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RESEARCH ARTICLE

ANTIOXIDANT POTENTIAL OF SOME WILD PEPPERS

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ABSTRACT

Wild relatives of the cultivars are now being given much attention by conservationists across the globe. The *Piper* genus is an extremely well known and widely distributed pantropical taxon of aromatic plants. The wild relatives of species *Piper nigrum* are reported from The Nilgiris, approximately 2,000 metres (6,600 ft) above sea level. The antioxidant assay of the fruits of *Piper schimidtii*, *Piper mullesua* and *Piper velayudhanii* were conducted following DPPH Radical Scavenging Assay and Total Antioxidant Capacity (TAC) assay by Phosphomolybdenum method. All the three wild pepper species fruits are found to possess credible antioxidant activity. Since both ethyl acetate and ethanol extract demonstrated antioxidant activity, indicate the fact that structurally similar compound exhibits the property in the fruits of the plant.

INTRODUCTION

The extensive use of medicinal plants by people all over the world increased the attention of the scientific community and the focussed research targets bioactive phytochemicals. Many countries in the world, that is, two-third of the world's population depends on herbal medicine for primary health care. Plants synthesize a vast range of structurally diverse secondary metabolites, and many are distributed among a very limited number of species within the plant kingdom (Fraga, 2010). The Nilgiris also known as Blue Mountains, are a range of mountains with as many as 24 peaks 2,000 metres (6,600 ft) above sea level, in the western end of Tamil Nadu state. It is located at the junction of the Eastern Ghats and the Western Ghats meet. This area nourishes a wide variety of endemic and exotic plants within a forest cover of 763sq. kilometers. The rich biodiversity of the region is designated as 'the hotspots of biological diversity' by UNESCO, harbours species of wild pepper alongside of other valuable plants. The *Piper* genus is an extremely well known and widely distributed pantropical taxon of aromatic plants, many of which have been used in the past as food and medicinal plants. *Piper* plants are rich in essential oils, which can be found in many tissues and organs: fruits, seeds, leaves, branches, roots and stems (Da Silva et al., 2017; Mgbeahurike et al., 2017).

The genus possess an array of biological activities such as antibacterial (Lugar et al., 2002) antioxidant (Lei et al., 2003), anti-inflammatory (Lin et al., 2006) etc. In the present investigation species of three wild peppers namely *Piper schimidtii*, *Piper mullesua* and *Piper velayudhanii* fruits were evaluated for their *in vitro* antioxidant properties.

MATERIALS AND METHODS

Collection and preparation of Plant materials: The wild *Piper* species were collected from the upper Nilgiris region of Nilgiris district, Tamil Nadu. The identity of the specimens are confirmed and certified by Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore district. Fresh fruits of *Piper schimidtii*, *Piper mullesua* and *Piper velayudhanii* are collected and shade dried and are milled to a coarse powder by using mortar and pestle. The powders are stored in sterile, air tight glass bottles and kept at room temperature for further analysis. Extraction of fruits for the present study was carried out by successive extraction method as described by Das et al. (2010). 100g of each leaf and fruit powders were charged into glass beakers then successively soaked with three organic solvents (petroleum ether, ethyl acetate and ethanol) successively for 72 hours. Each time before employing the solvent of higher polarity, the marc was dried. Each extract was stored in sterile containers at room temperature for phytochemical profiling.

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Fig. 1. *Piper mullesua*



Fig. 2. *Piper schmidtii*



Fig. 3. *Piper velayudhanii*

Antioxidant activities: The antioxidant assay of various extracts of the selected *Piper* species was carried out by DPPH Radical Scavenging Assay and Total Antioxidant Capacity (TAC) by Phosphomolybdenum method.

DPPH (Diphenyl Picryl Hydrazyl) Radical Scavenging Assay: *Chemical and reagents used:* 2, 2, Diphenyl-1-Picryl hydrazyl (DPPH), methanol, Dimethyl sulfoxide (DMSO), distilled water.

Procedure: The assay was carried out in a 96 well microtitre plate. To 200 μ l of DPPH solution, 10 μ l each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used are 1000 to 1.95 μ g/ml. The plates were incubated at 37 $^{\circ}$ C for 20 minutes and the absorbance of each well was measured at 490 nm, using ELISA reader against the corresponding test and standard blanks and the amount remaining DPPH was calculated. IC₅₀ (Inhibitory Concentration) is the concentration of the sample required to scavenge 50% of DPPH free radicals (Gudda Darangavvanahally et al, 2004; Sithisarn and Gritsanapan, 2005; Tirzitis and Bartosz, 2010).

$$\% \text{ of inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Estimation of total antioxidant capacity by Phosphomolybdenum method: *Chemicals and Reagents used:* Sulphuric acid (0.6M solution), sodium sulphate (28mM solution), ammonium molybdate (4mM). All these reagents are mixed (Total Antioxidant Capacity (TAC) reagent) and used for the study.

Procedure: 100 μ l of plant extract is dissolved in 1 ml of TAC reagent. Blank is maintained with distilled water replacing the TAC reagent. Absorbance is seen at 695 nm (Gudda Darangavvanahally et al, 2004, Sithisarn and Gritsanapan, 2005).

RESULTS AND DISCUSSION

DPPH⁺ radical scavenging activity: The results of the DPPH⁺ free radical scavenging activity of the selected wild *Piper* species are shown in figure 1 and table 1. In this assay, ascorbic acid used as reference control (standard). It was determined by measuring the concentration of sample necessary to decrease initial concentration of DPPH⁺ by 50% (IC₅₀) under the experimental condition. Therefore, lower value of IC₅₀ indicates a higher antioxidant activity. Ethanol extract of *P. schmidtii* (IC₅₀ - 24 \pm 0.43 μ g/mL), *P. mullesua* (19 \pm 0.0143 μ g/mL) and ethyl acetate extract of *P. velayudhanii* (23 \pm 0.01) showed maximum DPPH⁺ radical scavenging activity, which is comparable to the activity of reference control, ascorbic acid (IC₅₀ 2.69 \pm 0.05 μ g/mL). The free radical scavenging activity was found to be lower in petroleum ether extract of all the species. Proton radical scavenging action is an important attribute of antioxidants, which is measured by DPPH radical scavenging assay.

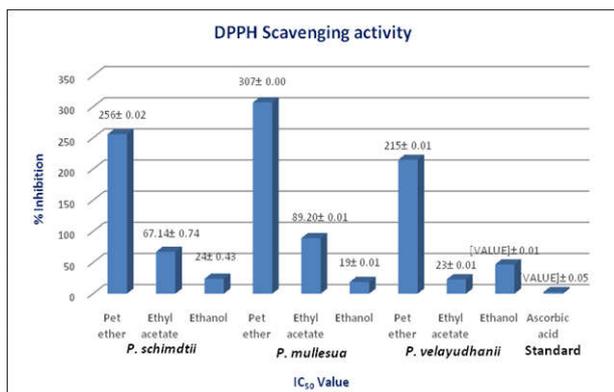
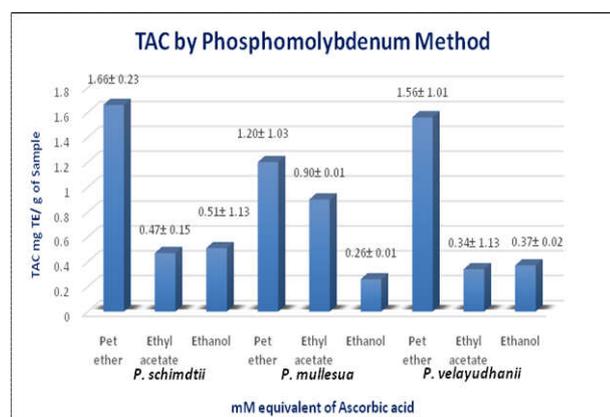
Total Antioxidant Capacity (TAC) by Phosphomolybdenum Method: Figure 2 and table 2 depicts the total antioxidant capacity obtained through the phosphomolybdenum assay for selected *Piper* fruit extracts in comparison with that of mM equivalent of Ascorbic acid, it used as reference. Overall, the petroleum ether extract of all the tested plants showed higher antioxidant activity (1.66 \pm 0.2mg TE/g in *P. schmidtii*, 1.20 \pm 1.03mg TE/g in *P. mullesua* and 1.56 \pm 1.01mg TE/g in *P. velayudhanii*) than other extracts. The ethanol extract of *P. mullesua* (0.26 \pm 0.01mg TE/g) showed a much lower antioxidant potential than other extracts. This result differs with the lowest antiradical effect of ethanol extract of *P. mullesua* and *P. schmidtii* and ethyl acetate extract of *P. velayudhanii* determined by the DPPH assay. In contrast, ethyl acetate extract of and *P. schmidtii* and *P. velayudhanii* showed much similar activity in this assay.

Table 1. DPPH⁺ radical scavenging activity of Fruit extracts of selected *Piper* species

Species	Extracts	IC ₅₀ (µg/mL)
<i>P. schmidtii</i>	Petroleum ether	256± 0.02
	Ethyl acetate	67.14± 0.74
	Ethanol	24± 0.43
<i>P. mullesua</i>	Petroleum ether	307± 0.00
	Ethyl acetate	89.20± 0.01
	Ethanol	19± 0.01
<i>P. velayudhanii</i>	Petroleum ether	215± 0.01
	Ethyl acetate	23± 0.01
	Ethanol	47± 0.01
Standard	Ascorbic acid	2.69 ± 0.05

Table 2. Total Antioxidant Capacity (TAC) by Phosphomolybdenum method in Fruit extracts of selected *Piper* species

Species	Extracts	TAC (mM equivalent of Ascorbic acid)
<i>P. schmidtii</i>	Petroleum ether	1.66± 0.23
	Ethyl acetate	0.47± 0.15
	Ethanol	0.51± 1.13
<i>P. mullesua</i>	Petroleum ether	1.20± 1.03
	Ethyl acetate	0.90± 0.01
	Ethanol	0.26± 0.01
<i>P. velayudhanii</i>	Petroleum ether	1.56± 1.01
	Ethyl acetate	0.34± 1.13
	Ethanol	0.37± 0.02

**Figure 1. DPPH⁺ radical scavenging activity of Fruit extracts of selected *Piper* species****Figure 2. Total Antioxidant Capacity (TAC) by Phosphomolybdenum method in Fruit extracts of selected *Piper* species**

The best antioxidant as demonstrated by the highest value of TAC compared to other extracts and standards used (ascorbic acid 2.69 ± 0.05mg TE/g). It is followed by the petroleum ether extract of *P. schmidtii* (1.66± 0.23mg TE/g), which is not

significantly different from the standard ascorbic acid. Generally, phenolic compounds of plant origin such as flavonoids and tannins believed to prevent the cells from oxidative stress, such compounds reported to possess great antioxidant potential in many research articles. The present antioxidant assays confirm the presence of flavonoids and tannins in secondary metabolite profiling in the respective plant extracts.

Overall, the antioxidant capacity of ethanol and petroleum ether extract of *Piper* species may well be related to the proportion of phenolic compounds that constitute it. The result of the current investigation demonstrates that the wild peppers tested do retain sufficiently good antioxidant activity in their seeds. The distribution of the antioxidant status in the seed of an endemic species may be considered as an adaptive strategy to escape from extinction and means to expand chemical ecological status.

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