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Evaluation of Phenolic Compounds and Vitamins from *Helicteres isora*. L fruits

Shireen Bano and K. D. Jadhao
P.G. Department of Botany,
Govt. Vidarbha Institute of Science and Humanities (Autonomous),
Amravati 444604.

Abstract

The study was conducted to evaluate the antioxidant property and nutritional property depending on phenolic compounds and vitamins content of *Helicteres isora* L. fruits, using spectrophotometer. Plant kingdoms are rich in active chemicals that can be used to treat a wide range of incurable illnesses. Since ancient times, herbal remedies have been employed as the main treatment in traditional medical systems. *Helicteres isora*, is commonly known as Marodphali, Marorphali, Enthani is used as a folk medicine to treat snake bite, diarrhea and constipation of new born baby. Phenolic and polyphenolic compounds either alone or in combination with vitamins, like vitamin C, act as antioxidants, in the human body that protect the tissues from the damaging effects of oxidative stress. These wild edibles not only provide the body's basic energy and metabolic needs, but they also serve as additional sources of vitamins and minerals, which the body needs in order to maintain healthy physiological homeostasis. Wild edibles have Nutritional qualities are comparable and occasionally superior to the domesticated variety. The main objective of this study is to evaluate the Secondary Metabolites and Vitamins like Total phenol, Bound phenol, Quinones, Total flavonoids, Tannins, Ascorbic acid, Riboflavin, Niacin, Thiamin. The findings were Total phenol (325 µg/g), Bound phenol (10300 µg/g), Quinones (1960 µg/g), Total flavonoids (300 µg/g), and Tannins (10 µg/g). where Vitamins such as Ascorbic acid (740 µg/g), Riboflavin (145 µg/g), Niacin (517.5 µg/g) and thiamin (470 µg/g). Phenolic compounds have been used for many therapeutic purposes due to their effects on inflammation also involve in defense against UV radiation and other characteristics of human diseases, which may guide future research.

Key words: *Helicteres isora*, Phenolic compounds, Vitamins, Spectrophotometer, Antioxidant.

1. Introduction

Antioxidants and active principles are abundant in medicinal plants. Supplemental antioxidants are utilized to assist the human body in lessening the oxidative damage caused by active oxygen and free radicals. The use of natural products by human being with the purpose of supplying physical and biological needs is an ancient practice, being the knowledge acquired transmitted throughout the generations. Chronic-degenerative diseases (CDD) represent a significant threat to public health Nevertheless, there aren't many effective pharmaceutical treatments for these conditions, and the ones that are somewhat limited. Plants are a significant source of bioactive chemicals against CDD because they have historically been utilized to treat a variety of illnesses. Phenolics are among these metabolites; they are associated with many biological properties (e.g., antioxidant and antimutagenic) of plant extracts (Cervellati *et al*. 2002; Zhu *et al*. 2014).

Traditionally, herbs have been utilized as an adjunctive therapy in conjunction with pharmaceuticals, if not as the primary means of promoting immunity for preventative purposes. Well-known for their pharmacological action and immunomodulatory effects are polyphenols (Szliszka and Krol, 2011). According to (Lakhanpal and Rai, 2007), polyphenols have been identified as immune regulators with anti-inflammatory properties. It has been demonstrated that consuming tannins extracted from apples can stop food allergies from developing, and this benefit might be linked to a rise in the percentage of γδ TCR T cells in intestine intraepithelial lymphocytes (Sato et al, 2010). Numerous studies have demonstrated the efficacy of

phytotherapy, including its capacity to treat infectious disorders (Zhang *et al*,2020). Medicinal plants are popular for their ample store of active ingredients as well as boast of several pharmacological properties including the modulation of components of the immune system. In order to combat the undesirable activities of pathogens including bacteria, viruses, and protozoa, an appropriate immune response is essential. In mammals without sufficient protection, many infections such the measles, chickenpox, Ebola, and human immunodeficiency virus can be fatal or have severe repercussions. Chemicals derived from plants have immunostimulatory properties that boost the immune system's defense against immune-depleting illnesses brought on by viruses like HIV (Ogunrinola *et al*, 2022).

Antioxidant is a molecule that prevent the other molecules from oxidation (Dold and Cocks, 2002). Numerous biological effects, such as antibacterial, antiviral, anticancer, anti-inflammatory, antiallergic, antithrombic, and vasodilatory properties, are known to be exhibited by natural antioxidants. Antioxidant activity gives rise to anti-carcinogenicity, anti-immunogenicity and antiaging activity (Ames *et al*, 1993 Yang *et al*, 2001). Antioxidants are responsible for the defense mechanisms of the organism against the pathologies associated with the attack of free radicals. Accordingly, consumption of antioxidants derived from plants plays a role in preventing degenerative diseases brought on by oxidative stress, including Parkinson's disease, cancer, Alzheimer's disease, and atherosclerosis (Valko *et al*, 2007). According to scientific evidence, antioxidants reduce the risk for chronic diseases, including cancer and heart disease (Marchioli *et al*, 2001).

Essential nutrients such as thiamine, riboflavin, and niacin are critical to the oxidation-reduction reaction that occurs during the body's synthesis of energy in living cells. Since thiamine and riboflavin cannot be synthesized by the human body, the requirement for these vitamins must be satisfied by diet. The body can generate a little quantity of niacin, but external nutrition is necessary to provide the major demand. Thiamine (B1), riboflavin (B2), niacin (B3) and ascorbic acid (C) are water soluble vitamins. Water soluble vitamins are crucial for maintaining good health in humans; lack of a sufficient amount of any of them can cause serious diseases (Akompong *et al*, 2000). The human being is one of the few mammals unable to synthesize vitamin C (Ashor *et al*, 2016). Vitamin C is essential for the formation of collagen, skin, ligaments, tendons and blood vessels as well as wound repair, and maintenance of cartilage, boons and teeth, it has three fundamental roles in human metabolism like enzyme cofactor, chemical reductant and antioxidant (Hayas and Roberts, 2003). Thiamin, riboflavin, niacin, and vitamin B_6 are essential micronutrients that are basically involved in energy metabolism; they may also prevent the occurrence of developmental abnormalities and chronic degenerative and neoplastic diseases (Mielgo-Ayuso *et al*, 2018). In fact, thiamin plays a critical role in the energy metabolism, and, therefore, in the growth, development, and function of cells (Ortigoza-Escobar, 2017). Riboflavin is an essential component of two coenzymes (flavin mononucleotide and flavin adenine dinucleotide) that are crucial for energy generation, cellular growth, cellular respiration, antibody production and development, and the metabolism of lipids, medications, and steroids. Niacin serves as the body's precursor for the coenzymes NAD and NADP, which are nicotinamide nucleotides. NAD is essential for energy-producing reactions and NADP for anabolic reactions (Mielgo-Ayuso *et al*, 2018).

Helicteres isora L. (Family: Sterculiaceae) distributed widely in forests throughout India and commonly known as East Indian screw tree. It is a medicinally important sub deciduous shrub or a small tree. *Helicteres isora* is an important medicinal plant possessing remarkable nutritional and therapeutic activities. The Indian System of Medicine (ISM) has traditionally employed different components of the plant to treat different types of illnesses. *H. isora* is a rich source of bioactive substances with medicinal properties, including tannins, polyphenols, and alkaloids. In addition, *H. isora* is said to be an excellent source of iron, calcium, phosphorus, proteins, fiber, and carbohydrates (Gayathri *et al*, 2010). The plant's fruits have antispasmodic properties and are used to treat intestinal gripping and flatulence in children (G Kumar *et al*., 2008). They are also astringent, stomachic, vermifugal, and vulnerary (Pohocha and Grampurohit, 2001), Pods are used to treat anthelmintic and in fever due to cold. Seeds aqueous extract administered in colic disorder and dysentery (Khare, 2008). Traditional medicine has utilized powdered dry fruits to treat a range of skin conditions, including dermatitis, eczema, acne, and others. It is used as a tonic following delivery and as an antipyretic, antidiarrheal, antidysentery, and anthelmintic for tapeworms. Applying the root juice externally is said to help treat scabies, intestinal infections, emphysema, diabetes, cough, and snake bites (Singh *et al*, 1985).

2. Material and methods:

2.1. Plant material

Helicteres isora L. fruits were collected from Semado, Melghat forest, Amravati District (Maharashtra state), India. Plant identification and authentication was carried out with the help of floras (Cook,1957; Dhore,1986;1988; Naik, 1998).

2.2. Preparation of plant material

Collected fruits were washed and dried under shade, finely powdered sample were stored in airtight container. 1gm of powder were taken for estimation of phenolic compounds and Vitamins and 100mg of powder were taken for estimation of Bound phenol and Tannins.

2.3. METHODS:

Estimation of Phenolics such as total phenol, Bound phenol, Quinones, Tannins, Total flavonoid, Ascorbic acid, Riboflavin, Niacin and Thiamin were done according to the methods prescribed by Thimmaiah (1999), Saranya *et al*, (2017), Nasreen et al, (2022) which are given below.

2.3.1. Estimation of total phenols

1gm of sample was grind with the help of mortar and pestle with 10 ml of 80% ethanol. And centrifuged at 10,000 rpm (20 minutes). Supernatant was collected and evaporated to dryness. after dryness residue was taken into a test tube and make up the volume with 5ml distilled water. 1 ml aliquot was pipette out in test tube, and make up the volume up to 3 ml with distilled water. 0.5 ml of double diluted Folin- Ciocalteu reagent was added. After 3 minutes, into each tube 2 ml of 20% Na_2CO_3 solution was added. Mixed thoroughly and tubes was kept in boiling water for 1 minute, then allowed to cool and absorbance was measured at 650 nm against reagent blank. reagent blank was prepared similarly without the extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol (100µg/ml). (Thimmaiah S. R. 1999).

2.3.2. Estimation of bound phenols

100mg sample was grind with 5ml of SDS solution. Centrifuged at 2,000g (5min) and supernatant was discarded. washed the residue successively once with 5ml of SDS solution, two times with water, twice with 5ml of ethanol and twice with 10ml of diethyl ether. After each washing centrifuged and supernatant was discarded. Residue was allowed to dry and suspended in 3ml of 0.5M NaOH. Then kept overnight at room temperature. Next day centrifuged and saved the supernatant. Supernatant was diluted 1:10 with 0.5M NaOH. Absorbance was measured at 290nm against the blank prepared similarly without the extract. Presence of bound phenols in the sample was calculated by using standard curve prepared from working standard catechol solution at different concentrations (0.1 to 1ml). (Thimmaiah S. R. 1999).

2.3.3. Estimation of Quinones

1gm sample was grind with the help of mortar and pestle by using chilled phosphate buffer (5ml for each gm of tissue). The supernatant was collected after centrifugation for 30 minutes this was used as enzyme extract. 3ml of buffer, 3ml of standard catechol and 1.5 ml of enzyme extract was pipetted in a test tube. It was shaken gently and then placed in water bath for incubation. 4ml of TCA (Trichloro acetic acid) reagent (without ascorbic acid) to one and 4ml of TCA reagent (with ascorbic acid) was added. Precipitate was filtered. Absorbance was measured at 400 nm against a reagent bank lacking only extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of working standard catechol. (Thimmaiah S. R. 1999)

2.3.4. Estimation of Tannins

Vanillin hydrochloride method was used to estimate tannin. 0.1 gm of sample was mixed in 5ml methanol after 20-28 hrs. centrifuged and supernatant was collected. 1ml of supernatant was pipette out into test tube and quickly added 5ml of vanillin hydrochloride reagent and mixed. After 20 min absorbance was read at 500nm. A reagent blank was prepared with vanillin hydrochloride reagent alone. A standard graph was prepared from working standard (100µg/ml) of catechin and amount of tannins was calculated. (Thimmaiah S. R. 1999).

2.3.5. Estimation of total flavonoid content

The Aluminium chloride method was used to determine the total flavonoid content. In brief, 1gm sample was grind with the help of mortar and pestle with 10ml methanol. centrifuged and supernatant was collected. 0.5ml of supernatant was pipette out into test tube. 0.5 mL of each extract (1:10 g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2.8 ml of distilled water was added. The reaction was left for completion for 30 min, and absorbance was measured at 415 nm against a methanolic blank (80% methanol). Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol (Saranya *et al*, 2017).

2.3.6. Estimation of Ascorbic Acid (Vitamin C)

Colorimetric method was used to estimate the total flavonoid content. For the extraction 1gm of sample was grind with the help of mortar and pestle with 4% oxalic acid. Centrifuged and supernatant was collected. Aliquot transferred into conical flask and few drops of Bromine water was added and volume was making up with 10 ml of 4% oxalic acid. For the estimation 1ml of sample extract was pipette out in flask and volume was make up to 3ml with distilled water, 1ml DNPH reagent was added and one to two drops of thiourea was added and kept it for incubation at 37^0 for 3h. After incubation, in aliquot, 7 ml of 80% sulphuric acid was added. Absorbance was measured at 540 nm against a reagent blank lacking only sample extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) from 100 µgm/ml standard ascorbic acid solution. (Thimmaiah S. R. 1999).

2.3.7. Estimation of Thiamine (vitamin B1)

1g of powder sample used to homogenized with ethanolic sodium hydroxide (10ml). Ethanolic sodium hydroxide (4.2 gm Sodium Hydroxide in 5ml DH2O and then 1L Ethanol (aldehyde free) were added in it). the solution was allowed to stand for 24 hrs in tightly stoppered bottle and then quickly decant the clear liquid in another suitable bottle. Centrifuged and supernatant was collected in 100ml flask. After addition of 10 ml of 0.1N Potassium Dichromate, color was developed. Absorbance was measured at 360 nm against a reagent blank lacking only sample extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1ml) standard Thiamine solution (Nasreen *et al*, 2022).

2.3.8. Estimation of Riboflavin (Vitamin B2)

1g sample was grind with the help of mortar and pestle with 10ml of 50% ethanol solution. The sample was placed in a shaker for 1 hr. Then filtered into flask. 2ml of extraction was pipette and placed into the volumetric flask. Then 2ml of (5%) Potassium Permanganate was added afterwards 2ml of 30% H_2O_2 was added and allowed to stand over a hot water bath (30min). 2 ml of Sodium Sulphate (40%) was added. This was made up to 10ml mark and absorbance was measured at 510 nm in spectrophotometer against a reagent blank lacking only sample extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) standard Riboflavin solution (Nasreen *et al*, 2022).

2.3.9. Estimation of Niacin (vitamin B3)

1g sample was treated with 10ml of 1N H_2SO_4 and shaken for 30 min. 3 drops of ammonia solution were added to the sample and filtered. 2ml Filtrate was pipette into a volumetric flask and 5 ml Potassium Cyanide (0.5g KCN dissolved in 100 ml cold DH2O kept in refrigerator) was added. This was acidified with 0.2 N H_2SO_4 (5ml) and at 470 nm wavelengths, absorbance was measured against blank lacking only sample extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) standard Niacin solution (Nasreen *et al*, 2022).

3. Results and Discussion

In the present investigation estimation of phenolic compounds as well as vitamins were performed by using spectrophotometer.

3.1. Content of Phenolic compounds in *H. isora* fruits

Results obtained from quantitative estimation of Phenolic compounds in *H. isora* are given in table 1. In the dry fruits of *H. isora*, amount of bound phenol (10300 µg/g) was highest as compare to Quinones (1960 µg/g), total phenol (325 µg/g) and total flavonoids (300 µg/g), while amount of Tannins (10 µg/g) was found to be lower. Okwu (2001) and Raquel (2007) reported that many studies have been described that the medicinal plants which are rich in phenolic compounds shows high antioxidant potential. they prevent or reverse damage in the body cells caused by aging, environmental factor and life style. Over the time this damage is linked to

an increased risk of several chronic diseases. But they are also possessing other biological properties like anti-aging, anti-atherosclerosis, anti-apoptosis, anti-carcinogen, anti-inflammation, cardiovascular protection and improvement of endothelial function (Brown and Rice-Evans,1988).

Flavonoids have been reported to exert a wide range of biological activities such as antioxidant, anti-inflammatory, antimicrobial, antiallergic (Cook and Samman, 1996., Krings and Berger, 2001., Han *et al*, 2007) anti-tumor, treatment of neurodegenerative diseases, and vasodilatory action (Murray, 1998., Williams *et al*, 2004). Phenolic compounds such as flavonoids, phenolic acids and tannins, act as inhibiter of α-glucosidase and α-amylase, which are responsible for the digestion of dietary carbohydrates to glucose and tannin, quinone and total flavonoid justifies the medicinal use of the plant, good source of antioxidant activity, anti-aging, anti-inflammatory, anticancer, anti-diabetic, antimicrobial, ant plasmodial, neurological, antitumor, and anti-HIV activity and modulate immune responses. (Lin *et al*,2016 and Shireen Bano and Jadhao, 2024).

These results suggest that the presence of higher amount of phenolic compound in the fruit it act as antioxidant, prevent the cell damage, anti-aging, anti- apoptosis, anti-carcinogen, anti-diabetic, cardiovascular protection, reduce the LDL cholesterol, anti-inflammation, improvement of endothelial function, antimicrobial, ant plasmodial, neurological, antitumor, and anti-HIV activity and modulate immune responses.

Table 1. Content of Phenolic compounds in *H. isora* fruits

Sr. no.	Phenolic compounds	Wt. of material	Total volume of extract	Volume taken for analysis	µg/g
1	total phenol	1g	10 ml	1 ml	325
2	Bound phenol	100mg	5 ml	1 ml	10,300
3	Quinones	1 g	5 ml	1.5	1960
4	Tannins	100mg	10ml	1ml	10
5	Total flavonoid	1g	10ml	0.5ml	300

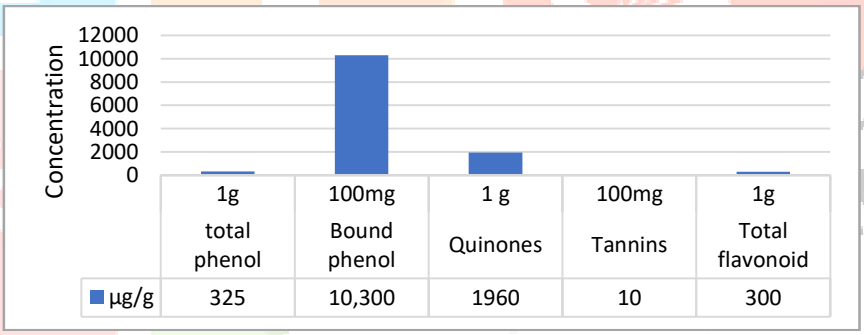

Figure 1. Content of Phenolic compounds in *H. isora* fruits

3.2. Content of vitamins in *H. isora* fruits

Ascorbic acid, riboflavin, niacin and thiamine content in dry fruits of *H.isora* are presented in the table 2. Among these water soluble vitamins, dry fruits of *H.isora* shows higher content of Ascorbic acid (740 µg/g**)** in comparison to other vitamins like Niacin(517.5 µg/g), Thiamin (470 µg/g) and Riboflavin (145 µg/g). Vitamin C is a potent antioxidant that facilitates the absorption of iron from plant-based foods and help the immune system to work properly, according to national institute of health (NIH) vitamin C helping to protect cells from damage caused by free radicals. free radicals formed when our bodies convert the food we eat into energy and also exposed to polluted environment and UV light from sun, the reduction of folic acid intermediates and the synthesis of cortisol. Its deficiency includes fragility to blood capillaries gum decay, scurvy (Bender, 2009). According to NIH taking vitamin c with or without other antioxidants doesn't seem to protect people from getting cancer.

Thiamine is unstable to heat (Thornalley, 2005) and destroyed by oxygen in the air and water, so heat may have effect on reduction in thiamine content in jute leaves (Tolonen, 1990). Thiamin (Vitamin B1) is responsible for the proper functioning of the brain, central nervous system, digestive system and heart (Ba, 2008). According to Lanska (2010), Riboflavin (Vitamin B2) promotes iron metabolism for the production of red blood cell. According to Combs (2007), Niacin (vitamin B3) has the ability to scavenge free radicals and protect the tissues from oxidative damage. Riboflavin is relatively heat stable (Merck Service Bulletin, 1976). Dry fruits are considered as rich source of riboflavin. Almond and cashew nut, classified under dry fruits, had

highest riboflavin and thiamine respectively (Agte *et al*, 2002)]. According to Melinda Ratini (2022) as a cholesterol treatment there are research shows that niacin can boost level of good HDL Cholesterol and lower the triglycerides as well as LDL Cholesterol.

These results suggest that the presence of ascorbic acid (vit C) in the fruits of *H. isora* which help in the absorption of iron from plant-based food, and riboflavin help iron metabolism for the production of red blood cell, antibody production and energy metabolism while thiamin support the central nervous system, proper function of the brain, digestive system and heart and niacin act as antioxidant, protect from cancer.

Table 2. content of vitamins in *H. isora* fruits

Sr. no.	Vitamins	Wt. of material	Total volume of extract	Volume taken for analysis	µg/g
1	Ascorbic acid	1g	10	1ml	740
2	Riboflavin	1 g	10	2ml	145
3	Niacin	1 g	10	2ml	517.5
4	Thiamin	1 g	10	10ml	470

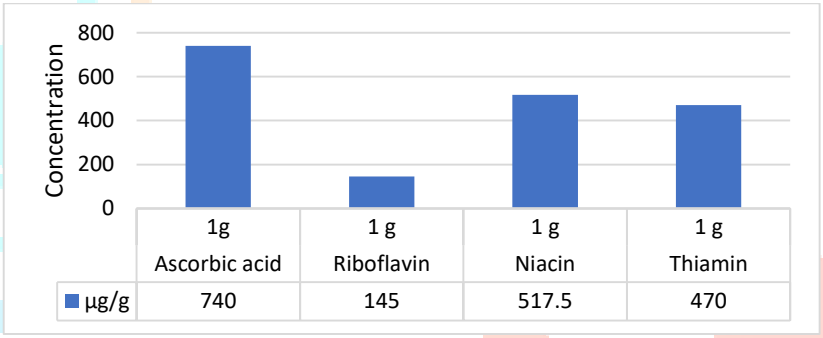

Fig 2. content of vitamins in *H. isora* fruits

4. Conclusion

In the present investigation it was observed that higher amount of bound phenol and ascorbic acid were found in the *H. isora* fruits. Phenolic compounds exhibit strong bio activities including antioxidant, anti-inflammation, Anticancer, and cardiovascular disease and the high phenolic content was positively correlated with free radical scavenging activity of the extracts. Highest amount of Ascorbic acid was found in *H. isora* fruits. Ascorbic acid (vitamin C) also a potent antioxidant, aided to protect cells from damage caused by free radical, help in the absorption of iron from plant-based foods and help the immune system to work properly. *Helicteres isora* as a medicinal plant possesses various therapeutical activities and nutritional benefits. The chemical analysis suggests the presence of various bioactive compounds and their application in pharmacological activities. there is a need to explore their pharmaceutical importance to develop drugs in curing various diseases.

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