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Screening and Quantitation of Phytochemicals and Nutritional Components of the Fruit and Bark of *Helicteres Isora*

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Abstract

Helicteres isora is a medium sized tree abundantly found in the hills and forests, well known for its use in traditional medicine. The fruit and bark are said to possess several medicinal values. Hence the present study was designed to screen and quantify selected phytochemicals (polyphenols, tannins, total carotenoids, flavonoids) and proximate principles (carbohydrates, protein, fibre, minerals such as calcium, phosphorus and iron). Results indicate that the fruit contained more amounts of polyphenols (317.7 mg/100g), ascorbic acid (80.0 mg / 100g) and carotenoids (1.7 mg / 100g) than the bark. The bark contained more amounts of tannins (205.1mg / 100g), flavonoids (42.0 mg / 100 g), α-tocopherol (44.0 mg / 100g) and reduced glutathione (184.6 mg / 100g) when compared to the fruit. Among the nutrients, the fruit contained more phosphorus (103.6 mg / 100g) and the bark contained appreciable quantities of total carbohydrates (41.8 mg / 100g), calcium (526.7 mg / 100g) and iron (35.2 mg / 100g) than the fruit. Appreciable quantities of the phytochemicals and nutraceuticals may attribute to the medicinal and nutritive values of *Helicteres isora*.

Key words: *Phytochemical, phenols, tannins, flavonoids, saponins, steroids, alkaloids, calcium, phosphorus, iron, Helicteres isora.*

1. Introduction

Plants with medicinal properties, the gift of Mother Nature to mankind, are in use for centuries in the traditional system of medicine like Ayurveda, Siddha and Unani, in India. Medicinal plants are nature's priceless gift to human beings. The herbal medicines are being used by about 80 per cent of the world population for primary health care, particularly in the developing countries[1]. These drugs are popular for its safety and efficacy and are used in the treatment of diseases that have long defied synthetic drugs[2].

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Helicteres isora, is a medium sized tree with brick red flowers, belonging to the family of *Sterculiaceae*. It is referred as Modimodika (Oriya), Modaphala (Bhuyan), Valampuri (Malayalam), Valampuri or Tirugupalai (Tamil). Flowers appear in the month of September to December, fruits in the month of January to March. The fruit and bark of the plant are used for treating various diseases and in particular for the treatment of diabetes[3] . The fruit is boiled with mustard oil, filtered and used for massaging legs of patients suffering from gout, twice a day for five days[4]. Extracted juice from the raw fruit of *Helicteres isora* is mixed with equal quantity of mustard oil or ground the fruit along with *Cyanodon dactylon* and mixed with turmeric paste, and is used for massaging the body of children to relive them of profound weakness[5] .

Hence based on the review about the therapeutic values of *Helicteres isora*, the present study was attempted to screen the phytochemicals, quantify them and analyze for selected nutritional components such as carbohydrates, protein, fibre, minerals like calcium, phosphorus and iron.

2. Materials and Methods

2.1 Collection of the fruit and bark

Dried fruits of *Helicteres isora* were obtained from the local market and the bark of the tree was collected from Velliyangiri hills, Coimbatore. The specimen was identified and confirmed by the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore.

2.2. Qualitative detection of phytochemicals

Qualitative analysis of the fruit and bark was carried out systematically to identify the phytochemicals. Protein was tested using biuret reaction and xanthoproteic reaction[6] , carbohydrates by Molisch's test[7], tannins by lead acetate test[8], flavonoids by Shinoda test[9], steroids and terpeniods by Salkowski's test and Lieberman-Burchard reation[10], alkaloids using Wagner's reagent[8], phenols using neutral ferric chloride reagent[11] and saponins using sodium bicarbonate[8] .

2.3. Quantification of phytochemicals

Phenols were extracted from the fruit and bark of *H.isora* using 80% ethanol, centrifuged and collected the supernatant. Residue re-extracted, centrifuged, pooled the supernatants and evaporated to dryness. Dissolved residue in distilled water and estimated phenols by the method of Malick and Singh using pyrocatechol as the standard[12]. Tannins were extracted by gently heating the powdered material of the sample with distilled water for 30 minutes, centrifuged at 2000rpm for twenty minutes and collected supernatants. Made up the volume with 100ml of water and estimated by the method of Schanderl [13]. Flavonoids were extracted by grinding the fruit and bark sample, in two steps, firstly with methanol:water (9:1) and secondly with methanol:water (1:1) using sufficient solvent, left for six to twelve hours and filtered to separate the extract. Combined the extracts and evaporated to about 1/3rd the original volume and estimated by the method of Cameron *et al.* [14]. Sample for carotenoids was prepared by extracting the fruit and bark of *H.isora* with petroleum ether repeatedly till the aqueous layer turned colourless and estimated by the method of Zakaria *et al* [15]. Tocopherols were estimated using Emmerie-Engel reaction as described by Rosenberg[16].

The sample was homogenized and allowed to stand overnight with 0.1 N H_2SO_4, filtered and used aliquots of filtrate for the estimation. Reduced glutathione was quantified by the method of Moron *et* al[17]. The sample was homogenized in 5% TCA, precipitated protein was centrifuged at 1000 rpm for ten minutes and supernatant was used for the estimation. The above sample after treatment with a pinch of charcoal was estimated for ascorbic acid by the method of Roe and Kuether[18].

2.4. Quantification of proximate principles

The fruit and bark were quantitatively analyzed for selected nutrients like carbohydrate, protein, fibre, and minerals such as calcium, phosphorus and iron. Total carbohydrates was estimated by the method of Hedge and Hofreiter[19] , protein by the method of Lowry *et* al [20] and fibre content by the method of Raghuramulu *et* al [21]. The ash for the determination of the mineral content was prepared by completely charring the sample over a low flame and heating in a muffle furnace for about three to five hours at 600^{o}C , cooled and obtained the ash. The ash was moistened with distilled water and added distilled HCl, evaporated to dryness on a boiling waterbath (twice), filtered and made up the volume to 100 ml. Calcium was estimated according to the method of Clark and Collip[22], phosphorus by the method of Fiske and Subbarao[23] and iron by the method of Oser[23].

3. Results and Discussion

3.1. Identification of the phytochemicals in the fruit and bark of Helicteres isora

The fruit and bark of Helicteres *isora* was screened qualitatively for the presence of various phytochemicals, the observation and results are depicted in Table I.

3.2. Quantification of phytochemicals

The amount of polyphenols, tannins, flavonoids, carotenoids, tocopherol, reduced glutathione were quantified as per the methods described and the values are expressed as mean ± SD (Table2). It is evident from the results (Table II) that the fruit and bark of *Helicteres isora*are very good sources of polyphenols, tannins, flavonoids and vitamin E than carotenoids and glutathione.

Ascorbic acid plays a central role in pulmonary function, immune response, prevention of coronary heart diseases, cancer and cataract. It also enhances the absorption of non-heme iron from food. It acts as the main radical acceptor from vitaminE itself acts as a scavenger of oxygen radicals and thus inhibits lipid peroxidation[24]. Phenolic compound have been shown to exhibit cellular defense mechanism in atherogenesis and cancer. Polyphenols are a major group of antioxidative compounds, more powerful than vitamin E after it becomes oxidized. They offer protection against LDL oxidation and inhibition of platelet aggregation. A wide array of phenolic substances present in dietary and medicinal plants has been reported to possess powerful antimutagenic activity apart from the antioxidant property. Recently increasing evidences support the hypothesis that the phenolic compounds could play an essential health promoting role[25].

The pharmacological effects of flavonoids include CNS activity, cardiotonic, lipid lowering, antiulcer, hepatoprotectiv, anti-inflammatory, antineoplastic, antimicrobial antioxidant and hypoglycemic activity. Dietary intake of flavonoids containing foods potentially lowers the risk of certain free radical related pathophysiology[26]. Certain tannins (ellagitannins from *Lagerstroemia speciosa*) stimulate glucose uptake. They exhibit insulin like activity acting as glucose transport activators of fat cells [27]. Number of epidemiological studies has demonstrated that increased intake of natural antioxidants like vitamin A, C, E, flavonoids is very promising in reducing the level of free radicals, severity of diabetic complications, risk of cardiovascular diseases, cancer and many chronic degenerative diseases [28].

Table 1. Phytochemical Screening of the Fruit and Bark of *Helicteres isora*

Phytochemicals	**Observation**	**Inference**
Carbohydrate	Reddish violet ring at the junction of two liquids was obtained in Molisch's test	+
Proteins	Violet color obtained in biuret reaction and deep orange color developed in xanthoproteic reaction	+
Polyphenols	Blue color developed with ferric chloride	+
Tannins	White precipitate with lead acetate was obtained	+
Flavonoids	Deep blue color	+
Alkaloids	Yellow brown precipitate	+
Saponins	A honey comb like froth formed	+
Steroids	The upper layer red and the sulphuric layer showed an yellow color with a green fluorescence	+

+ indicates Presence The fruit and bark of *Helicteres isora* contained all the phytochemicals tested.

Table 2. Phytochemical Content in *Helicteres isora*

Compound	**Fruit (mg/100g)**	**Bark (mg/100g)**
Polyphenols	317.7 ± 4.2	269.3 ± 2.8
Tannins	180.4 ± 2.3	205.1 ± 1.86
Flavonoids	33.0 ± 0.65	42.0 ± 0.24
Carotenoids	1.7 ± 0.03	1.0 ± 0.01
α – tocopherol	10.6 ± 0.16	44.0 ± 0.94
Reduced glutathione	148.3 ± 1.4	184.6 ± 2.3
Ascorbic acid	80.0 ± 2.3	67.7 ± 3.1

Values are mean ± SD of triplicates

Table 3. Nutritional Components of *Helicteres isora*

Parameter	Fruit	Bark
Total carbohydrates (g/100g)	23.5± 0.25	41.8 ± 0.12
Protein (g/100g)	2.1 ± 0.04	2.3 ± 0.02
Fibre (g/100g)	1.0 ± 0.002	1.5 ± 0.004
Calcium (mg/100g)	45.2 ± 0.10	526.7 ± 4.82
Phosphorus (mg/100g)	103.6 ± 1.2	50.1 ± 1.23
Iron (mg/100g)	23.5 ± 0.34	35.2 ± 0.43

Values are mean ± SD of triplicates

The fruit and bark of *Helicteres isora* are found to contain appreciable quantities of carbohydrate, protein and the essential mineral nutrients necessary for human health maintenance such as calcium, phosphorus and iron. Calcium is important for ossification and iron is necessary for normal hemopoiesis. The bark is found to be rich in all these nutrients except phosphorus compared to the fruit. The fibre content of the fruit and bark of *Helicteres isora* was found to be 1.0 and 1.5 g respectively. Intake of fibre improves the body's handling of glucose and the hormone insulin, perhaps by slowing down the digestion and absorption of carbohydrate. This high level of crude fibre may be helpful in slowing down the carbohydrate absorption and thereby preventing hyperglycemia.

Certain inorganic mineral elements like potassium, zinc, calcium, traces of chromium and magnesium play an important role in the maintenance of normal glucose-tolerance and in the release of insulin from beta cells of islets of langerhans. The fruit and bark are rich in calcium. This may facilitate the efficient release of insulin from beta-cells more efficiently [29]. Increased dietary intake of Ca is currently recommended for the general population to lower the risk of hypertension and osteoporosis. Dietary supplementation of Ca also lowers serum cholesterol [30]. Hence, the fruit and bark may also have hypolipidemic properties due to the good content of calcium.

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