Introduction

Antioxidants are important in the prevention of human diseases. Naturally occurring antioxidants in leafy vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compounds, possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and ageing\(^1\)-\(^3\). Antioxidant compounds may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation\(^4\). They are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxianisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years. The researchers have focused on natural antioxidants and numerous crude extracts and pure natural compounds have been reported to possess antioxidant properties\(^5\).

Research Paper

In-vitro antioxidant activity of hot aqueous extract of Helicteres isora Linn. fruits

P K Basniwal\(^1\)*, M Suthar\(^1\), G S Rathore\(^1\), R Gupta\(^1\), V Kumar\(^1\), A Pareek\(^2\) and D Jain\(^3\)

\(^1\)L B S College of Pharmacy, Udai Marg, Tilak Nagar, Jaipur-302 004, Rajasthan, India
\(^2\)L M College of Science and Technology (Pharmacy Wing), Shastri Nagar, Jodhpur-342 003, Rajasthan
\(^3\)School of Pharmaceutical Sciences, Rajiv Gandhi Technological University, Bhopal-462 036, M P, India

*Correspondent author; E-mail: pawan.basniwal@gmail.com; Tele Fax : 0141-2620517

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Abstract

The antioxidant activity of the aqueous (hot) extract of Helicteres isora (AEHI) (Family—Sterculiaceae) fruits was investigated in various in vitro models. The total polyphenolic content of the extract was found 7.04% of AEHI, when compared to gallic acid and total flavonoids content was 2.4 mg/g of AEHI, when compared to rutin. Hydrogen peroxide radicals were inhibited at IC\(_{50}\) = 165 µg/ml, while ascorbic acid inhibited at 187.33 µg/ml. AEHI inhibited the nitric oxide radical at IC\(_{50}\) = 820 µg/ml, when it was compared with rutin as standard antioxidant with IC\(_{50}\) = 68.52 µg/ml. Superoxide (SO) radical’s inhibition was compared with quercetin and IC\(_{50}\) value was found more than 1000 µg/ml. Moreover, the results were observed in a concentration dependent manner. Present in vitro studies clearly indicated that the aqueous (hot) extract of H. isora has significant antioxidant activity.

Keywords: Helicteres isora, Indian Screw Tree, Total flavonoids, Superoxide, Antioxidant.

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\textbf{Helicteres isora} (Family-Sterculiaceae), commonly known as East Indian Screw Tree large shrub or small tree occurs often gregariously, throughout India, South East Asia, the southern part of China and in dry deciduous forests, up to 1500 m on the hill slopes\(^6\)-\(^7\). It is one of the Jamu raw materials used in traditional folk medicine in Indonesia; it is called “Buah Kayu Ules or Ulet-Ulet” in Java island and is used for treating gastrospasm, as an anthelmintic for tapeworm in Indonesia and as an antispasmodic, antipyretic, anti diarrhoeal and antidysenteric in Saudi Arabia and as a tonic compound after childbirth in the Malayan Islands\(^8\)-\(^9\). In traditional use, the root juice is claimed to be useful in cough, asthma, diabetes, empyema, intestinal infections, a cure for scabies when applied topically and a favourite cure for snakebites. Fruits are demulcent, mildly astringent and useful in gripping and flatulence\(^10\)-\(^12\).

A potent inhibitory activity of aqueous (hot) extract of \textit{H. isora} fruits was reported against reverse transcriptase from avian myeloblastosis virus (AMVRT)\(^13\) and anti-human immunodeficiency virus-type-1 (anti-HIV-1)\(^14\). Six neolignans, the Helicetins...
A–F were isolated and reported in aqueous (hot) extract of the fruits\textsuperscript{15} and the plant also reported with flavonoid glucuronides\textsuperscript{16}. However, no data are available in the literature on the antioxidant activity of \textit{H. isora} fruits. Therefore, the present work was aimed to investigate its antioxidant activity by various in \textit{vitro} models.

**Materials and Methods**

**Instruments and chemicals**

Shimadzu UV-160A spectrophotometer (Shimadzu Corporation, Japan) was used for the measurement of absorbance of solution mixtures. L-Ascorbic acid, gallic acid, quercetin, rutin, potassium ferricyanide, ethylenediaminetetra acetic acid (EDTA), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), napthylethylenediamine dihydrochloride (NEDD), sulphanilamide, sodium nitroprusside, aluminum chloride and ferric chloride were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

**Plant material and extract preparation**

The fruits were purchased, from the local market of Ootacamund and authenticated by Director, Survey of Medicinal Plants and Collection Unit, Ootacamund, Tamil Nadu, India and were shade dried, coarsely powdered and extracted with hot water by maceration process. The extract was filtered and concentrated in vacuum and kept in a vacuum desiccator for complete removal of solvent. The yield of extract (AEHI) was found 1.8%. The dried extract thus obtained was used for the assessment of antioxidant activity by various in \textit{vitro} assays.

**Total phenolic content**

Total soluble phenolic of AEHI was estimated by Folin–Ciocalteau reagent method\textsuperscript{17} using gallic acid as a standard phenolic compound. One ml of stock solution (1g/ml) of AEHI was diluted up to 46 ml with distilled water and 1.0 ml of Folin Ciocalteau reagent was added and mixed thoroughly. 3.0 ml of 2% sodium carbonate was added to solution mixture after 3 min and allowed to stand for 2h with intermittent shaking. The absorbance of the blue colour was observed at 760 nm. The concentration of total phenolic compounds in the extract was estimated as gallic acid which was obtained by extrapolating the absorbance of gallic acid on standard gallic acid graph (absorbance = 0.0008 X gallic acid). The concentration of total phenols was expressed as mg/g of dry extract\textsuperscript{18}.

**Total flavonoid content**

Total soluble flavonoid of AEHI was estimated by aluminium chloride colorimetric method\textsuperscript{19}. 0.5ml of stock solution (1g/ml) of AEHI, 1.5 ml methanol, 0.1ml aluminium chloride (10%), 0.1 ml potassium acetate (1M) were added to reaction test tube and volume was made up to 5ml with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Total phenolic content was calculated by extrapolating the absorbance of reaction mixture on standard curve of rutin in the range of 1-10µg/ml. The total flavonoid content was expressed as equivalent to rutin in mg/g or % w/w of the extracts.

**Nitric oxide radical scavenging activity**

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide\textsuperscript{20-22} which interacts with oxygen to produce nitrite ions which can be estimated by use of Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide\textsuperscript{21,22}. Sodium nitroprusside (5µM) in phosphate-buffered saline (PBS) was mixed with 3.0 ml of different concentrations (10–320µg/ml) of the drug and incubated at 25°C for 150 min. This solution was treated with Griess reagent (1% sulphanilamide, 2% H\textsubscript{3}PO\textsubscript{4} and 0.1% napthylethylenediamine dihydrochloride). The absorbance of resulting chromophore from diazotization and subsequent coupling with napthylethlenediamine was observed at 546nm. Control reaction was also carried out in between potassium nitrite and Griess reagent and absorbance was measured. The percentage of nitric oxide radical scavenging was calculated as, NO scavenged (%) = [(A\textsubscript{cont} - A\textsubscript{test})/A\textsubscript{cont} ] X 100, (A\textsubscript{cont} = absorbance of control reaction & A\textsubscript{test} = absorbance in presence of sample of extract). Rutin was used as standard for nitric oxide radical scavenging activity.

**Superoxide anion radical scavenging activity**

Measurement of superoxide
anion scavenging activity of the AEHI was based on the method described by Liu et al\textsuperscript{23} with slight modification. Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT). The superoxide radicals were generated in 3.0 ml of Tris-HCl buffer (16 µM, pH 8.0) containing 1.0 ml of NBT (50 µM) solution, 1.0ml NADH (78 µM) solution and sample solution of the extract (50-300 µg/ml) in water. The reaction initiated by addition of 1.0ml of phenazine methosulphate (PMS) solute on (10µM) to the mixture. The reaction mixture was incubated at 250°C for 5 min and the absorbance was measured at 560 nm against blank samples. L-Ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The % inhibition of superoxide anion generation was calculated as, SOD scavenged (%) = [(A\textsubscript{cont} - A\textsubscript{test})/A\textsubscript{cont}] x 100, (A\textsubscript{cont} = absorbance of control reaction & A\textsubscript{test} = absorbance in presence of sample of extract). Quercetin was used as standard for H\textsubscript{2}O\textsubscript{2} radical scavenging activity.

**H\textsubscript{2}O\textsubscript{2} radical scavenging activity**

The ability of AEHI to scavenge H\textsubscript{2}O\textsubscript{2} was determined according to the method of Ruch R J et al\textsuperscript{24}. A solution of H\textsubscript{2}O\textsubscript{2} was prepared in phosphate buffer (pH 7.4). H\textsubscript{2}O\textsubscript{2} concentration was determined spectrophotometrically by measuring absorption with extinct coefficient for H\textsubscript{2}O\textsubscript{2}. AEHI (10-320 µg/ml) in distilled water was added to H\textsubscript{2}O\textsubscript{2} solution (0.6 ml, 40 µM). After 10 min, absorbance of H\textsubscript{2}O\textsubscript{2} was measured at 230 nm against blank solution containing the phosphate buffer without H\textsubscript{2}O\textsubscript{2}. The % of H\textsubscript{2}O\textsubscript{2} scavenging effect of both extract and standard compound was calculated as, H\textsubscript{2}O\textsubscript{2} scavenged (%) = [(A\textsubscript{cont} - A\textsubscript{test})/A\textsubscript{cont}] x 100, (A\textsubscript{cont} = absorbance of control reaction & A\textsubscript{test} = absorbance in presence of sample of extract). L-Ascorbic acid was used as standard for H\textsubscript{2}O\textsubscript{2} radical scavenging activity.

**Results and Discussion**

**Total phenolic compounds and flavonoids**

Phenolic compounds are known as powerful chain breaking antioxidants\textsuperscript{25} and phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups\textsuperscript{26}. These compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity\textsuperscript{27}. The phenolic compounds may contribute directly to antioxidative action. It was suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1g daily from a diet rich in fruits and vegetables\textsuperscript{28}. The total phenolic content of AEHI was found 7.04%, which was equivalent of gallic acid, one of the components responsible for the antioxidant activity. Flavonoids present in food of plant origin are also potential antioxidants\textsuperscript{29, 30}. Most of the beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities\textsuperscript{31}. Studies have shown that certain flavonoids exhibit hypoglycaemic effect\textsuperscript{32}. The total flavonoids content of AEHI was found to be 2.4 mg/g and equivalent to rutin.

**Inhibition of nitric oxide and H\textsubscript{2}O\textsubscript{2} radical**

Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by content of AEHI. IC\textsubscript{50} value of AEHI was found to be 825 µg/ml with 3.68 standard deviation while rutin showed IC\textsubscript{50} = 68.5µg/ml with 2.27 standard deviation. Comparison of nitric oxide radical inhibitory activity with rutin, AEHI showed mild antioxidant activity.

Hydrogen peroxide itself is not very reactive, but it may be toxic to cell due to increase in hydroxyl radical concentration in the cells\textsuperscript{33}. Thus, removal of H\textsubscript{2}O\textsubscript{2} as well as O\textsubscript{2} leads to survival of the cell life and its components. The scavenging ability of AEHI on hydrogen peroxide is comparable with that of standard ascorbic acid. IC\textsubscript{50} value of AEHI was 165µg/ml with 2.95 standard deviation for inhibitory activity of hydrogen peroxide radical, while its standard ascorbic acid showed IC\textsubscript{50} = 187µg/ml with 3.45 standard deviation. These finding shows that AEHI is more potent than its standard ascorbic acid for hydrogen peroxide radical inhibitory activity.

**Superoxide anion radical**

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. AEHI has exhibited mild superoxide radical scavenging activity having IC\textsubscript{50} more than 1000 µg/ml, while quercetin was found more potent having IC\textsubscript{50} = 155µg/ml with 3.16 standard deviation.
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Conclusion

The aqueous (hot) extract of *H. isora* Linn. (AEHI) exhibited strong antioxidant activity by inhibiting nitric oxide and scavenging superoxide anion and hydrogen peroxide radicals when compared with different standards such as L-ascorbic acid, quercetin and rutin. In addition to this, the AEHI contains a significant amount of phenols and flavonoids, which play a major role in controlling oxidation. Thus, this easily available plant can be used as a potential antioxidant. However, further work is required on the isolation and identification of the antioxidant components present in it.

References


