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Full Length Original Research Paper

In-Vitro Antioxidant and Phytochemical screening of various extracts of *Vernonia cinerea* Leaves

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Abstract: The free radical scavenging potential of the leaves of *Vernonia cinerea* was studied by using different solvents i.e. Methanol, ethanol, petroleum ether, benzene, acetone, ethylaceteate chloroform and Aqueous extracts. Preliminary phytochemical analysis reveals the presence of flavonoids, tannins, saponins, steroids, triterpenoids, glycosides, and phenolic constituents. *In vitro* antioxidant potential of leaves were evaluated by DPPH reduction and nitric oxide radical scavenging activity. The extracts exhibited IC_{50} values of 25.14 ± 1.23, 31.18 ± 1.08, 212.60 ± 3.03, 92.46 ± 2.79, 45.20 ± 3.92, 79.17 ± 1.05, 98.54 ± 2.87, and 128.38 ± 1.65 µg/ml, Ascorbic acid, which was used as a standard, showed an IC_{50} of 74.12µg/ml respectively in DPPH. 32.25 ± 01.12, 31.87 ± 01.04, 89.26 ± 04.62, 65.45 ± 03.20, 61.82 ± 01.23, 78.10 ± 03.08, 67.40 ± 01.33 and 71.18 ± 02.86 µg/ml, ascorbic acid, the standard, showed an IC_{50} of 28.41µg/ml, respectively in nitric oxide inhibition assays. These observations confirm that different extract of *Vernonia cinerea* leaves have quantitatively different constituent and outline its importance toward antioxidant activity.

Keywords: *Vernonia cinerea*, DPPH, nitric oxide, Free radical scavenging.

Introduction

Vernonia cinerea leaves belong to the family Asteraceae. It is an annual or short lived perennial to 50cm with ovate leaves herbs commonly known as 'Sahadevi' has been in using as indigenous Indian Medicine from time immemorial. It is found in India, Srilanka, Brazil, Russia, etc. It is used as a tonic, stomachic, and a stringent and is also a known cure for tridosa, consumption, asthma, and bronchitis [1]. The flower extract of the plant was used in adjuvant induced arthritis [2]. Further, the phenolic constituents of *Vernonia cinerea* were found to be effective inhibitors of the oxidative burst of activated polymorphonuclear leukocytes and therefore may also contribute to the anti inflammatory activity [3]. So far, no other biological investigations have been carried out on this plant.

Free radicals are responsible for aging and causing various human diseases. A study shows the antioxidant substances which scavenge free radicals play an important role in the prevention of free-radical-induced diseases. By donating hydrogen radicals, the primary radicals are reduced to non-radical chemical compounds and are then converted to oxidize antioxidant

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radicals [4]. There is extensive evidence to implicate free radicals in the development of degenerative diseases [5]. Free radicals have been implicated in causation of ailments such as diabetes, liver cirrhosis and nephrotoxicity etc [6].

The principal agents responsible for the protective effects could be the presence of antioxidant substances that exhibit their effect in absorbing and neutralizing free radicals, Quenching singlet and triplet oxygen or decomposing peroxides [7]. Hence in the present study, the successive extract of *Vernonia cinerea* was screened for invitro antioxidant properties using standard procedures.

MATERIALS AND METHODS

Plant Material

Vernonia cinerea leaves were collected in the month of April 2012 from the Western ghats of Anamalai hills, Valparai taluk of Coimbatore district, Tamil Nadu State, India. They were identified by Dr. V. S. Ramachandran, Professor, Department of Botany and Voucher specimens were deposited at the herbarium.

Preparation of Extracts and Standards

The fresh leaves were dried under shade. Powdered, pass through a 40-mesh sieve and stored in closed vessel for further use. The powder (180g) was extracted successively with methanol, ethanol, petroleum ether (40–60°C), benzene, acetone, ethyl acetate, chloroform, and Aqueous in a soxhlet extractor for 18 hrs. The extracts were concentrated under reduced pressure at low temperature (40–50°C). The extractive values were 34.5, 26.3, 0.9, 1.8, 18.4, 4.5, 20 and 12.5% respectively. Solution of ascorbic acid used as a standard for *in vitro* studies were prepared in distilled DMSO. The extract was subjected to qualitative chemical tests for alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, glycosides, vitamin C, fixed oils and fats and phenolic constituents respectively.

DPPH radical inhibition assay

The antioxidant capacity of *Vernonia cinerea* extracts was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH [8]. For assessment of DPPH radical scavenging activity DPPH solution was prepared by dissolving 4 mg DPPH in 100 ml methanol. A dilution series were prepared for ascorbic acid and extract. After that 5ml of sample solution was mixed with 0.5 ml DPPH solution and incubated for 30 min at room temperature in dark condition and absorbance was taken at 517 nm and calculated the % inhibition of DPPH radical.

Nitric oxide radical inhibition assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which can be estimated with oxygen to produce nitrite ions, which can be estimated by the use of Griess Illosvoy reaction [8]. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric oxide [9]. The reaction mixture (3ml) containing sodium nitroprusside (10mM. 2ml), phosphate buffer saline (0.5) and extract or standard solution (0.5ml) was incubated at 25°C for 150 minutes. After incubation, 0.5ml of the reaction mixture containing nitrite was pipette 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 1-naphthylamine (5%) was added, mixed and allowed standing for 30 min. a pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540nm against the corresponding blank solutions.

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The IC_{50} value is the concentration of sample required to inhibit nitric oxide radical.

Results and Discussion

The various extracts of *Vernonia cinerea* leaves exhibited antioxidant activity in the DPPH and the nitric oxide radical inhibition assay as evidenced by the low IC_{50} values (Table-1 & 2). The extracts i.e. methanol, ethanol, petroleum ether (40 - 60°C), benzene, Acetone, ethyl acetate, chloroform and Aqueous exhibits IC_{50} values of 25.14 ± 1.23, 31.18 ± 1.08, 212.60 ± 3.03, 92.46 ± 2.79, 45.20 ± 3.92, 79.17 ± 1.05, 98.54 ± 2.87 and 128.38 ± 1.65 µg/ml, respectively in DPPH and 32.25 ± 01.12, 31.87 ± 01.04 89.26 ± 04.62, 65.45 ± 03.20, 61.82 ± 01.23, 78.10 ± 03.08, 67.40 ± 01.33 and 71.18 ± 02.86, µg/ml, respectively in nitric oxide radical inhibition arrays. These values were found to be less than those obtained for the reference standard ascorbic acid.

Table 1: Antioxidant activity of *Vernonia cinerea* leaves extracts using DPPH method

S. No.	Test Compounds	IC_{50} Values ± SE* (µg/ml)
1	Methanol	25.14 ± 1.23
2	Ethanol	31.18 ± 1.08
3	Petroleum ether(40-60°C)	212.60 ± 3.03
4	Benzene	92.46 ± 2.79
5	Acetone	45.20 ± 3.92
6	Ethyl acetate	79.17 ± 1.05
7	Chloroform	98.54 ± 2.87
8	Water	128.38 ± 1.65
9	Ascorbic acid	74.12 ± 2.23

*Average of 10 determinations

Among the eight extracts of *Vernonia cinerea* leaves and standard tested for antioxidant activity using DPPH method. The methanol and ethanol extracts showed the maximum antioxidant activity with IC_{50} values of 25.14 ±1.23µg/ml and 31.18 ± 1.08µg/ml, respectively, where as petroleum ether (40-60°C) and Aqueous extract showed lowest antioxidant activity with an IC_{50} value of 212.60 ± 3.03µg/ml and 128.38 ± 1.65µg/ml. The known antioxidants ascorbic acid exhibited IC_{50} values of 74.12 ± 2.23µg/ml respectively.

Among the various extracts of *Vernonia cinerea* leaves and standard tested for antioxidant activity using nitric oxide radical inhibition method, the ethanol and methanol showed maximum antioxidant activity with IC_{50} values of 31.8 ± 01.04µg/ml and 32.25 ± 01.12µg/ml, respectively, where as Petroleum ether (40-60°C) showed lowest antioxidant activity with an IC_{50} value of 89.26 ± 04.62µg/ml. The known antioxidants ascorbic acid exhibited IC_{50} values of 28.41 ± 01.16µg/ml.

Table 2: Antioxidant activity of *Vernonia cinerea* leaves extracts using nitric oxide radical inhibition assay

S. No.	Test Compounds	IC_{50} Values ± SE* (µg/ml)
1	Methanol	32.25 ± 01.12
2	Ethanol	31.87 ± 01.04
3	Petroleum ether(40-60°C)	89.26 ± 04.62
4	Benzene	65.45 ± 03.20
5	Acetone	61.82 ± 01.23
6	Ethyl acetate	78.10 ± 03.08
7	Chloroform	67.40 ± 01.33
8	Water	71.18 ± 02.86
9	Ascorbic acid	28.41 ± 01.16

*Average of 10 determinations

The phytochemical tests (Table-3) indicated the presence of flavonoids in methanol, ethanol, acetone, ethyl acetate and chloroform extracts. Tannins in methanol, Saponins in methanol, ethyl acetate and aqueous, Steroids in petroleum ether (40–60°C) and benzene, Triterpenoids and glycosides in ethanol, methanol and chloroform, Vitamin C in methanol, Fixed oils and fats in petroleum ether (40-60°C), Phenolic constituents in methanol, and ethyl acetate extracts respectively.

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Table 3: Phytochemical analysis of the successive extracts of *Vernonia cinerea*

Sl. No	Chemical Constituents	Methanol	Ethanol	Pet. Ether (40-60°C)	Benzene	Acetone	Ethyl Acetate	Chloroform	Aqueous
1.	Aklaloids	-	-	-	-	-	-	-	-
2.	Flavonoids	+	+	-	-	+	+	+	-
3.	Tannins	+	-	-	-	-	-	-	-
4.	Saponins	+	-	-	-	-	+	-	+
5.	Steroids and Sterols	-	-	+	+	-	-	-	-
6.	Triterpenes	+	+	-	-	-	-	+	-
7.	Vitamin C	+	-	-	-	-	-	-	-
8.	Fixed Oils and Fats	-	-	+	-	-	-	-	-
9.	Glycosides	+	+	-	-	-	-	+	+
10.	Phenolic constituents	+	-	-	-	-	+	-	-

A large number of flavonoids and triterpenoids including these are known to possess antioxidant properties. Many of these phytochemicals possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of cancer in several human populations [11]. Thus *Vernonia* plays a role in scavenging the free radical attack on biomembranes. Hence the role of *Vernonia cinerea* is probably due the presence of flavonoids and triterpenoids.

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