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Full Length Research Paper

The phytochemical and antioxidant screening of *Justicia wynaadensis*

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The plant *Justicia wynaadensis* is consumed by the local community of Kodagu district, Karnataka, India for its medicinal properties. In our present study, we have identified and estimated polyphenols and flavonoids in the plant extract. The medicinal properties of *J. wynaadensis* extract may be due to the presence of the above mentioned phytochemicals. The observed high antioxidant property could be related to the presence of phenolics, flavonoids as well as catalase and peroxidase enzyme activities. The plant *J. wynaadensis* may be exploited as a source of natural antioxidant and as a possible food supplement.

Key words: *Justicia wynaadensis*, antioxidants, phenolics, flavonoids, 1,1-diphenyl-2-picryl hydrazyl (DPPH), enzyme activity.

INTRODUCTION

Justicia wynaadensis, belonging to Acanthaceae family, is reported to be endemic to the regions of Western Ghats, from South Canara, Coorg (Kodagu) to Wynaad, East Nilgiris and South Malabar Hills in South India, up to 3000 ft in evergreen forests and on waste lands (Gamble, 1967). A survey among the local populace revealed that, the plant under study locally called Maddhu thoppu is believed to acquire the medicinal property during the Hindu calendar month of Kataka or Adi (July to August). The plant is believed to have maximum medicinal property when harvested on the 17th of this month (first week of August). The juice from the stem and leaves of this plant, is extracted either by soaking in water or boiling in water. The deep purple coloured extract thus obtained is consumed, generally as a sweet dish by the local community. This traditional practice is believed to keep the people healthy throughout the year.

Extensive literature survey has shown that not much work has been done on this plant. The only publication is the patent on the cholesterol lowering properties of *J. wynaadensis* by Subbiah et al. (2002) which reports that the plant extract lowers cellular cholesterol and cholesteryl ester concentration. Their studies also have shown a novel inhibitory effect on the uptake of ox-LDL by human macrophage cell line.

Our work involved the estimation of polyphenols and flavonoids in the leaf and stem of *J. wynaadensis* and the study of catalase and peroxidase activity. The present investigation also involved the evaluation of antioxidant property and reducing property of the plant extract.

MATERIALS AND METHODS

Collection of plants

The plant was collected (the aerial parts, that is stem and leaves) from Kodagu District of Karnataka State, India during first week of August, 2008. The stems were separated from leaves. Fresh leaves were used for the estimation of reducing power and for the enzyme assay. The stems and leaves were oven dried at a constant temperature of 45°C and used for phytochemical analysis.

Extraction of phytochemicals

The phytochemicals were extracted from five gram each of the dry powdered leaf and stem sample by the method described by Harborne (1998).

Determination of total phenolics

Total phenolic content in the plant material was determined by

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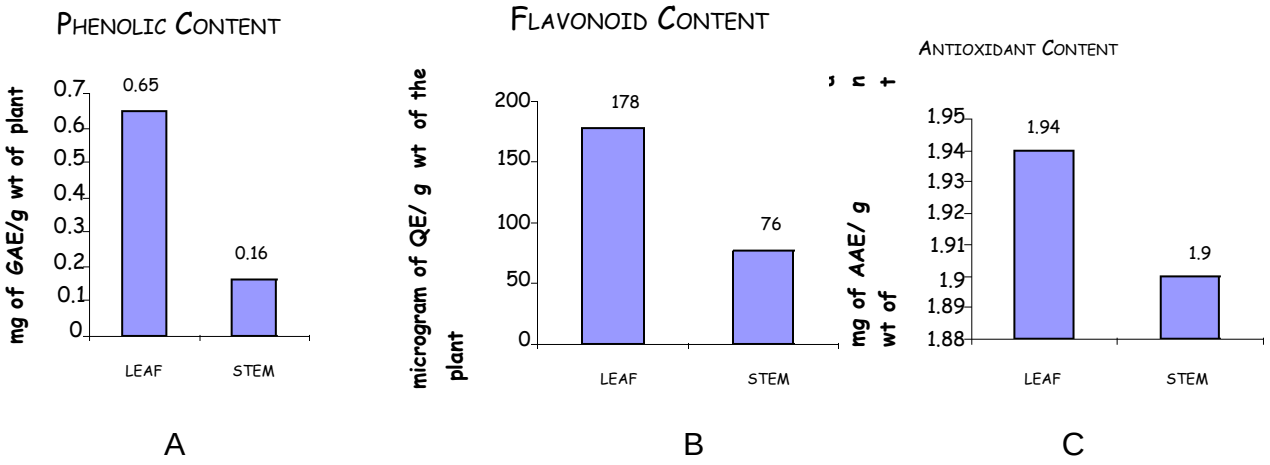

Figure 1. A) Total phenolic content, B) Flavonoid content and C) Antioxidant property.

slightly modifying the Folin-Ciocalteau method (Pourmorad et al., 2006). The total phenolic content was expressed in terms of gallic acid equivalents (GAE) (Figure 1).

Determination of flavonoids

Flavonoid content in the plant material was determined by modified aluminium chloride colourimetric method (Pourmorad et al., 2006). The flavonoid content was expressed in terms of quercetin equivalents (QE) (Figure 1).

Antioxidant activity – DPPH method

Antioxidant activity of the leaf and stem extract was determined by DPPH method (Pourmorad et al., 2006) (Figure 1).

Reducing power assay

For the measurement of the reducing power, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of *J. wynaadensis* using the method of Rajeshwar et al. (2005).

Enzyme assay

Catalase and peroxidase assay on the plant extract was carried out by the method of Sadasivam and Manickam (2008).

RESULTS AND DISCUSSION

It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested daily from a diet rich in fruits and vegetables (Rajeshwar et al., 2005). The total phenolic content present in the plant *J. wynaadensis* was found to be 0.65 ± 0.08 mg and 0.16 ± 0.01 mg of gallic acid equivalent (GAE)/g weight of leaf and stem respectively (Figure 1A).

The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health - they have been reported to have antiviral, anti-allergic, anti platelet, anti-inflammatory, antitumor and antioxidant activities (Hsu, 2006). Total flavonoid content in the plant *J. wynaadensis* was 178 and 76 µg Quercetin Equivalent (QE) / g weight of leaf and stem respectively (Figure 1B).

Scientific research shows positive links between accumulated free-radical damage and age-related diseases such as atherosclerosis, alzheimer, and osteoarthritis (Pendry et al., 2005). The results of this study showed the antioxidant activity in leaf to be 1.94 mg AAE /g and that in stem to be 1.90 mg AAE/g (Figure 1C).

For the measurements of the reducing activity, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of *J. wynaadensis* by the method of Rajeshwar et al. (2005). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Reducing power of the *J. wynaadensis* leaves and stem extract was found to be significant (Figure 2).

Enzymes such as catalase and peroxidase attenuate the generation of reactive oxygen species by removing potential oxidants or by transforming reactive oxygen species into relatively stable compounds. It has been suggested that these enzymes could be supplemented to combat the effects of aging or to ward off serious illness (Vaya et al., 2006). Extract of the plant *J. wynaadensis* showed catalase activity as well as peroxidase activity.

Maximum catalase activity seen in 1 g of the fresh leaves was 0.0012 µmoles of Hydrogen Peroxide per sec. Maximum peroxidase activity seen in 1 g of freshleaves was 0.00095 µmoles of hydrogen peroxide per second. These enzymes could also contribute to the antioxidant activity of this plant (Figure 3).

Further studies are in progress in our laboratory to isolate the active components. It would be interesting and

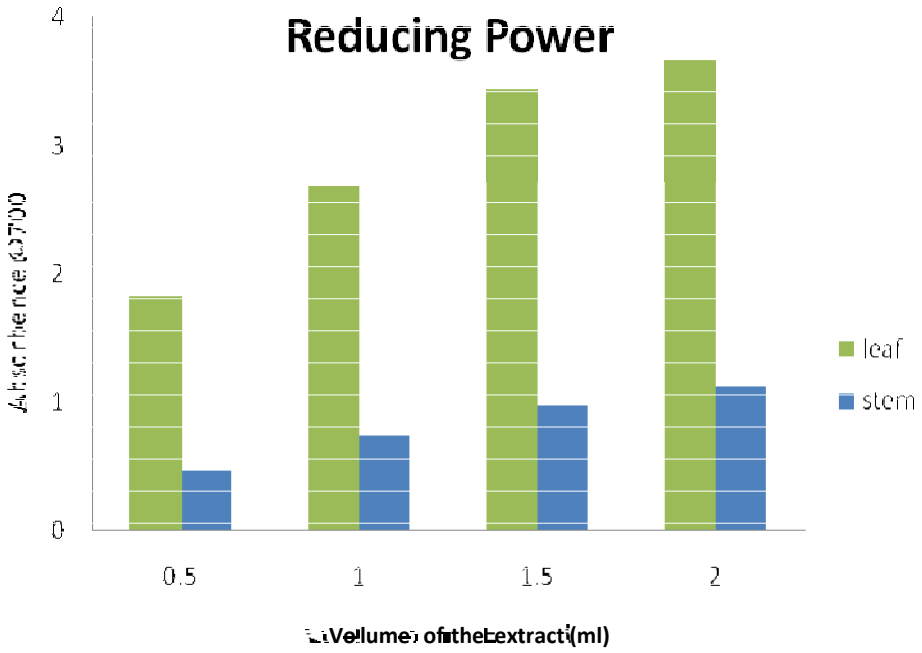

Figure 2. Reducing power of leaf and stem extract.

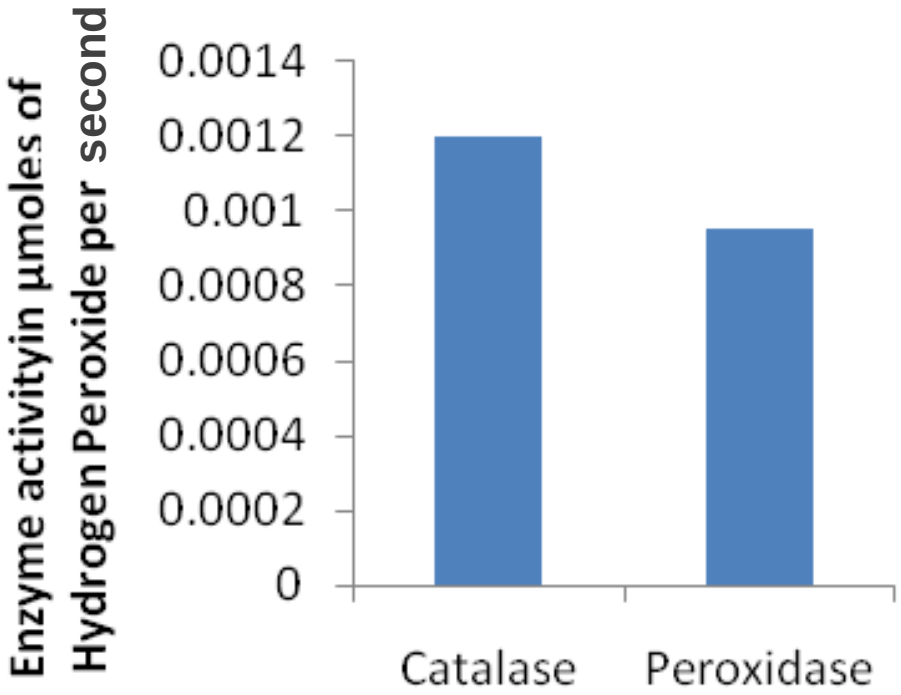

Figure 3. Enzyme activities in leaf extract.

worthwhile to further investigate the potential effectiveness and usage of *J. wynaadensis* as a natural antioxidant and as a food supplement in preventing diseases and damages caused by the free radicals.

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