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Antioxidant potential of bark extract of *Holigarna Arnottiana Hook.f*

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Abstract

Holigarna Arnottiana Hook.f belongs to Anacardiaceae. In this study, we use ethanolic extract of *H.arnottiana,* by hot and cold extraction method to compare the antioxidant potential. DPPH scavenging activity and Ferric Reducing Antioxidant Potential (FRAP) were analysed (10µg-100µg). Methanolic extract obtained by cold extraction showed higher activity (64.10±0.23 at 100 µg for FRAP assay and 0.425±0.21 at 100 µg for DPPH activity) in both DPPH scavenging activity and ferric reducing antioxidant potential. The test was conducted against the standard BHA and there was an increased potential with respect to the extract concentration.

Keywords: *Holigarna Arnottiana Hook.f*, Ethanolic extract, Antioxidants, DPPH, FRAP

INTRODUCTION

Oxidative stress is known to implicate the initiation and progression of human degenerative diseases, including atherosclerosis, diabetes, dysfunction of immune systems, and cancer. Therefore, it is plausible that the alleviation of oxidative stress can decrease the incidence, or progression, of oxidative stress-associated diseases (Lee *et al.,* 2001). Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are often generated as by-products of biological reactions or from exogenous factors. In vivo, some of these ROS play positive roles in cell physiology; however, they may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and DNA mutations leading to cancer, degenerative, and other diseases (Ames, 1998; Finkel, 2000). Anacardiaceae is an angiosperm family known to produce allergenic substances in the resin canals of primary and secondary phloem associated with the veins of leaves and other parenchymatous tissues. *Holigarna arnottiana* is a tall deciduous tree, up to 50 feet in length and 9 feet in girth, found only in Western Ghats from Konkan southwards. In Anacardiaceae, toxic phenols are likely a defence against pests, because they are capable of restricting the growth of pathogenic fungi such as *Alternaria sp*. Free radicals are simply defined as any species capable of independent existence that contains one or more unpaired electrons and these active byproducts are generally reactive oxygen species as well as reactive nitrogen species. These compounds play a dual role as both toxic and beneficial to health. At low or moderate amount, it exerts beneficial effects on cellular response and immune function and at high levels, they generate oxidative stress that can damage all cells.

Materials and methods

The bark of *H. arnottiana* was selected for this study. The bark was dried, powdered finely and extracted through soxhlet extraction

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with ethanol (HA-I). We also extracted the material with ethanol in the magnetic stirrer for 4 hours (HA-II), after that extract was filtered and evaporated in the rotary evaporator.

Preparation of the sample

The residue obtained after evaporated of ethanolic extracts (HA-I & HA-II) was partitioned between water (150 ml) and chloroform (3 x *300* ml). After evaporation, chloroform yields a residue which contains Urushiol-oily organic allergen found in *H. arnottiana* (Elsohlyp *et al.,* 1981). The water - containing part is evaporated and used for the antioxidant studies.

Ferric Reducing Antioxidant Power (FRAP)

Various concentrations of sample (10µg, 50µg and 100µg) were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 mins. Next, 2.5 mL of 10% trichloroacetic acid (w/v) were added. 5mL of above solution was mixed with 5 mL of distilled water and 1 mL of 0.1% of ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Butylated hydroxy anisole (BHA) was used as standard antioxidant (Barreira *et al.,* 2008).

Free radical scavenging activity

Different concentrations (10µg, 30µg and 50µg) of sample and Butylated hydroxy anisole (BHA) were taken in different test tubes. The volume was adjusted to 500µl by adding Methanol. Five millilitres of a 0.1 mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH) was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at RT for 20 min. The absorbance of the samples was measured at 517nm (Kumar *et al.,* 2008). Radical scavenging activity was calculated using the following formula:

$$\% \text{ free radical scavenging activity} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100.$$

Results and discussions

In both, FRAP assay and DPPH assay HA-II showed highest activity which is in a dose dependent manner At higher concentration, the free radical scavenging activity is similar to the standard used is showed in (Fig.1 & 2).

The reaction mechanism of the hydroxyl radical with biomolecules has been a subjected towards particular interest, due to its diverse action on biological systems. A study was conducted on the antioxidant activity of *H. arnottiana* showed high antioxidant potential.

Fig.1.Ferric reducing antioxidant

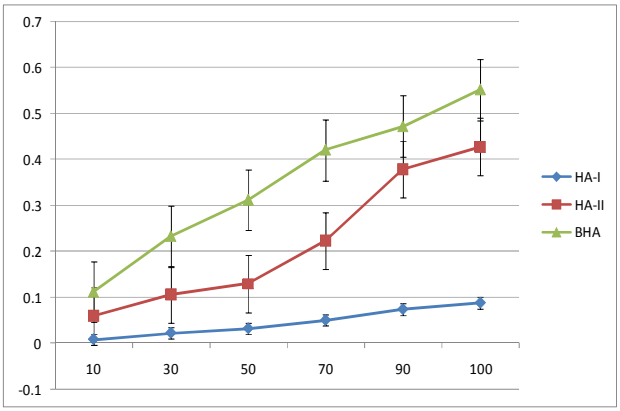
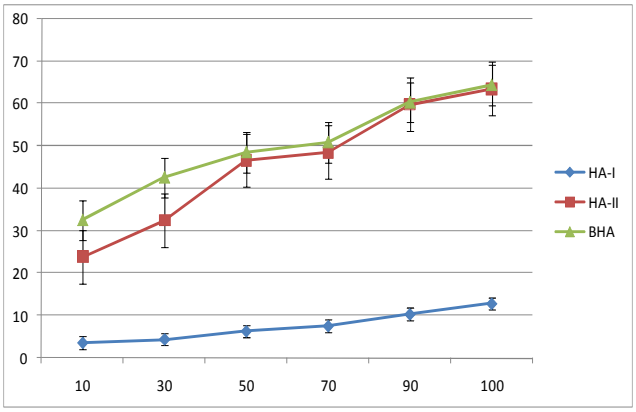


Fig.2. Free radical scavenging activity



In conclusion, the results of the study showed that the ethanol extract of *H. Arnottiana* has a potent antioxidant property. Further isolation and characterization of active compounds from *H. Arnottiana* may show promising therapeutic effects.

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