

Phytochemical Screening and Antioxidant Activity of *Garcinia xanthochymus*; Wild Fruit from Western Ghats

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ABSTRACT: Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. The qualitative and quantitative analysis is very essential for identifying the compounds present in the medicinal plants. Fruits contain high levels of biologically active components that impart health benefits beyond basic nutritional value. There are so many wild underutilized fruits which make up the list. This study would help in prospecting of such wild underutilized fruits. This present communication deals with the antioxidant profile and phytochemical screening of wild *Garcinia xanthochymus* grown in Hosanagara, Karnataka, India; which is a part of Western Ghats. This wild fruits are consumed in India are underutilized and inexpensive rich source of several nutrients. Their chemical composition and medicinal properties have already been studied and recorded. However the available information regarding their antioxidant activity profile and phytochemicals are fragmentary. Therefore this wild fruit pulp is selected for the present investigation. Ethanolic extract of *Garcinia xanthochymus* fruit pulp was subjected to various antioxidant assays namely total phenolics, total flavonoids, total antioxidants, DPPH. The result of present investigation revealed that the preliminary phytochemical analysis of ethanolic fruit pulp extract of *Garcinia xanthochymus* is bestowed with the presence of several bioactive compounds such as carbohydrates, proteins, amino acids, saponins, tanins, terpenoids, flavonoids, etc. This documentation on wild edible fruit gauged essential and enhance potential interest in the use of wild fruits for improving the efficacy of different products as nutritional and pharmacological products.

KEYWORDS: Antioxidants, Wild fruits, *Garcinia xanthochymus*, Phytochemicals.

I. INTRODUCTION

India is home to over 47513 plants species, with concentrated spots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island, in which 3000 plant species have got medicinal value. Western Ghats alone is gifted with more than 613 medicinal plant species... Lot many plants and plant products are been used directly or/and indirectly by Indians with or without knowing their pharmaceutical value since antiquity. Nutritionists recommend fruits and vegetables due to their health benefits as they are rich with dietary fibers, vitamins and minerals, in particular phytochemicals. Most of the phytochemicals show antioxidant activity. Epidemiological evidence highlights that antioxidant content of fruits and vegetables can help in preventing several diseases. An antioxidant is a substance that removes potentially damaging oxidizing agents in a living organism. These powerful substances, which mostly come from the fresh fruits, and vegetables we eat, prohibit (and in some cases even prevent), the oxidation of other molecules in the body. The benefits of antioxidants are very important to good health, because if free radicals are left unchallenged, they can cause wide range of illness and chronic diseases. Synthetic antioxidants such as BHA, BHT, PG and TBHQ are commonly used in food formations. Their safety is questioned due to their toxicity, liver damage and carcinogenesis. However, due to their toxicity, interest in natural antioxidants has intensified (Shahidi, 2000). Within the biologically active components, natural antioxidants have attracted interest because of their safety and potential therapeutic effect (Rufino et al. 2011). Wild food is also an abundant source of these things. The unrestricted urbanization over the

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natural environment and deterioration of forest weaned away people from consumption of wild food. *Garcinia xanthochymus* is one among those which is a wild fruit found to be exotic or underutilized. *Garcinia xanthochymus* being a member of clusiaceae family is distributed in evergreen, semi evergreen, moist deciduous and in lower hill forests up to 1400 meters. A 20m tall with a dense pyramidal crown; bark blackish or dark grey, exuding gum; latex milky or pale green turning yellow on exposure. White or cream colored flower. Fruit is a berry, subglobose, as large as middle sized apple, smooth, very short-pointed at apex, deep yellow when ripe. The fruit has a juicy pulp with a pleasant acrid flavor. Locally known as jarige, jeerige, devarige, devajarige, deerkanahannu, bettahunase, etc. Xanthochymol, isoxanthochymol, cambogin, volkensiflavone, morelloflavone, biflavones GB-1 and GB-1a, maclurin, 1,5-dihydroxyxanthone and 1,7-dihydroxyxanthone have been isolated from fruits (Rastogi & Mehrotra, 1993). Xanthochymol present in the fruit is antibacterial against streptococcus faecalis and klebsiella pneumonia; action is better than tetracycline (Asolkar et al., 1992). It is made into preserves and jams. It can be used in place of tamarind for curries and in the preparation of vinegar. The exudation from the under-ripe fruit, which is yellow in colour, and the bark are used in Assam for dyeing. Fruit cathartic, used in abdominal disorders, vomiting, piles, anaemia, fever, giddiness and cardiac diseases; sherbet from dried fruits is used in biliousness (Yusuf et al. 2009). Most of the *Garcinia* species have some therapeutic properties as they are rich source of secondary metabolites including xanthenes, flavonoids, benzophenones, lactones and phenolic acid (Deachathai et al., 2005). Even though this fruit is used in folk medicines, and many reports report the medicinal value and other significant values, basic data like physico-chemical characteristics, phytochemical content, antioxidant activity is least documented. Hence this investigation portrays the phytochemical content and total antioxidant capacity of *Garcinia xanthochymus*.

II. MATERIAL AND METHODS

Collection of plant material

The fruits of *Garcinia xanthochymus* were procured from the Nagarakodige village of Hosanagara, Karnataka, India in the month of January 2017.



Figure-1: *Garcinia xanthochymus* Tree



Figure-2: *Garcinia xanthochymus* fruit

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Identification and confirmation of plant

The collected fruits were identified and authenticated by the taxonomist from the Department of Botany, Sir M.V. Government Science College, Bhadravathi, Karnataka as *Garcinia xanthochymus* Hook. f. ex J. Anders. (Fl. Brit. India 1: 269 1874.)

Plant Extract Preparation

Fresh fruits of *Garcinia xanthochymus*, were washed in running tap water followed by washing with distilled water to remove the surface impurities like dust particles. The fruit was ground well. About 750g of fruit pulp was submerged into sufficient volume of ethanol in an air-tight flat bottomed container for seven days for extraction in the room temperature with occasional shaking and stirring. The extract was then filtered using muslin cloth at first and through Whatmann No.1 filter paper. The filtrate was dried on an electrical water bath and concentrated. The crude extract obtained was stored in desiccators for maximum of three days for complete dehydration. The yield of crude extract obtained from the sample is 140g/750g of paste. The dried extract was stored in labeled air tight vials in a freezer until further use.

Preparation of test and standard solution

The fruit extract of experimental sample and standard phenolic (catechol), standard flavonoids (rutin), and standard antioxidant (BHA) were dissolved separately in Dimethyl sulfide (DMS) in different concentration and used for further study.

Qualitative phytochemical screening

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in ethanolic extracts of fruit extract. (Harborne, 1984; Trease and Evans, 1989; Kokate et al. 1990 and Khandelwal, 2006).

1. Test for carbohydrates:

Molisch's test: To 2ml of extract add few drops of alpha naphthol solution in alcohol and add 2ml of concentrated sulphuric acid along the sides of the test tube. Observation is made for purple ring formed at the junction of two liquids which indicates the presence of carbohydrates.

2. Test for proteins: Biuret test:

To 3ml of test solution add 40% sodium hydroxide and 1% copper sulphate solution. Observation was made for the appearance of violet or pink colour which indicates the presence of protein.

3. Test for amino acids: Ninhydrin test: 3ml of test solution was treated with 5% Ninhydrin reagent and kept in boiling water bath for 10 minutes. Observation was made for the formation of blue colour.

4. Test for alkaloids: Evaporate the alcoholic extracts separately. To the residue add dilute HCl. Shake well. It was cooled and filtered. With the filtrate, perform following tests.

a. Mayer's test: 2-3ml filtrate with few drops Mayer's reagent gives precipitate.

b. Wagner's test: 2-3ml filtrate with few drops Wagner's reagent gives reddish brown precipitate.

5. Test for tannins:

a. Ferric chloride test: To 1-2 ml of the extract, few drops of 5% w/v ferric chloride solution was added. A green colour indicates the presence of gallotannins, while brown colour indicates the presence of pseudo tannins.

b. KOH test: 1ml of freshly prepared 10% KOH was added to 1ml of each extract in different test-tubes. Dirty white precipitate formation confirms the presence of tannins.

6. Test for flavonoids:

a. Shinoda's test: To a test tube containing 0.5 ml of the extract add 10 drops of dilute hydrochloric acid followed by a few fragments of magnesium ribbon were added. Formation of pink, reddish or brown colour indicates the presence of flavonoids.

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b. Lead acetate test: To 2ml of extract add few drops of 10% lead acetate solution observation were done for yellow precipitate.

C. Zinc-Hydrochloric acid reduction test: To 2ml of extract add zinc dust and few drops of hydrochloric acid, observation was done for magenta red colour.

7. Test for saponins:

a. Emulsion test: 5ml of each extract in distilled water was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil are shaken vigorously after which it was observed for the formation of a stable emulsion.

b. Fehling Test: 2 ml of each of the Fehling solution A and B was added to 3 ml of each leaf extract. The mixture was boiled for 5 minutes. Observed for the formation of brick precipitate.

8. Test for terpenoides:

a. Salkowski test: To 5 gm each of the extract was added to 2 ml of chloroform and 2 ml of concentrated sulphuric acid was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

b. Liebermann-Burchard's test (LB test): 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet colored ring indicated the presence of terpenoids.

9. Test for glycosides:

a. Bromine water test: Test solution when dissolved in bromine water gives yellow precipitate.

b. Legal test: Test solution was treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives

10. Anthraquinone test: 0.5gm of direct extract + 5ml CHCl₃ shaken for 5 minutes and filtered + equal volume of NH₃, gives pink or red in lower layer

Determination of Total Flavonoids

Known volume of sample was pipetted out in series of test tubes and volume was made up to 0.5 ml with distilled water. Sodium nitrite (5%; 0.03ml) was added to each tube and incubated for 5 min. at room temperature. Aluminium chloride solution (10%; 0.06ml) solution was added and incubated for 5 min. at room temperature. Sodium Hydroxide solution (1 M; 0.2ml) solution was added and total volume was made up to 1 ml with distilled water. Absorbance was measured at 510nm against a reagent blank. Standard curve using different concentrations of rutin was prepared. From the standard curve, concentration of flavonoids in the test samples was determined and expressed as mg of rutin equivalent.

Determination of Total Phenols

Known amount of sample were pipetted out in series of test tubes and volume was made up to 3 ml with distilled water. Folin-Ciocalteu reagent (0.5ml) was added to each tube and incubated for 3 min. at room temperature. Sodium carbonate (20%; 2ml) solution was added, mixed thoroughly and the tubes were incubated for 1 min. in boiling water bath. Absorbance was measured at 650nm against a reagent blank. Standard curve using different concentrations of standard phenol -catechol was prepared. From the standard curve, concentration of phenols in the test samples was determined and expressed as mg of catechol equivalent

Total Antioxidant Activity by Phosphomolybdenum Method

It is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of a green phosphate-Mo (V) complex at acidic pH. The antioxidant capacity is expressed as ascorbic acid

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equivalent (AAE). Various concentrations of samples (10µg, 50µg and 100 µg) were taken in a series of test tubes. To this, 1.9 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. and allowed to cool. The absorbance of the aqueous solution of each was measured at 695 nm against a blank. Antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. Butylated hydroxyl anisole (BHA) was used as reference standard. The values are expressed as ascorbic acid equivalents in µg per mg of extract.

Free Radical Scavenging Activity Using DPPH

Different concentrations (10µg, 50µg and 100µg) of sample in Dimethyl sulfoxide (DMSO), were taken in a series of test tubes. The volume was adjusted to 500µl by adding Methanol. Five milliliters of a 0.1 mM methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH; from Sigma –Aldrich, Bangalore) 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 room temperature for 20 min. The absorbance of the samples was measured at 517 nm. Butylated Hydroxy Anisole (BHA) was used as reference standard. Free Radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = [(\text{control OD} - \text{sample OD}) \times 100] / \text{Control OD}$$

Nitric oxide radical Scavenging Activity

Various concentrations (10µg, 50µg and 100µg) of samples and Butylated hydroxy anisole (BHA) were taken in different test tubes and made up to 3ml with 0.1M phosphate buffer (pH 7.2). Sodium Nitroprusside (5mM) prepared in buffered saline (pH7.2) was added (1 ml) to each tube. The reaction mixture was incubated for 30 min at RT. A control without the test compound, but with an equivalent amount of methanol was maintained. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1-Naphthylethylenediaminedihydrochloride). The absorbance of the samples was measured at 546 nm. Nitric oxide radical scavenging activity was calculated using the following formula:

$$\% \text{ NO radical scavenging activity} = [(\text{control OD} - \text{sample OD}) \times 100] / \text{Control OD}$$

III. EXPERIMENTAL RESULTS

The qualitative analysis of ethanolic fruit pulp extract of *Garcinia xanthochymus* showed the presence of several phytochemical constituents such as carbohydrates, proteins, amino acids, saponins, tanins, phenols and flavonoids. At the same time, phytochemical constituents like cardiac glycosides and carotenoids were not detected.

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Table-1: Phytochemicals detected in ethanol fruit extract of *Garcinia xanthochymus*.

Chemical group	Test	Ethanollic fruit extract of <i>Garcinia Xanthochymus</i>
Carbohydrates	Molisch's test	++
Proteins	Biuret test	++
Amino acids		Ninhydrin test ++
Alkaloids	Mayer's test	+
	Wagner's test	+
	Hagner's test	+
Tannins	Ferric chloride test	+
	KOH test	+
	Gelatin test	+
Flavonoids	Shinoda's test	++
	Lead acetate test	++
	Zinc-hydrochloric acid reduction test	++
Saponins	Emulsion test	+++
	Foam test	+++
	Fehling' test	+++
Terpinoids	Salkowski test	+
	Liebermann-burchard's test	+
Glycosides	Bromine water test	-
	Legal test	-
Anthraquinone	Anthraquinone test	+

+ +=very abundant; ++=abundant; +=detected; -=not detected

The quantitative analysis of ethanolic fruit pulp extract of *Garcinia xanthochymus* shows the following:

1. Total phenolics:

Phenolics are colorless crystalline solid and aromatic compound. Phenolics are non-polymeric phytochemical compounds containing multiple phenol substructures. They are largest groups among phytochemicals account for many antioxidant properties. It is proved that sterically hindered phenolics are primary antioxidants and react rapidly and are termed 'radical scavengers'. Our study showed positive result for phenolics in this sample extract.

Total phenolics = 0.51 µg/ml catechol equivalent

Ref: Malick, C.P. and Singh, M.B. (1980). In: Plant Enzymology and Histochemistry. Kalyani Publishers. New Delhi. P 286

2. Total flavonoids:

Chemically, flavonoids have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C). They are largest groups among naturally occurring phenolic. They involved in pharmacological activities like antimicrobial, anti allergic, anti-inflammatory, antioxidant, anti-fungal, anti-viral, anti-cancer, anti-diarrheal, etc. Our study revealed that flavonoids amount is more among phenolic in sample extract.

Total flavonoids = 17.11 µg/ml rutin equivalent

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Ref: Helmja K., Vaher M., Gorbatšova J. and Kaljurand M. (2007). Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis. Proc. Estonian Acad. Sci. Chem., 56; (4) 172–186.

3. Free radical scavenging assay:

DPPH is a dark colored crystalline compound composed of stable free radical molecule. It monitors the chemical reaction involving free radical; most notably it's a common antioxidant assay. DPPH is the well-known, powerful free radical scavenger; the rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction, DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction and identification of radicals. Our experimental data of sample revealed the potentiality of radical scavenging activity of DPPH. At lower concentration it showed more activity.

Table-2: % free radical scavenging activity of ethanol fruit extract of *Garcinia xanthochymus* and BHA

% free radical scavenging		
Concentration	Sample	BHA
10µg	10.55	8.18
50µg	16.71	29.90
100µg	31.93	45.73

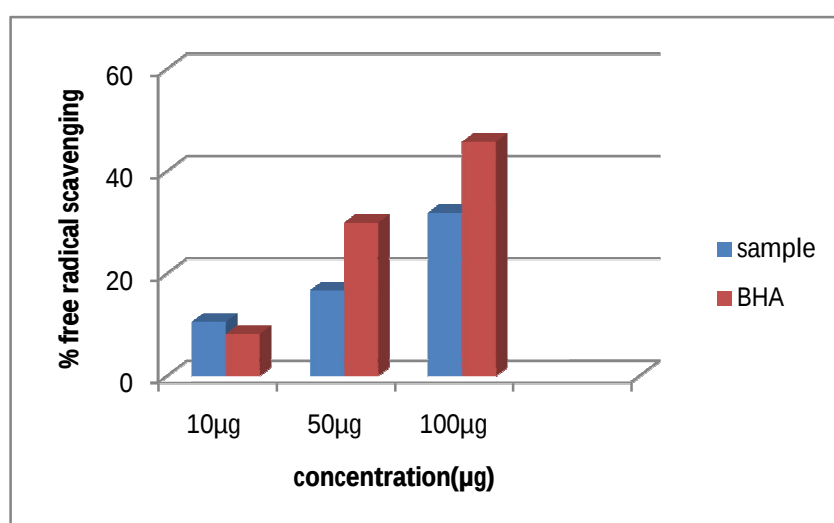


Figure-3: % free radical scavenging activity of the sample and BHA. The graph shows higher potency of free radical scavenging activity of sample as compared to BHA at lower concentration. **Ref:** Kumar S., Kumar D., Manjusha, Saroha K., Singh N. and Vashishta B.(2008): Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad.methanolic fruit extract, Acta. Pharm. 58; 215–220.

4. Nitric oxide radical scavenging activity:

Nitric oxide is major radical produced inside human body during some biological reaction; it is an important cellular signaling molecule involved in many physiological and pathological processes. It is a powerful vasodilator with a short

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half-life of a few seconds in the blood. Nitric oxide is highly unstable molecule, on oxidation it forms into nitrogen dioxide. Symptoms of short-term nitrogen dioxide exposure include nausea, dyspnea and headache. Long-term effects could include impaired immune and respiratory function. Our sample extract showed good result of nitric oxide free radical scavenging activity. The principle involved in this assay is that extract competes with oxygen to react with nitride oxide; flavonoids are actively involved in this process.

Table-3: % Nitric oxide scavenging activity of ethanol fruit extract of *Garcinia xanthochymus* and BHA

Concentration	% NO radical scavenging	
	Sample	BHA
10µg	54.59	65.41
50µg	67.03	85.57
100µg	95.14	96.76

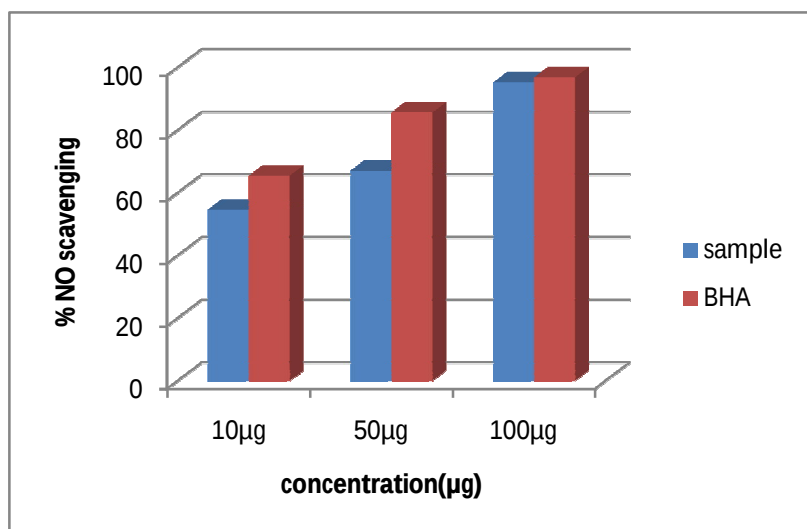


Figure-4: % NO scavenging activity of sample and BHA. The graph shows similar potency of Nitric oxide scavenging activity of sample as compared to BHA at higher concentration. **Ref:** Kumar S., Kumar D., Manjusha, Saroha K., Singh N. and Vashishta B.(2008): Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.)Schrad.methanolic fruit extract, Acta. Pharm. 58; 215–220.

5. Total anti-oxidant assay:

Total antioxidant capacity is the term used to describe the ability of antioxidants in different foods to clean harmful free radicals in the blood and cells. Often referred to by the acronym TAC, it plays vital role in life sustenance of an organism. Oxygen radical absorbance capacity scale (ORAC) is the scale used to measure TAC, our study revealed the positivity of TAC of extract.

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Table-4: Total antioxidant activity of ethanol fruit extract of *Garcinia xanthochymus* and BHA

Concentration	Total antioxidant	
	Sample	BHA
10 μ g	29.70	30.65
50 μ g	39.70	46.10
100 μ g	45.75	59.50

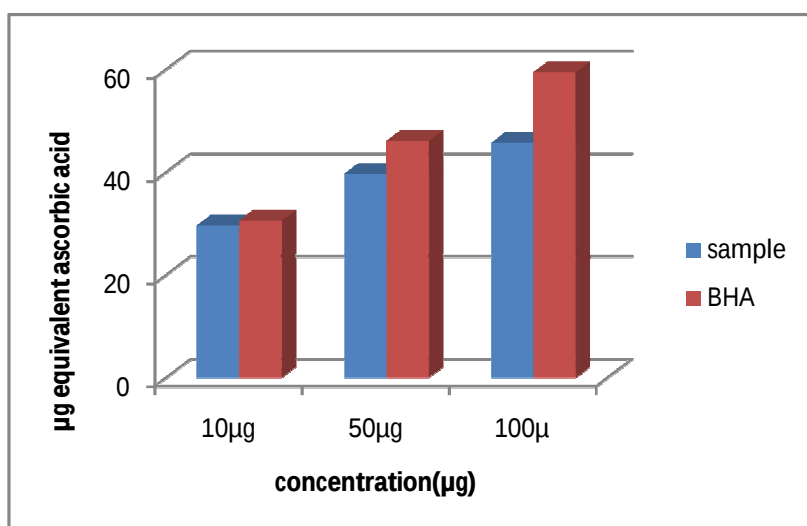


Figure-5: Total antioxidant activity of sample and BHA in μ g equivalent ascorbic acid. The graph shows the similar potency of antioxidant capacity of the samples as that of the BHA, suggesting its potential usage as a natural antioxidant. **Ref:** Prieto P., Pineda M. and Aguilar M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E1. *Analytical Biochemistry*. 269: 337-341.

IV. CONCLUSION

Fruits and vegetables cultivated in one geographic location often tend to have a different capacity from fruits and vegetables grown in another. Environmental components largely contribute to Total Antioxidant Capacity, phytochemical component and many more. The biochemical test of *Garcinia xanthochymus* fruit extract revealed that it is the good source of flavonoids. Also has considerable amount of other phenolic compounds. Since both have significant contribution for antioxidant property, it gave an excellent result in DPPH model and nitric oxide scavenging activity. It also exhibits the high amount of saponins and positive result for other phytochemicals. It assures an advanced food style, and since it is from Western Ghats (Hottest Biodiversity Hotspot) it awakens the value of conservation.

Future: *Garcinia* could be used as a natural antioxidant source. Being a saponin rich fruit, it gives a hope to treat cancer.

V. CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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BIOGRAPHY



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