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Estimation of bioactive components and antioxidant activity of garcinia gummigutta fruit rind

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Abstract
The aim of the in-vitro research was to determine the bio-active components and antioxidant activity of Garcinia gummigutta dry and wet rinds. The bio active components such as total flavanoids were analysed by Aluminium chloride colorimetric method, total tannins by Ferri cyanide and ferric chloride colorimetric method and total poly phenols by Folin ciocalteu's reagent method respectively. Antioxidant activity of Garcinia gummigutta fresh and dry rinds were determined using DPPH scavenging activity by taking Gallic acid as the standard. The bio active components were high in fresh rind i.e total flavanoids, w/w 2.48 (per cent), total tannins, w/w 2.76 (per cent) and total poly phenols, w/w 1.1 (per cent) than in dry rind 0.20 (per cent), 0.10 (per cent) and 0.04(per cent) respectively. The antioxidant activity of fresh rind was 66.30 (per cent), 73.65 (per cent) and 81.44 (per cent) at the dose of 20mg, 40mg and 60mg. Hence, the traditionally claimed medicinal benefits of gummigutta might be due to its potential antioxidant property. However, further studies to be carried out on animal models using their biological tissues before exploiting commercially.

Keywords: Garcinia gummigutta, Total favonoids, Total tannins, Total polyphenols, DPPH scavenging activity

Introduction
Medicinal plants, medicinal herbs, or simply herbs have been identified and used from prehistoric times. Plants make many chemical compounds for biological functions, including defense against insects, fungi and herbivorous mammals. Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and also have harmful side effects just like conventional drugs. The earliest historical records of herbs are found from the Sumerian civilization, where hundreds of medicinal plants including opium are listed on clay tablets. The Ebers Papyrus from ancient Egypt describes over 850 plant medicines. Drug research makes use of ethno botany to search for pharmacologically active substances in nature, and has in this way discovered hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes, the alkaloids, glycosides, polyphenols, and terpenes. Medicinal plants are widely used to treat disease in non-industrialized societies, not least because they are far cheaper than modern medicines. The annual global export value of pharmaceutical plants in 2012 was over US$2.2 billion. (Lichterman, B. L., 2004)
For thousands of years mankind is using plant sources to alleviate or cure (kamboj, 2000) [4]. Novel chemical compounds synthesis from the plant active constituents, which are of potential use in medicine and other usefull application. Herbal remedies are popular remedies for diseases used by a vast vast majority of the world's population (Pal and Shukla, 2003) [9]. Herbal plants having many pharmacologically active compounds like flavonoids, alkaloids, tannin, steroids,glycosides, phenols, fixed oils, which is stored in their specific parts of leaves, bark, flowers, seed, fruits, root etc.3. Biophytum sensitivum (family- Oxalidaceae) having different pharmacological activities such as dengu, anticancer, anti-inflammatory, chemoprotective, anti-diabetic and wound healing activities of their different parts.

There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging etc. A free radical is defined as any atom or molecule possessing unpaired electrons (Krishnaswamy, 1996). Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS), reactive nitrogen species (RNS). ROS is composed of superoxide anion (O2·), hydroxyl (OH·), hydro-peroxyl (OOH·), peroxyl (ROO·), alkoxyl (RO·) radicals non free radicals are hydrogen peroxide (H2O2), hypochlorous acid (HOCl), ozone (O3) singlet oxygen (1O2). RNS are mainly nitric oxide (NO·), peroxynitrite (ONOO·) nitrogen dioxide (NO2). Antioxidants can

greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells prevent damage to lipids, proteins, enzymes, carbohydrates DNA.

A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases.

Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are

important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging (Pal *et al*, 2009) [10].

Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions1. Free radicals have been implicated in the etiology of several major human ailments, including cancer, cardiovascular diseases, neural disorders, diabetes and arthritis 2–4. Due to the recent trends in nutrition towards development of healthy foods in the form of 'functional foods', one of the desirable properties in a dietary component is considered to be its antioxidant effect (Bhaskar, 2010 and Natarajan *et al*, 2010) [1, 8].

The mechanisms involved in many human diseases such as hepato toxicities, hepato carcinogenesis, diabetes, malaria, acute myocardial infarction, skin cancer include free radicals-induced lipid peroxidation as the main source of membrane damage. The antioxidant action can also be measured by inhibition of damage caused by free radicals (Lachnicht, 2002 and Devasagayam, 2003) [7, 2]. In our studies, ascorbate–Fe2+, a system relevant to endogenous oxidative damage was used for rat liver mitochondria. Modulation of diseased states such as cardiovascular ailments, neurological disorders, cancer and diabetes using dietary components, including fruits and vegetables, natural products and medicinal plants as a possible therapeutic measure has become a subject of active scientific investigations (Hertog, 1993 and Surh, 2003) [3, 11].

The very concept of food is changing from a past emphasis on health maintenance to the promising use of food to promote better health to prevent chronic illnesses. The advent of functional foods may allow us to improve public health. 'Functional foods' are those that provide more than simple nutrition; they supply additional physiological benefits to the consumer. In India also, the demand for functional foods is increasing in recent years. In this aspect, foods rich in preparations from Malabar Tamarind and its syrup can be considered as functional foods. The assays used for testing the antioxidant capacities of G. gummigutta extract act at various levels of antioxidant activity at which the damage caused by free radicals can be prevented. Thus these experiments could be useful in detecting the mechanism of prevention of free-radical damage.

Garcinia gummigutta (dried rind known as 'Uppage') is an Indian spice used in many parts of the country for making several vegetarian and non-vegetarian 'curry' preparations, including the popular 'solkadhi'. Many therapeutic effects of the fruit have been described in traditional medicine based on Ayurveda. It is an appetizer and a good liver tonic; to improve appetite and to allay thirst; as a cardiotonic and for bleeding, piles, dysentery, tumours and heart diseases. One of the ingredients of G. gummigutta is the presence of hydroxycitric acid (HCA), has been patented for use as an hypocho lesterolaemic agent. HCA is a potential anti-obesity agent9. It suppresses fatty acid synthesis, lipogenesis, food intake and induces weight loss (Sannigrahi *et al*., 2009) [10]. Garcinol, a polyisoprenylated benzophenone purified from G. indica fruit rind, displays antioxidant, anti-cancer and anti-ulcer properties (Pal *et al* and Kuluvar *et al*., 2009) [6]. Apart from HCA and garcinol, Malabar tamarind also contains other compounds with potential antioxidant properties. These include citric acid, malic acid, polyphenols, carbohydrates, anthocyanin pigments and ascorbic acid (Bhaskar and V. Rajalakshmi, 2010) [1].

Therefore, the objective of our present study is to determine the bioactive components and antioxidant activity of fresh and dry fruit rind of Garcinia gummigutta.

Materials and Methods

A. Antioxidant Activity

In this study free radical scavenging activity of methanolic fruit rind extract of G. gummigutta was determined by *in vitro* assay models such as DPPH free radical and Gallic acid was used as standard.

A. DPPH radical scavenging activity

Principle

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses colour stochiometrically depending on the number of electrons taken up 9.

Procedure

DPPH radical scavenging activity was measured using the method of Kiranmai *et al.*; with some modifications. 2 ml of reaction mixture containing 1 ml of DPPH (100 μM in methanol) 1 ml of test solution, at various concentrations of the extract fractions was incubated at 37°C for 30 min absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the following equation

Percentage inhibition = (1− absorbance of test/absorbance of control) × 100

b. Estimation of bio active components

A. Total flavonoids

Procedure: Flavonoids give yellow colour when treated with aluminium chloride reagent. The content of flavonoids in plant extracts can be estimated using this principle.

Reagent: Aluminium chloride reagent (1% w/v solution): Weigh accurately about 1g of aluminium chloride in 100ml volumetric flask. Add 70ml – 80ml of 95% ethanol (to be prepared freshly) and dissolve by sonication. Make the volume up to the mark with same solvent (to be prepared freshly). Unless otherwise specified in the individual STP, weigh accurately about 0.5 g of finely powdered test substance in to 250 ml flat bottomed flask and add 70 to 80 ml of methanol. Extract content by refluxing on a water bath at 80°C ± 2°C for 30 minutes and decant the dissolved extract in to a 100ml volumetric flask. Wash the residue with 10ml of methanol and transfer to the same 100ml volumetric flask. Make up the volume to 100 ml with methanol. Filter the extract through what mann no 1 filter paper, discard the first 25 ml of the filtrate and use the subsequent filtrate for the analysis.

Standard Preparation: Unless otherwise specified in the individual STP, weigh accurately about 100mg of standard rutin in 100ml volumetric flask. Add 70 to 80 ml methanol and dissolve by sonication. Make up to volume with methanol to 100ml. Pipette out 5 ml of this solution in 100ml volumetric flask and make up the volume with methanol.

Method

Standard: Unless otherwise specified in the individual STP, pipette out 1ml of the standard rutin solution in 10ml volumetric flask. Add 1ml of aluminium chloride reagent and mix. Make up the volume to 10 ml with ethanol (unless otherwise specified in the individual STP). Measure the optical density of the standard solution (S) against the reagent blank at 410nm (unless otherwise specified in the individual STP) exactly at 15 minutes after the addition of reagent. Reagent blank prepared by dissolving 1ml of aluminium chloride with ethanol and make up the volume to 10 ml with ethanol.

Samples: Unless otherwise specified in the individual STP, pipette out 1ml each of test solution in 10 ml volumetric flask. Add 1 ml of aluminium chloride reagent and mix. Make up the volume to 10 ml with ethanol. Measure the optical density of the test solution (T) against the reagent blank at 410 nm exactly at 15 minutes after the addition of reagent.

Blank solutions: Prepare standard blank (SB) and sample blank (TB) solutions by diluting 1ml (unless otherwise specified in the individual STP) of respective standard and sample to 10 ml with ethanol and read the optical density at 410 nm after 15 minutes.

Calculation

Subtract the corresponding blank values from the standard and test absorbance values
(S – SB; T – TB). Calculate the flavonoid content with respect to Rutin and express as % w/w of flavonoids.

$$\frac{\text{Absorbance of sample } (T-TB)}{\text{Absorbance of Std } (S-SB)} \times \frac{\text{Weight of Standard (mg)}}{100} \times \frac{5}{100} \times \frac{\text{Volume of Std taken for reaction}}{\text{Volume of sample taken for reaction}} \times \frac{\text{Total volume of sample}}{\text{Weight of sample (mg)}} \times \text{\% Purity of Std}$$

B. Estimation of tannins

Principle: tannins give greenish – blue colour with potassium ferri cyanide and ferric chloride reagents. The colour product can be read at 720 nm and the amount of tannin can be quantified using Tannic acid as standard.

Reagent

1. Prepare 1% potassium ferri cyanide in water.
2. Prepare 1% ferric chloride in water.

Sample preparation

Extract 100 mg of sample in 50 ml of water at 100°C using water-bath for 1 hour. Filter into a 100 ml volumetric flask, wash the residue with water and makeup the volume with water.

Standard preparation

Weigh accurately about 100 mg of standard tannic acid in 100 ml standard flask and makeup to volume with water (standard stock solution). Pipette out 1 ml from the above solution and makeup to 100 ml with water (Standard solution).

Procedure

Standard: Take 1 ml of standard solution in 10 ml volumetric flasks. Add 1 ml of potassium ferri cyanide, and 1 ml of ferric chloride. Mix well and make the volume up to 10 ml with water. Exactly 30 minutes after addition of the reagents read the optical density at 720 nm against reagent blank. Reagent blank is prepared by diluting 1 ml of potassium ferri cyanide and 1 ml of ferric chloride to 10 ml with water.

Test solution: Take 0.2 ml of test solution and makeup to 10 ml with water and measure the absorbance (TB) against water.

Note: All the optical density readings should be taken exactly 30 minutes after addition of the reagents.

Calculation

Subtract the reading of test solution from test blank and calculate the content of tannic acid from the standard curve and express as % w/w of tannins.

$$\frac{\text{Abs of spl (T – TB)}}{\text{sample Abs of std}} \times \frac{\text{weight of std (mg)}}{100} \times \frac{1}{100} \times \frac{\text{Volume of std taken for reaction}}{\text{volume of spl taken for reaction}} \times \frac{\text{total vol of}}{\text{Weight of spl}}$$

= Total tannins % w/w

C. Determination of polyphenols

Standard Preparation

Weigh accurately about 30.0 mg of Gallic acid R.S. Carefully transfer in to a 50 ml volumetric flask. Dissolve the contents with water and sonicate. Make up to the mark with water. Take 5 ml of the above solution in to another 50 ml volumetric flask. Make up to the mark with water. Take 2 ml from the above solution in to 25 ml volumetric flask. Add 2 ml of 1N Phenol reagent and 10 ml of sodium carbonate solution. Make up to the mark with water.

Sample preparation

Weigh accurately about 25 mg of the sample in a 50 ml volumetric flask. Dissolve the contents with water and sonicate. Make up to the mark with water. Take 5 ml of the above solution in to another 50 ml volumetric flask. Make up to the mark with water. Take 2 ml from the above solution in to 25 ml volumetric flask. Add 2 ml of 1N Phenol reagent and 10 ml of sodium carbonate solution. Make up to the mark with water. Keep the samples and standard preparation for 1 hour at room temperature and measure the absorbance at 760 nm against water blank.

Calculation

$$\textbf{Total Polyphenol} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \frac{\text{Standard Weight}}{\text{Sample weight}} \times \text{Purity of Standard} \times 100$$

Results and discussion

Table 1: Total Antioxidant estimation of Garcinia gummigutta

S. No	Parameters	Unit
1.	Total flavonoids, w/w	2.48%
2.	Total Tannins, w/w	2.76%
3.	Total Polyphenols, w/w	1.1%

Table 2: Anti oxidant activity (Free radical scavenging activity) by DPPH of fresh and dry fruit rinds of Garcinia gummigutta

Concentration of the Sample →	20mg	40mg	60mg
↓ Name of the Sample	Antioxidant (DPPH method) % activity		
Standard (Gallic acid)	65.40	79.20	81.90
Fresh rind	66.32	73.65	81.44
Dry rind	85.68	87.05	90.60

Dry fruit rind of G. gummigutta posses more free radical scavenging property than fresh rind. While the standard gallic acid and fresh rind shows relatively same antioxidant activity. The potential antioxidant property of G. gummigutta is due to the presence of some of the bioactive components such as flavonoids, polyphenols and tannins. This has the capability to fight against the free radicle activity and thus prevents several metabolic disorders.

Conclusion

The metabolic extract of whole plant extract of *BG. gummigutta* which possess antioxidant property. Hence further investigation and proper isolation of more active principles might help in the findings of new lead compounds which will be effective against free radical mediated diseases. *Garcinia gummigutta* (Malabar tamarind) is an Indian spice, the fruit rind of which is used in cooking and has several medicinal properties. We have examined the antioxidant activity of fruit fresh and dry rind. The high antioxidant activity of murugalu adds one more positive attribute to its known medicinal properties and hence its use in cooking, home-remedies and general value added products may be promoted.

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