was a moderate inhibition of the superoxide radical, with the maximum inhibition 66.1% at 1 mg mL^{-1} extract concentration (TABLE 1). Superoxide radical O_2 is a highly toxic species, which is generated by numerous biological and photochemical reactions. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyze the superoxide radical breakdown. Superoxide dismutase (SOD) is effective in reducing leukocyte adhesion in inflammatory conditions. The potassium superoxide assay was used to measure the superoxide dismutase activity of *P. zeylanica* extract.

There was a minimum inhibition of the nitric oxide formation with the maximum inhibition 59.4% achieved with 1 mg mL^{-1} extract concentration (TABLE 1). Incubation of solutions of sodium nitroprusside in phosphate buffer saline (PBS) at 25 °C for 2 h resulted in linear time-dependent nitrite production, which was reduced by the *P. zeylanica* extract. The scavenging of nitric oxide by the extract was concentration-dependent.

Since *P. zeylanica* showdantioxidant activity, its use in the traditional systems of medicine for its anti-inflammatory and anti-ulcer activity may in part be due to its free radical scavenging ability.

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