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RESEARCH ARTICLE

FREE RADICAL SCAVENGING ACTIVITY OF LEAVES OF *VOLKAMERIA INERMIS*

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ABSTRACT

Volkameria inermis is an evergreen shrub of 3m tall, distributed throughout the tropical and inter tropical regions of India. It belongs to the family Lamiaceae. The petroleum ether, chloroform, ethyl acetate, ethanol and aqueous leaf extracts of *Volkameria inermis* were screened for free radical scavenging activity against DPPH, superoxide, hydroxyl, hydrogen peroxide and nitric oxide. The scavenging activity was found to be dose dependent. Of all the extracts the ethanolic extract showed maximum scavenging activity. Hence, from the present study it can be concluded that the leaves of *Volkameria inermis* possess good radical scavenging activity and it can be used as an easily available source of natural antioxidants.

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INTRODUCTION

Free radicals are molecules with an unpaired electron due to which they are highly unstable, trying to capture electrons from nearby molecules and cause deterioration of cells. This chain reaction continues and causes damages to cell which includes damage to DNA, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivate specific enzymes by oxidation of co-factors. Free radicals cause many human diseases like cancer Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease, fibrosis, etc. Antioxidants combat the free radicals formed and terminate the chain reaction before vital organs are damaged (Sarma *et al.,* 2010). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) are widely used because they are cost effective and cheaper than naturalones. However, studies have reported that synthetic antioxidants are toxic and restrictions have been imposed on their use. Hence, researchers have focused their interest on plant derived natural antioxidants (Narayanaswamy and Balakrishnan, 2011). Antioxidants derived from plant materials terminate the harmful effects of free radicals and protect the human system from various diseases. Hence, there is a growing interest all over the world in using the medicinal plants as a source of antioxidants.

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Volkameria inermis belongs to the family Lamiaceae, commonly known as wild jasmine. The pharmacological activities of the plant are anti microbial, anti nematidalcidal effects and anti hepatotoxic activity (Chethana *et al.,* 2013). However the free radical scavenging activity of *Volkameria inermis* has not been reported so far, hence the present study deals with the *in vitro* free radical scavenging activity carried on the leaves of *Volkameria inermis*.

MATERIALS AND METHODS

(i) Collection of plant materials

The leaves of *Volkameria inermis* was collected from Tamil Nadu Agriculture University campus (TNAU) Coimbatore, Tamil Nadu, India. The sample was identified and authenticated by The Botanical Survey of India, TNAU, Coimbatore. The authentication number is BSI/SRC/5/23/ 2015 /Tech/2082.

(ii) Preparation of the extracts

To ten gram of the dried, powdered leaf the solvents were added in increasing polarity petroleum ether, chloroform and ethanol (10g/100ml). Plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24 hours. After 24 hours the extract was filtered and the filtrate was concentrated using flash evaporator and stored in air tight containers at 4°C (Santhi *et al.,* 2011). Apart from the solvent extracts, a fresh aqueous extract was also prepared.

(iii) Chemicals

Hydrogen peroxide, potassium hydrogen buffer, 1,1 diphenyl-2-picyrl-hydrazyl (DPPH), ethylene diamine tetra-acetic acid (EDTA), sodium nitroprusside were obtained from Himedia and Merck. The solvents used were of analytical grade.

(iv) Determination of DPPH scavenging activity

The DPPH scavenging activity was studied by the method proposed by Mensor *et al.* (2011). The different solvent extracts and crude aqueous extract (20µl corresponding to 10mg) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was then allowed to react at room temperature for 30 minutes. The DPPH methanol solution was used as positive control and methanol alone acted as blank. After incubation, the discolourisation of the purple colour was read at 518nm in a spectrophotometer. The percent inhibition was calculated using the following formula;

$$\text{Inhibition (\%)} = \frac{\text{A(Control) - A(Sample)}}{\text{A(Control)}} \times 100$$

(Vi) Determination of superoxide scavenging activity

Superoxide scavenging activity was studied by the method of Mc Cord and Fridovich (1968). The assay tubes contained plant sample with 0.2ml of EDTA, 0.1ml NBT, 0.05ml riboflavin and 2.55ml of phosphate buffer. DMSO instead of plant sample was treated as the control. The initial absorbance was recorded at560nm. The tubes were uniformly illuminated using a fluorescent lamp for 30 minutes and the absorbance was read at 560nm. The difference in optical density is the production of superoxide ion and the percentage inhibition was calculated using the formula;

$$\text{\% Superoxide Scavenging} = \frac{\text{A (After illumination) – A (Reference)}}{\text{A (Control)}} \times 100$$

Viii) Determination of hydroxyl scavenging activity

The hydroxyl radical scavenging activity was studied by the procedure of Elizabeth and Rao (1990). The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of ferric chloride, 0.1ml of H_2O_2, 01ml of ascorbate, 0.1ml buffer and plant extract. The volume was made upto 1ml with water. The test tubes were capped tightly and incubated at 37°C for one hour. The reaction mixture was terminated by the addition of 1.0ml of TBA. The test tubes were incubated in water bath for 20 minutes . The intensity of pink colour formed was measured at 532nm.

$$\text{Hycroxyl radical scavenging activity (\%)} - \frac{\text{A (Control) – A (Sample)}}{\text{A (Control)}} \times 100$$

(Vii) Determination of hydrogen peroxide scavenging activity

The ability of the plant extract to scavenge hydrogen peroxide was determined by the method proposed by Ruch *et al*. (1989). Plant extracts at a concentration of 10mg in 10µl was added to 0.6ml of H_2O_2. The final volume was made upto 3ml with phosphate buffer. After 10 minutes the absorbance value was read at 230nm against the blank containing phosphate buffer without H_2O_2. The percent inhibition was calculated using the formula;

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{\text{A (Control) – A (Sample)}}{\text{A (Control)}} \times 100$$

(V) Determination of nitric oxide scavenging activity

The antioxidant effect was studied using nitric oxide scavenging activity according to the method proposed by Green *et al.*(1982). The reaction mixture containing plant extract and 3ml of sodium nitroprusside was incubated at 25°C for 150 minutes. After incubation 0.5ml of Griess reagent was added. Control contains all the reaction mixtures except the plant sample. The absorbance was read at 546nm. The percent inhibition was calculated using the formula;

$$\text{Nitric oxide scavenging activity (\%)} = \frac{\text{A (Sample) - A (Sample)}}{\text{A (Control)}} \times 100$$

RESULTS AND DISCUSSION

1) DPPH Scavenging Activity

The DPPH scavenging activity was found to be increased with the increase in the concentration of the extracts ranging from 20-100 µg/ml. The results obtained were shown in figure 1. Of all the extracts the ethanolic leaf extract of *Volkameria inermis* showed the highest DPPH scavenging activity followed by aqueous, chloroform, petroleum ether and ethyl acetate.

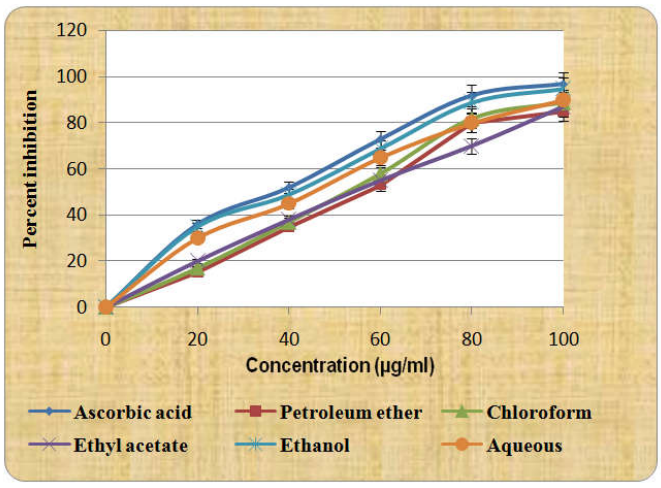

Figure 1.DPPH Scavenging Activity of Leaves of *Volkameria inermis*

The DPPH is the commonly used reagent to evaluate the free radical scavenging activity of antioxidants. The DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants Lagnika *et al.,* 2011. The dose dependent DPPH radical scavenging activity and the IC_{50} value of ethanolic extract of *Volkameria inermis* was found to be 41.5 µg/ml and standard ascorbic acid was found to be 37.5 µg/ml (Table 1). The reducing ability of the extract depends on the presence of reductants, which have been shown to exert antioxidant action by breaking the free radical chain reactions by donating a hydrogen atom. This increased

scavenging capacity of the extract may be due to the presence of high levels of phytochemical constituents. Similar dose dependent DPPH scavenging effect was also observed in *Syzygium cumini* seed ethanolic extract when compared with ascorbic acid (Banerjeem and Narendhirakannan, 2011). Methanolic extracts of *Conocarpus erectus* leaves, stems, fruits and flowers showed high free radical scavenging activity toward DPPH radical (Abdel-Hameed *et al.,* 2012).

Table 1. IC_{50} Value of Extracts of the Leaves of *Volkameria inermis*

Solvents	IC_{50} Value (µg/ml)				
	DPPH	Superoxide	Hydroxyl	Hydrogen Peroxide	Nitric oxide
Standard	37.5	53.0	62.0	88.0	80.0
Petroleum Ether	58.0	86.0	85.0	50.0	40.0
Chloroform	53.0	63.0	70.0	59.0	53.0
Ethyl acetate	55.0	67.0	78.0	61.0	48.8
Ethanol	41.5	55.0	64.0	84.0	87.7
Aqueous	47.0	60.0	68.0	79.0	65.5

2) Superoxide Scavenging Activity

Superoxide anion is an oxygen-centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions. Overproduction of superoxide anion radical, contributes to redox imbalance and are associated with harmful physiological consequences. Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, which are very harmful to the cellular components in a biological system (Sivanandam *et al.,* 2012).

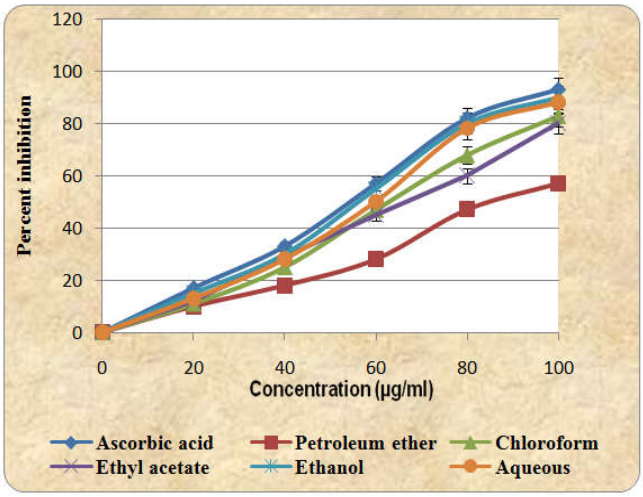

Figure 2. Superoxide Scavenging Activity of Leaves of *Volkameria inermis*

The dose dependent scavenging activity of superoxide radical of different extracts of *Volkameria inermis* was shown in figure 20. The IC_{50} values were found to be higher for ethanolic extract 55.0 µg/ml followed by aqueous 60.0 µg/ml against the standard ascorbic acid 53.0 µg/ml (Table 1). Thus, the extract could maintain redox homeostasis in body by inhibiting superoxide anion production. Similar dose dependent inhibition of superoxide generation was observed by free radical scavenging activity of methanolic extract of *Vitis vinifera, Oroxylum indicum* and Policosanol isolated from *Saccharum officinarium* (D'Mello *et al.,* 2012).

3) Hydroxyl radical Scavenging Activity

Hydroxyl radical was produced from either metal catalyzed Haber–Weiss reaction or Fenton reaction. These radicals are the major active oxygen species responsible for lipid peroxidation resulting various deleterious biological effects to induce carcinogenesism and mutagenesis. In this process, the ferric ion is reduced by superoxide, with subsequent oxidation of ferrous ion by H_2O_2 forming hydroxyl radical there by initiating the series of oxidative reactions (Manian *et al.,* 2008; Ramkumar *et al*., 2009).

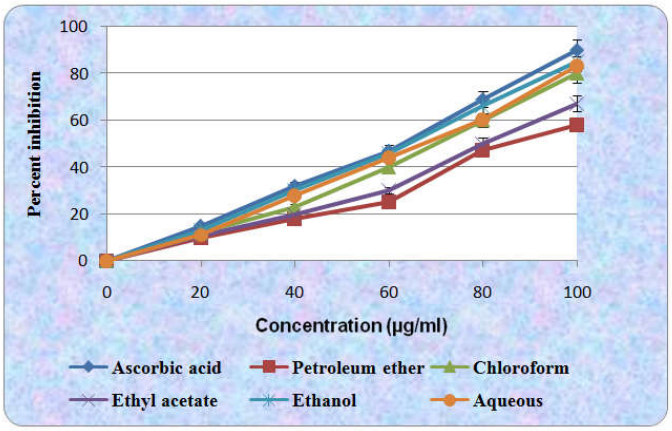

Figure 5. Hydroxyl Radical Scavenging Activity of Leaves of *Volkameria inermis*

The hydroxyl radical scavenging activity of the ethanolic leaf extract and standard ascorbic acid was found to be dose dependent (figure 21) and their IC_{50} values were found to be as 64.0 µg/ml and 62.0 µg/ml respectively (Table 1). Similar dose dependent inhibition of hydroxyl generation was observed by free radical scavenging activity of *Cucumis trigonus* (Balakrishnan and Kokilavani, (2011).

4) Hydrogen peroxide

Hydrogen peroxide is not a radical species but play a role to contribute oxidative stress through Fenton reaction. Hydrogen peroxide can easily cross the cell membranes. In *in vivo* condition hydrogen peroxide reacts with iron complexes inside the cell to generate highly reactive hydroxyl radicals and which inturn causes toxic effects (Nishaa *et al.,* 2012).

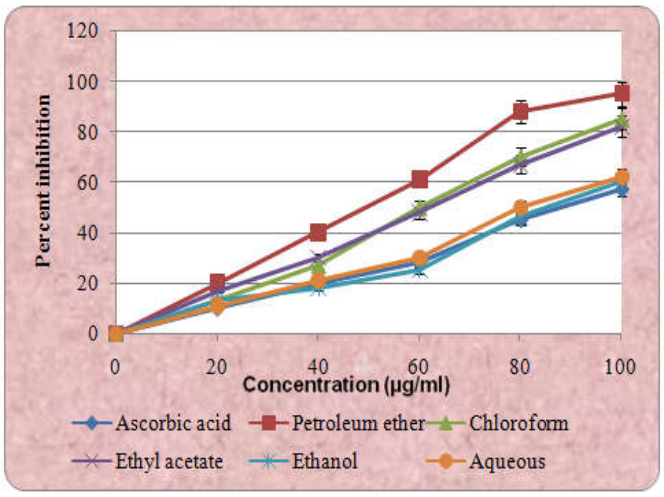

Figure 4. Hydrogen peroxide Scavenging Activity of Leaves of *Volkameria inermis*

The hydrogen peroxide radical scavenging activity of the ethanolic leaf extract and standard ascorbic acid was found to be dose dependent as shown in figure 22 and their IC_{50} values were found to be as 84.0 µg/ml and 88.0 µg/ml respectively

(Table 1). Phytochemical constituents present in the extract might be responsible for the neutralization of H_2O_2 into water by denoting electrons. Similar dose dependent H_2O_2 scavenging effect was also observed by the organic fractions of *Garcinia kola* and *Njavara rice bran* (Okoko, 2009) ethanolic leaf extracts of *Ziziphus mucronata* (Kwape and Chaturvedi, (2012).

5) Nitric oxide scavenging activity

Nitric oxide radicals are produced by macrophages under inflammatory condition. Because of its mutagenic nature nitric oxide interferes with DNA and causes various carcinomas and inflammatory diseases. Nitric oxide reacts with superoxide radical and forms highly reactive metabolite peroxynitrite (ONOO–) anion which is highly toxic to humans (Shajeela *et al.*, 2012).

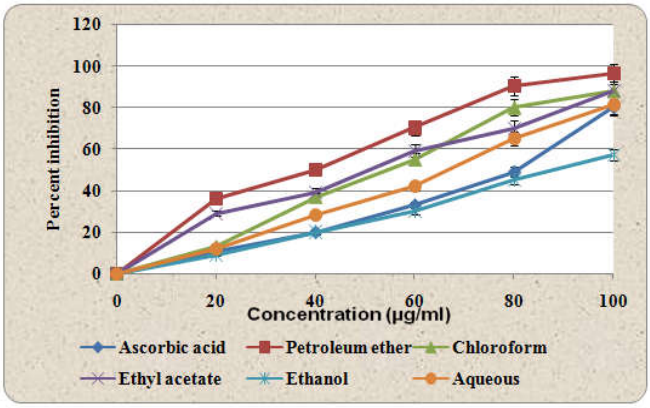

Figure 5. Nitric oxide Scavenging Activity of Leaves of *Volkameria inermis*

The nitric oxide scavenging activity of ethanolic leaf extract of *Volkameria inermis* increased in a dose dependent manner as shown in figure 5.The radical scavenging activity of ethanolic leaf extract of *Volkameria inermis* was compared with the standard ascorbic acid. The extract showed a dose dependent nitric oxide scavenging activity. The IC_{50} value of ethanolic leaf extract and standard was found to be 87.7µg/ml and 80.0 µg/ml respectively (Table 1). The leaf extract was found to be more active than that of ascorbic acid. Grace *et al*., 2012, observed a dose dependent inhibition of nitric oxide in the leaf extract of *Lantana camara*. Similarly, the ethyl acetate and ethanolic fraction of *Pisonia grandis* showed a scavenging of nitric oxide against the standard ascorbic acid (Jayakumari *et al.*, 2012).

Conclusion

The present study reveals that the different extracts namely petroleum ether, chloroform, ethyl acetate, ethanol and aqueous showed a potent antioxidant activity against DPPH, superoxide, hydroxyl ,hydrogen peroxide and nitric oxide scavenging activity. Of all the extracts the ethanolic leaf extract of *Volkameria inermis* showed a strong antioxidant activity. Hence, it can be concluded that the leaves of *Volkameria inermis* showed a good antioxidant potential and can be used for medicinal purpose. However, future studies have to be carried out to evaluate the antioxidant capacity of the extracts in *in vivo* system using animal models.

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