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Research Article

# EVALUATION OF ENZYMIC AND NON-ENZYMIC ANTIOXIDANT LEVELS IN *VERNONIA CINEREA* EXTRACTS

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**ABSTRACT**

**Objective**: The objective of this study was to evaluate the enzymic and non-enzymic antioxidant levels in various solvent extracts of *Vernonia cinerea* leaves.

**Methods:** The fine powder of leaf (180 g) was extracted successively with methanol, ethanol, petroleum ether (40–60°C), benzene, acetone, ethyl acetate, chloroform, and aqueous in a Soxhlet extractor for 18 h. The extracts were concentrated under reduced pressure at low temperature (40–50°C), and the extracts were analyzed for the antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), peroxidise, glutathione S-transferase (GST), glutathione peroxidase (GPx), polyphenol oxidase, glutathione (GSH) reductase, and glucose-6-phosphate dehydrogenase and non-enzymic antioxidants such as Vitamin A, C, E, reduced GSH, and total phenol.

**Results:** Significant activities of enzymic antioxidants such as CAT (23.68 µ mole of $H_2O_2$ decomposed/min/mg protein, SOD (19.75 inhibition of 50% nitrite form/min/mg protein), and GST (73.28 µ mole of 1-chloro-2,4-dinitrobenzene conjugate formed/min) were observed higher in the methanolic extracts. Whereas, ethanolic extract exhibits maximum activity of GPx (1.054 µ mole of GSH utilized/min) and Px (102.1 µ mole of pyrogallol oxidized/min/mg protein). Total GSH (172.3 µM/g), Vitamin E (23.76 µM/g), and total phenols were significantly predominant in the ethanolic extracts followed by methanol and ethyl acetate extracts.

**Conclusion:** *V. cinerea* seems to be a promising plant in respect of its antioxidant potential, there is a lot more to be done to understand the mechanisms behind these effects as well as to employ them as possible therapeutic agents.

**Keywords:** *Vernonia cinerea*, Antioxidants, Glutathione, Total phenol.

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## INTRODUCTION

Free radicals are chemically unstable atoms that cause damage to lipid cells, proteins, and DNA, as a result of imbalance between the generations of reactive oxygen species (ROS) and the antioxidant enzymes [1]. Free radicals and ROS are well-known inducers of cellular and tissue pathogenesis, leading to several human diseases such as asthma, cancer, cardiovascular disease, cataract, diabetes, gastrointestinal inflammatory disease, liver disease, muscular degeneration, and other inflammatory process [2]. ROS are continuously produced during cell metabolism and under normal conditions; they are scavenged and converted to nonreactive species by different intracellular enzymatic and non-enzymatic antioxidant system [3]. Antioxidants can be classified into two major classes, i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously which include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The non-enzymatic antioxidants include alpha-tocopherols, carotenoids, ascorbic acid, flavonoids, and tannins which are obtained from natural plant sources. A wide range of antioxidants from both natural and synthetic origin has been proposed for the use in the treatment of various human diseases [4].

*Vernonia cinerea* (L.) less belonging to the family *Asteraceae* is an annual plant widely distributed in India, Bangladesh, Sri Lanka, and Malay Island [5]. *V. cinerea* is one of the species of *Vernonia* found in Nigeria, and it is known as a weed [6]. The plant is extensively used in indigenous medicine in stomach aches, and for cold, asthma and bronchitis [7]. The Ayurveda Pharmacopoeia of India recommends the plant for the treatment of intermittent fever, filariasis, blisters, boils, and vaginal discharges. The root of the plant is used traditionally for the treatment of all types of eruptive boils, and the juice is used for faster healing of accidental wounds, filariasis, and toxic viral fevers. The seeds are used in dysuria and to treat colic in the form of decoction [8]. Young leaves of the plant are used for the treatment of tonsillitis [9]. The leaf juice extract is used to treat skin diseases and the leaf extract for treating dysentery in children. Besides these, the plant is used in smoking cessation, cough, fever, malaria, urinary calculi, and leprosy [10]. The plant possesses hepatoprotective [11], antibacterial [12], antioxidant [13], anthelmintic, anti-inflammatory, analgesic, antipyretic [14,15], antiflatulent, antispasmodic [7], and antidiuretic properties [16].

Epidemiologic studies that analyze the health implications of dietary components rely on the intake estimates in sample populations found in databases that list the component's content in commonly consumed foods. Therefore, the availability of appropriate and complete food composition data is crucial. Due to the chemical diversity of antioxidant compounds present in plants, complete databases on plant antioxidant content are not yet available. Differences between each solvent type extracts could be explained by: (a) Various substitution patterns (mainly hydroxylation/glycosylation) of constituents, (b) various constituents ratio, and (c) various total contents of antioxidant active substances in extracts [17]. Therefore, the present study has been undertaken to evaluate the antioxidant status in different extracts of *V. cinerea* leaves.

## METHODS

### Plant collection

*V. cinerea* leaves were collected in September 2015 from the Western Ghats of Anamalai Hills, Valparai taluk of Coimbatore district, Tamil Nadu, India. They were identified and authenticated by the experts

at the Department of Botany, Kongunadu Arts and Science College, Coimbatore, where the voucher specimen has been deposited.

**Preparation of the plant extract**

The fresh leaves were washed with distilled water and shade dried for a week and powdered coarsely by hand. Then, they were finely powdered mechanically using an electric grinder and passed through 40 mesh sieve and stored in airtight containers. The powder (180 g) was extracted successively with methanol, ethanol, petroleum ether (40–60°C), benzene, acetone, ethyl acetate, chloroform, and aqueous in a Soxhlet extractor for 18 h. 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, 14.5, 20, and 12.5%, respectively.

**Enzymic and non-enzymic antioxidant assays**

The catalytic activities of antioxidant enzymes CAT [18], SOD [19], peroxidise [20], glutathione S-transferase (GST) [21], GPx [22], polyphenol oxidase (PPO) [23], glucose-6-phosphate dehydrogenase [24], and enzyme protein [25] were evaluated in eight different solvent extracts.

The non-enzymic antioxidants, namely Vitamin A [26], C [27], E [28], reduced glutathione (GSH) [29], and total phenol [30] were also estimated in selected extracts of *V. cinerea*.

**RESULTS AND DISCUSSION**

Table 1 summarizes the activities of SOD, CAT, GPx, GST, and Px were observed higher in the methanolic and ethanolic extracts of *V. cinerea* compared to that of other extracts. Free radical scavenging enzymes such as SOD, CAT, GPx, GSH reductase, and GST are the first line of defense against oxidative injury decomposing $O_2^{\bullet}$ and $H_2O_2$ before interacting to form the more reactive hydroxyl radical (OH•), the equilibrium between these enzymes is an important process for the effective removal of oxidative stress in intracellular organelles [31].

Significant activities of G6PD were observed higher in the ethanolic extract when compared to that of other extracts (Table 1). G6PD is a cytosolic NADP-dependent enzyme. This generates NADPH which is necessary for the regeneration of reduced GSH from oxidized GSH. Maintenance of GSH in the reduced state is an important function of G6PD. The PPO activities from Table 1 were found to be higher in methanol and ethyl acetate extracts compared to that of other extracts.

There is good evidence, suggesting the protective effect of SOD and CAT which are scavenger enzymes involved in free radical scavenging mechanism on lipid peroxidation [32]. CAT is an iron-containing enzyme catalyzing the dismutation of $H_2O_2$ into $O_2$ and $H_2O$. It is a major antioxidant enzyme in curtailing the peroxidative damage in biological system. The enzyme is found in all aerobic eukaryotes and is important in the removal of $H_2O_2$ generated in peroxisomes by oxidases and also in glyoxylate cycle and purine catabolism. Various isoforms are also reported in cytosol and mitochondria [33]. GPx, a selenium enzyme, catalyzes the destruction of $H_2O_2$ and lipid hydroperoxides by reaction with reduced GSH to form GSH disulfide and the reduction product of hydroperoxide [34]. GST is a family of isoenzymes which participate in the conjugations of GSH with the products of metabolites of xenobiotic substances such as cytochrome p450 systems, increasing their elimination from organisms in the form of mercapturates [35]. Pxs are a group of enzymes that catalyze oxidation-reduction reactions. As such, they are classified as oxidoreductases. Pxs reduce $H_2O_2$ to water while oxidizing a variety of substrates. Thus, Pxs are oxidoreductases which use $H_2O_2$ as electron acceptor for catalyzing different oxidative reactions.

Polyphenols exhibit a wide range of biological effects as a consequence of their antioxidant properties. They inhibit low-density lipoprotein (LDL) oxidation *in vitro* [36]. Moreover, LDL isolated from volunteers supplemented with red wine or red wine polyphenols exhibited reduced susceptibility to oxidation [37]. Thus, polyphenols probably protect LDL oxidation *in vivo* with significant consequences in atherosclerosis and also protect DNA from oxidative damage with important consequences in the age-related development of some cancers [38]. The enzymic protein concentration from Table 1 was found to be high in methanol, ethanol, and chloroform extracts compared to that of other extracts.

**Non-enzymic antioxidants**

Total GSH (Table 2) was predominant in the methanol and ethanolic extracts. The levels of Vitamin A and Vitamin C were found to be higher in the ethyl acetate extract, whereas chloroform and ethanol extracts exhibited high levels of Vitamin E. High levels of GSH and GPx play an important role in the prevention of lipid peroxidation. GSH is an important constituent of detoxification mechanism operating in biological system. GSH can function as an antioxidant in many ways. It can react chemically with singlet oxygen, superoxides, and hydroxyl radicals, and therefore, function directly as free radical scavenger. GSH may stabilize membrane structure by removing acyl peroxides formed by lipid peroxidation [39]. Ascorbic acid functions as the main water-soluble antioxidant protecting tissue from oxidative damage. It acts as a direct scavenger of free radicals and act as a reductant in enzymatic reactions [40].

Vitamin A and related retinols have been reported to increase immunity to tumors by several mechanisms including enhancement of cytotoxic T lymphocyte activity, which has been exploited in the treatment of HIV infection [41]. A number of observations have long supported the concept that Vitamin C may act as an antioxidant [42]. It acts as the main radical acceptor from Vitamin E [43]. It suppresses the formation of carcinogens such as nitrosamines and quinines [44]. A preparation containing green tea extract, ascorbic acid, sunflower seed extract, carotene, and natural Vitamin E has been designed as a model "universal antioxidant" that may offer protection through its scavenging action on a wide range of free radicals both water soluble and fat soluble [45].

The tocopherols, specifically, α-tocopherol (Vitamin E), have been studied extensively in mammalian research as membrane stabilizers

**Table 1: Enzymic antioxidant levels in various extracts of *V. cinerea***

| Extracts | CAT | SOD | GPx | GST | Px | PPO | G6PD | Enzymic protein |
|---|---|---|---|---|---|---|---|---|
| Methanol | $23.68^{a}$ | $19.75^{a}$ | $0.920^{b}$ | $73.28^{a}$ | $96.96^{a}$ | $2.654^{a}$ | $4.037^{e}$ | $0.468^{a}$ |
| Ethanol | $22.60^{a}$ | $19.08^{a}$ | $1.054^{a}$ | $69.93^{a}$ | $102.1^{a}$ | $1.494^{b}$ | $7.284^{a}$ | $0.476^{a}$ |
| P.E | $6.975^{f}$ | $11.50^{d}$ | $0.301^{e}$ | $26.90^{e}$ | $38.94^{de}$ | $0.256^{d}$ | $1.006^{g}$ | $0.096^{d}$ |
| Benzene | $1.058^{e}$ | $5.602^{e}$ | $0.100^{g}$ | $12.95^{f}$ | $34.90^{e}$ | $0.420^{c}$ | $1.181^{f}$ | $0.091^{d}$ |
| Acetone | $17.12^{b}$ | $13.50^{c}$ | $0.366^{d}$ | $32.04^{d}$ | $61.69^{b}$ | $1.594^{b}$ | $4.162^{e}$ | $0.293^{c}$ |
| Ethyl acetate | $17.12^{b}$ | $14.77^{b}$ | $0.508^{c}$ | $55.32^{b}$ | $48.40^{d}$ | $2.545^{a}$ | $5.053^{b}$ | $0.349^{b}$ |
| Chloroform | $11.05^{c}$ | $13.10^{bc}$ | $0.302^{e}$ | $39.63^{c}$ | $64.96^{b}$ | $1.503^{b}$ | $4.283^{d}$ | $0.475^{a}$ |
| Aqueous | $9.517^{d}$ | $15.28^{b}$ | $0.257^{f}$ | $26.78^{e}$ | $57.24^{c}$ | $1.653^{b}$ | $5.672^{c}$ | $0.298^{c}$ |

Values are expressed as mean of three replicates. Means followed by a common letter are not significantly different at the 5% level by DMRT, CAT - 1µ mole of $H_2O_2$ decomposed/min/mg protein, SOD - inhibition of 50% nitrite form/min/mg protein, GPx - 1µ mole of GSH utilized/min, GST - 1µ mole of 1-chloro-2,4-dinitrobenzene conjugate formed/min, Px - 1µ mole of pyrogallol oxidized/min/mg protein, PPO - 0.01 O.D change/min/mg protein, G6PD - 0.01 O.D change/min/mg protein, Enzymic protein - mg/g. *V. cinerea: Vernonia cinerea*, DMRT: Duncan's multiple range test, CAT: Catalase, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GST: Glutathione S-transferase, Px: Peroxidase, PPO: Polyphenol oxidase

**Table 2: Non-enzymic antioxidant levels in various extracts of *V. cinerea***

| Extracts | Total GSH | Vitamin A | Vitamin C | Vitamin E | Total phenols |
|---|---|---|---|---|---|
| Methanol | 163.9[a] | 8.426[b] | 2.244[b] | 20.60[b] | 71.11[a] |
| Ethanol | 172.3[a] | 7.829[c] | 1.428[d] | 23.76[a] | 70.04[a] |
| P.E | 84.23[e] | 5.406[f] | 1.377[e] | 10.30[e] | 34.55[e] |
| Benzene | 122.54[c] | 5.917[e] | 0.918[f] | 15.05[c] | 42.24[d] |
| Acetone | 97.08[d] | 6.820[d] | 1.836[c] | 10.30[e] | 31.90[f] |
| Ethyl acetate | 59.53[f] | 9.671[a] | 3.417[a] | 11.09[d] | 46.55[c] |
| Chloroform | 136.6[b] | 7.994[c] | 0.816[g] | 22.17[a] | 33.60[e] |
| Aqueous | 101.5[d] | 5.671[f] | 0.852[g] | 10.38[e] | 50.07[b] |

Values are expressed as mean of three replicates. Means followed by a common letter are not significantly different at the 5% level by DMRT, Total GSH - µM/g, Vitamin A and E - µg/g, Vitamin C - mg/g, Total phenol - µg/g. *V. cinerea: Vernonia cinerea*, DMRT: Duncan's multiple range test, GSH: Glutathione

and multifaceted antioxidants that scavenge oxygen free radicals, lipid peroxyradicals, and singlet oxygen [46]. Vitamin E is the important hydrophobic chain-breaking antioxidant that protects membrane and plasma lipoprotein from free radicals [47]. Due to its dietary importance, tocopherol levels have been documented extensively in plant tissue [48]. The total phenols were predominant in the methanol and ethanolic extracts. The presence of phenolic compounds in the plant indicates that these plants may have antimicrobial agents. These antioxidants contribute significantly toward the biological activities such as hypoglycemic, antidiabetic, antimicrobial, antioxidant, anti-inflammatory, anticarcinogenic, antimalarial, and antileprosy activities [49].

## CONCLUSION

The results of this study showed that the level of antioxidants significantly varies with different solvent extracts. *V. cinerea* seems to be a promising plant in respect of its antioxidant potential; there is a lot more to be done to understand the mechanisms behind these effects as well as to employ them as possible therapeutic agents.

## CONFLICTS OF INTEREST

None.

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