Evaluation of nutritional composition and antioxidant properties of underutilized *Ficus talboti* King fruit for nutraceuticals and food supplements

Karuppusamy Arunachalam · Thangaraj Parimelazhagan

Revised: 24 January 2012 / Accepted: 3 February 2012 / Published online: 21 February 2012

© Association of Food Scientists & Technologists (India) 2012

Abstract This study investigated change in the chemical components and antioxidant activity of *Ficus talboti* fruit for the development of functional foods. Proximate compositions of crude protein, ash and fat were 23.28, 10.47 and 3.86% respectively. Macro-nutrient contents were found to be higher in the fruit when compared to micronutrients. The analysis also showed the presence of almost all the essential and non-essential amino acids especially Leucine, Phenylalanine and tyrosine in higher amount. Linolenic acid content was higher than stearic acid among the fatty acids in the fruit. Total phenolic content (35.4 ± 0.32 g GAE /100 g) was found in higher amount in acetone extract. The IC₅₀ values of 1,1-diphenyl-2-picrylhydrazyl, hydroxyl and superoxide radical scavenging of acetone extract were 346, 252 and 652 µg/mL respectively. Thus, *F. talboti* fruit can be used as a healthy and functional food ingredient which will ensure dietary diversity and food security among the marginalized and poor communities.

Keywords *Ficus talboti* · Amino acids · Fatty acids · Minerals · Antioxidant

Introduction

Many medicinal plants exhibit specific medicinal actions without serving a nutritional role in the human diet and may be used in response to specific health problems (Shirin and Jamuna 2001). Fig is among such plants in which recent

investigations have indicated that this has been cultivated for over 11,000 years, possibly predating cereal grains (Kislev et al. 2006). A number of *Ficus* species are being used as food and for medicinal properties in Ayurvedic and Traditional Chinese Medicine (TCM) especially amongst people where these species grow. Edible fruits, vegetables and whole grains contain many components that are beneficial to human health. Research supports that some of these foods, as part of an overall healthful diet, have the potential to delay the onset of many age-related diseases. The balance between pro-oxidants and antioxidants is an important strategy for maintaining health (Yen et al. 2008). Pro-oxidant is a substance that can induce oxidative damage to various biological targets such as nucleic acids (e.g., base modification, single- and double- strand breaks), lipids (e.g., peroxidation, fatty acid loss) and protein (e.g., oxidation of specific amino acid residues, formation of carbonyls). In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value (Ajila et al. 2007).

Ficus talboti King belongs to the family Moraceae. It is a wild, infrequent and endemic to India. It is a large evergreen tree without aerial roots. The barks are used for various purposes such as an astringent medicine, for cooling agent, haemostatic, laxative, in improving complexion, in cleaning vagina and useful in *pitta* and *kapha*. They are used in diabetes, diarrhoea, leucorrhoea, menorrhoea, nervous disorder, and vaginal diseases. It is also widely used in the treatment of skin diseases, ulcer and soreness in the mouth (Nadkarni 1994; Khare 2007). Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference. *F. talboti* fruits are orally taken by the local peoples in Sozhavanthan area Madurai District, for stomach ulcer. No other previous

K. Arunachalam · T. Parimelazhagan (✉)
Bioprospecting Laboratory, Department of Botany,
School of Life sciences, Bharathiar University,
Coimbatore 641 046, Tamil Nadu, India
e-mail: drparimel@gmail.com

studies on this plant for phytochemical and pharmacological research have been reported. Hence the present study has been undertaken to evaluate the nutritional and antioxidant properties of *F. talboti* fruit. The study could be helpful to develop medicinal preparation or nutraceuticals and functional foods.

Materials and methods

Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH[•]), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2'-bipyridyl, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), acetylcholineesterase, acetylcholin iodide, α -glucosidase, *p*-nitrophenyl glucopyranoside, α -amylase, starch (soluble) and 5,5'-dithiobis 2-nitrobenzoic acid were purchased from Sigma (Bangalore, India). Potassium ferricyanide, ferric chloride and potassium persulfate were purchased from Himedia (Mumbai, India). Ethylenediamine tetra acetic acid (EDTA) disodium salt and hydroxylamine hydrochloride were obtained from Merck (Mumbai, India). All other reagents used were of analytical grade.

Collection of plant material and extraction

The fruits of *F. talboti* were collected in the month of April 2008, from local areas of Madurai district, Tamil Nadu, India. The fruits were cleaned thoroughly, shade dried at room temperature and powdered. The powdered sample was extracted successively with petroleum ether, chloroform, acetone and methanol using Soxhlet apparatus. The solvents of the respective extracts were removed under reduced pressure, using a rotary vacuum-evaporator (Yamato, RE300, Tokyo, Japan) and the remaining residual moisture was removed by lyophilization (Vir Tis Benchtop K, Model - #4KBTXL-75, New York, USA). The freeze-dried extracts thus obtained were used directly for the assessment of antioxidant capacity through various chemical assays.

Analysis of proximate composition and minerals

The proximate composition of *F. talboti* fruit was determined by AOAC (1990a). Moisture and ash content were determined gravimetrically by desiccation at 105°C and incineration at 550°C in an electric furnace (F-2F; Kong Soung Co., Seoul, Mumbai), respectively. Crude protein content was obtained by micro-Kjeldahl method. Lipids were extracted using a Soxhlet-extraction apparatus (Dong Ha-Tech., Seoul, Kolkata). The minerals of *F. talboti* fruits were measured by wet digestion method. Ashes of RL were added to HCl: H₂O (1:3) and dried in water bath. Sample solutions were subsequently analyzed by

inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo Jarrell Ash, Franklin, MA, USA).

Amino acid analysis

Amino acids were determined as per the method of Ishida et al. (1981). 100 mg of finely homogenized sample was taken in a borosil test tube. 10 ml 6 N HCl was added into the test tube. The tubes were sealed after filling with nitrogen gas and the contents of the tube were digested by keeping at 120°C for 24 h in an oven. The test tubes were cooled and then the contents were filtered using Whatmann No 1 filter paper. The test tubes were rinsed with distilled water and then filtered. The filtrate was evaporated in a vacuum flash evaporator. Deionised water was added into the tubes and the evaporation was allowed to continue until the contents are acid free. The amino acids were dissolved in 0.05 N HCl. Samples were filtered through a membrane filter of 0.45 μ m and 20 μ l of this was injected to an amino acid analyzer (HPLC- LC 10 AS) equipped with cation exchange column packed with a strongly acidic cation exchange resin i.e., styrene di vinyl benzene co polymer with sulphonic group. The mobile phase of the system consists of two buffers, Buffer A and buffer B. Gradient system was followed for the effective separation of amino acids. The oven temperature was maintained at 60°C. The total run was programmed for 60 min. The amino acid analysis was done with non-switching flow method and fluorescence detection after post-column derivatization with o-phthaldehyde. In the case of proline and hydroxyl proline, imino group is converted to amino group with hypochlorite. Amino acid standard was also run to calculate the concentration of amino acids in the sample. The amount of each amino acid is expressed as g/100 g protein. Essential amino acids score was calculated with reference to the FAO/WHO reference amino acid pattern (FAO/ WHO/ UNU 1985)

$$\text{Amino acid score} = \frac{\text{Test amino acid}}{\text{Reference amino acid}} \times 100$$

Analysis of fatty acids

Fatty acid compositions of *F. talboti* fruit was determined by AOAC (1990b). For lipid extraction, 50 g of fruits were homogenized in 150 mL chloroform:methanol (1:2, v/v) mixture and reacted at 100°C for 30 min filling N₂, and then filtrated through Whatman No. 1 filter paper. Extracted lipid from each sample was saponified in 0.5 N NaOH methanolic solutions and esterified in BF₃-methanol. Fatty acids were determined using a gas chromatography (Varian star 3600; Varian Inc., Walnutcreek, CA, USA) equipped with flame ionization detector and Omagawax 205 fused-silica bond capillary column (30 m×0.32 mm, i.d.×0.25 μ m

film thickness). Temperature of oven, injection port, and detector were 140, 250, and 260°C, respectively, and nitrogen flow rate was 50 mL/min.

Estimation of total phenolics and tannins

The total phenolic content of *F. talboti* fruit extracts were determined by Folin ciocalteu method. Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP). The amount of total phenolics and tannins were calculated as gram gallic acid equivalents (GAE) from the calibration curve (Makkar et al. 2007).

Estimation of total flavonoid content

The total flavonoid content of sample extracts were determined by the use of a slightly modified colorimetric method described previously Zhishen et al. (1999). A 0.5 mL (1 mg/mL of methanol) extract was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% NaNO₂ solution. After 6 min of incubation, 0.15 mL of 10% AlCl₃ solution was added and allowed to stand for 6 min, and then 2 mL of 4% NaOH solution was added to the mixture. Immediately distilled water was added to bring the final volume of 5 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Rutin was used as standard compound for the quantification of total flavonoid. All the values were expressed as gram rutin equivalent (RE) per 100 g of extract.

Radical scavenging activity using DPPH• method

The DPPH• radical scavenging activity of the different extracts were measured according to the method of Blies (1958). IC₅₀ values of the extracts i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was also calculated.

Total antioxidant activity assay using radical cation (ABTS•⁺)

ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS•⁺) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to 30°C to give an absorbance at 734 nm of 0.70±0.02 in a 1 cm cuvette (Re et al. 1999). After the addition of 1 mL of diluted ABTS•⁺ solution to 10 µL of extracts or Trolox standards (final concentration 0–15 µM)

in ethanol, OD was taken at 30°C exactly 30 min after the initial mixing. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts. The concentration of *F. talboti* fruit extracts that produced between 20 and 80% inhibitions of the blank absorbance was determined and adapted.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of the sample extracts were estimated according to the procedure described by Pulido et al. (2000). FRAP reagent (900 µL), prepared freshly and incubated at 37°C, was mixed with 90 µL of distilled water and 30 µL of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37°C for 30 min in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 mL of 20 mmol/L TPTZ solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L FeCl₃ · 6H₂O and 25 mL of 0.3 mol/L acetate buffer (pH 3.6). At the end of incubation, the absorbance readings were taken immediately at 593 nm, using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2,000 µmol/L (FeSO₄·7H₂O) were used for the preparation of the calibration curve. The parameter Equivalent Concentration (EC₁) is defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L FeSO₄·7H₂O. EC₁ was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/L concentration of Fe (II) solution, determined using the corresponding regression equation.

Metal chelating activity

The chelation of ferrous ion by the different extracts from *F. talboti* fruit was estimated by the method of Dinis et al. (1994). Briefly extracts and standard BHT were added to a solution of 2 mmol/L FeCl₂ (0.05 mL). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm.

Hydroxyl radical scavenging activity

The scavenging activity of extracts on hydroxyl radical was measured according to the method of Klein et al. (1991). Various mass (100–500 µg/mL) of extracts in test tubes were added with 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of DMSO (0.85%

DMSO (v/v) in 0.1 M phosphate buffer, pH 7.4) sequentially. The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80–90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1.0 mL of ice-cold TCA (17.5% w/v). Subsequently, 3.0 mL of Nash reagent was added to each tube and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectrophotometrically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated by the following formula:

$$\% \text{ HRSA} = \frac{[(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100}{\text{Control OD}}$$

IC₅₀ values of the extracts were also calculated.

Assay of nitric oxide scavenging activity

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different extracts and incubated at room temperature for 150 min. The same reaction mixture without the extract but the equivalent amount of the solvent served as the control. After the incubation period, 0.5 mL of Griess reagent (1% sulfanilamide, 2% H₃PO₄ and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm (Sreejayan and Rao 1997).

Assay of superoxide radical (O₂^{•−}) scavenging activity

The assay was based on the capacity of the extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system (Beauchamp and Fridovich 1971). Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EDTA, 0.1 mg NBT and 1 mL sample solution (20–100 µg/mL). Reaction was started by illuminating the reaction mixture with different concentrations of sample extracts for 90 s. immediately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes with reaction mixture kept in dark served as blanks. The percentage inhibition of superoxide anion generation was calculated using the following formula: % of inhibition = [(A₀−A₁)/ A₀] $\times$ 100 where A₀ is the absorbance of the

control, and A₁ is the absorbance of the extract. IC₅₀ values of the extracts were also calculated.

Statistical analysis

The data on different parameters were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($p < 0.01$) using statistical (Stat soft Inc., Tulsa, USA). Values expressed are means of three replicate determinations $\pm$ standard deviation.

Results and discussion

Proximate composition

Proximate compositions of *F. talboti* fruit are shown in Table 1. The crude protein was found to be high (23.28%) compared to fat (3.86%) and ash content (10.47%). According to Pearson et al. (1976) plant food that provide more than 12% of its calorific value from protein are considered good source of protein. In accordance with these, the fruit selected for the present study *F. talboti* fruit might serve as an efficient source of protein rich food.

Minerals

The mineral contents of *F. talboti* fruit are shown in Table 1. It has been found that fruit was rich in Ca, K, and Mg. The values for the Ca, K, and Mg increased significantly from 1,774, 1,322, and 361 mg/100 g, respectively. Calcium has been reported to be effective in building of skeletal structures and muscle functioning while magnesium is important in the ionic balance and enzyme co-factors (FAO 1990). It has been shown that *F. talboti* fruit also had some minerals such as Ca, K and Mg, which were beneficial to human body so that they were thought to be used as food materials useful in health.

Aminoacid analysis

The aminoacid composition of the *F. talboti* fruit sample along with the essential aminoacid requirements pattern suggested by FAO (1990) is shown in Table 1. The aminoacid profile of the sample revealed that the proteins of the fruit contained adequate levels of aminoacid as compared FAO (1990). The amount of amino acids, Histidine (6.8 g/ 100 g protein), leucine (9.7 g/ 100 g protein), iso leucine (7.8 g/ 100 g protein), phenylalanine + tyrosine (9.6 g/ 100 g protein), threonine (7.2 g/ 100 g protein), tryptophan (1.8 g/ 100 g protein), and valine (8.2 g/ 100 g protein), were found to be higher, whereas the aminoacid

Table 1 Chemical composition *F. talboti* fruit

Proximate Composition (%)		Minerals (mg/100 g)	Fatty acids	Composition (%)	Amino acids (g/100 g protein)	<i>F. talboti</i> fruit	Reference pattern FAO/WHO/ UBU (1985)	
Moisture	6.9±0.03	Ca	1,774.8±95.86	Caprylic acid	0.1±0.011	Histidine	6.8	1.9
Crude lipid	3.8±0.04	K	1,322.8±35.81	Capric acid	0.9±0.01	Lysine	3.2	5.8
Crude protein	23.3±0.37	Mg	361.5±1.82	Myristic acid	0.8±0.01	Leucine	9.7	6.6
Crude ash	10.5±0.35	Fe	16.8±0.55	Palmitic acid	11.8±0.07	Isoleucine	7.8	2.8
		Na	14.6±6.10	Palmitoleic acid	2.1±0.29	Methionine + cysteine	2.2	2.5
		Zn	4.1±1.12	Stearic acid	4.3±0.01	Phenylalanine + tyrosine	9.6	6.3
		Cu	0.7±0.03	Oleic acid	08.5±0.34	Threonine	7.2	3.4
				Linoleic acid	30.4±0.93	Tryptophan	1.8	1.1
				Linolenic acid	33.1±1.04	Valine	8.2	3.5
				Arachidic acid	0.2±0.01			
				Behenic acid	1.5±0.16			
				Others	6.4±0.09			

FAO/WHO/UNU (1985). Amino acid reference pattern of protein for children (2–5 years old) diet. Values are % of protein. Each amino acid in the reference pattern was presumed to score a value=100. Values for each cultivar is expressed relatively to the reference pattern. Results were mean ± SD ($n=3$)

lysine (1.2 g/100 g protein), methionine + cysteine (g/100 g protein), recorded lower level but appreciable content than the reference pattern of FAO (1990). Amino acids in the form of proteins make up the greatest portion of our body weight. Apart from this, cysteine was found to have the ability to quench DPPH radical (Yokozawa et al. 1998). Arginine plays vital roles in nutrition and metabolism. It is classified as an essential amino acid in infants and young children and as a conditionally essential amino acid in adults at times of trauma or disease. The chemical score and amino acid index are widely used for screening potential protein foods. Amino acid deficiency can be met by consuming large amounts of legumes, or by taking a mixture of legumes, or by employing the complementarily that exists between high sulphur amino acid cereals and legumes.

Fatty acids

Fatty acids compositions of *F. talboti* fruit are shown in Table 1. A total of 11 fatty acids ranging from C8 to C22 were identified. In *F. talboti* fruit, higher content of unsaturated fatty acids such as linoleic acid (30%) and linolenic acid (33%) rather than that of saturated fatty acids such as palmitic acid (11%) and steric acid (4%) were recorded. The fatty acid composition of a diet alters the composition of membrane phospholipids, which in turn changes membrane functions (Field et al. 1985). The plant contains unsaturated fatty acids of physiologic and nutritional significance such as oleic and linoleic acids which could be contributory to the nutritional needs of the consumers.

Total phenolics, tannins and flavonoids

Phenolic compounds contribute directly to the antioxidant activity of fruits extract and therefore, it is quite important to evaluate the total phenolic content in tested extracts. These phenolic components are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers. The total phenolic content of the extracts was determined using Folin Ciocalteu's reagent and the results are presented in Table 2. The results demonstrated the content of phenolics was in the range of 1.6 to 35.4±0.32 g GAE/100 g extract. Among the extracts, low level of phenolics were recorded in solvents with low polarity (1.6 and 2.2 GAE g/100 g for petroleum ether, and chloroform respectively), whereas high polar solvents contained relatively higher amounts of phenolics (35.4 and 24.0 GAE g/100 g extract for acetone and methanol, respectively). As for total phenolic contents, high polar extracts also had the higher tannin contents. The low polar solvent like petroleum ether and chloroform gave negative results for tannins, i.e., tannins was not detected in these extracts. However, acetone and methanol (5.0 GAE g/100 g extract) had the highest tannin contents (5.4 GAE g/100 g extract).

Investigation for the content of flavonoids showed that *F. talboti* fruit has considerable amount of flavonoids. Flavonoids are one of the most diverse and widespread group of natural compounds and are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. The total flavonoid content of *F. talboti* fruit varied from 13.9 to 15.2 g RE / 100 g extract (Table 2).

Table 2 Chemical quality characteristics and antioxidant activity of various solvent extracts from *F. talboti* fruit ($n=3$)

Sample	Petroleum ether	Chloroform	Acetone	Methanol	BHT	BHA
Total phenolics (g GAE/100g extract)	1.6±0.12 ^c	2.2±0.24 ^b	35.4±0.32 ^a	24.0±0.41 ^a		
Tannins (g GAE/100g extract)	N.D	N.D	5.4±0.56 ^a	5.0±0.67 ^a		
Flavonoids (g RE/100g extract)	N.D	N.D	15.2±1.71 ^a	13.9±0.32 ^a		
FRAP1 (mmol Fe(II)/g extract)	121.3±2.34 ^c	265.3±12.75 ^c	876.3±45.63 ^b	687.3±46.37 ^c	1342.3±235.67 ^a	486.3±63.34 ^d
ABTS2 (μmol trolox/g extract)	587.2±37.65 ^c	2889.1±24.63 ^d	5173.8±81.67 ^c	4866.7±43.38 ^c	1645.3±83.65 ^a	8052.3±79.37 ^b
Metal chelating activity ³ (mg EDTA/g extract)	7.0±3.17 ^d	20.2±5.16 ^a	6.8±1.16 ^d	8.5±0.37 ^d	11.5±0.93 ^c	16.5±1.28 ^b
Nitric oxide IC50 values of free radical scavenging activity (μg/mL)	926.3±0.82 ^a	795.7±4.25 ^b	652.1±0.83 ^d	707.6±1.56 ^c	621.3±5.26 ^d	726.9±8.53 ^c
Superoxide IC50 values of free radical scavenging activity (μg/mL)	1236.4±5.65 ^a	961.3±5.17 ^c	652.0±2.03 ^d	702.5±1.83 ^d	297.2±6.37 ^e	312.6±0.82 ^e
Hydroxyl radical IC50 values of free radical scavenging activity (μg/mL)	415.9±6.05 ^a	436.2±18.53 ^a	252.1±6.57 ^b	267.8±3.26 ^b	312.3±2.06 ^b	278.6±4.17 ^b

Values are mean of three replicate determinations ± Standard deviation. Mean values followed by different superscript in a column are significantly different ($p<0.05$), ND Non Detectable

¹⁾ - Concentration of substance having ferric-TPTZ (2, 4, 6- tripyridyl -s- triazine) reducing ability equivalent to that of mmol Fe(II)/g extract

²⁾ - Total antioxidant activity (μmol equivalent Trolox performed by using ABTS⁺ radical cation). ³⁾ - mg of ascorbic acid equivalent per gram extract. FRAP- Ferric Reducing Antioxidant Power, ABTS- 2, 2'-azino-bis- (3-ethyl benzothiazoline)-6-sulfonic acid disodium salt, IC₅₀- Inhibition concentration 50, BHA- Butylated hydroxy anisole, BHT- Butylated hydroxy toluene

The recovery of polyphenol from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, solvent polarity will play a key role in increasing phenolic solubility (Naczak and Shahidi 2006). Therefore, it is hard to develop a standard extraction procedure suitable for phenol extraction of all the fruit samples. From the results shown in Table 2, it is evident that the recovery of phenolic compounds was dependent on the solvents used and their polarity. The acetone and methanol gave the highest yield of total phenolic contents. This is due to the fact that wide range of phenols can dissolve in aqueous methanol mixtures. On the other hand, acetone- water mixtures are good solvent systems for the extraction of polar antioxidants (Luximon-Ramma et al. 2003).

DPPH radical scavenging activity

DPPH is a commercial oxidizing radical which can be reduced by antioxidants. The disappearance of the DPPH radical can be monitored by decreased optical density at 517 nm (Blies 1958). The DPPH radical scavenging activity of all the extracts from *F. talboti* fruit increased with increasing concentration. The concentration of the extract necessary to decrease initial concentration of DPPH by 50% (IC₅₀ value) under experimental condition was calculated and presented in Figure 1. The highest DPPH radical scavenging activity was observed in acetone fraction. In the present study, the IC₅₀ values of petroleum ether, chloroform, acetone and methanol extracts of *F. talboti* fruit

exhibited 1986, 1,185, 349 and 491 μg/ mL respectively. The DPPH radical scavenging activity of extracts was in the order: acetone > methanol > chloroform > petroleum ether. The DPPH radical scavenging activity of the extracts showed a high correlation with total phenolic contents ($R^2=0.8801$). The presence of tannins and flavonoids in acetone and methanol extracts also contributed to high DPPH radical scavenging activity of these extracts.

It has been reported that antioxidants such as cystein, glutathione, ascorbic acid, tocopherol, phenolics, flavonoids and aromatic amines reduce and discolour DPPH through their hydrogen donating ability. It appears that radical scavenging activity of *F. talboti* fruit extracts is also due to their

Fig. 1 DPPH radical scavenging activity of various solvent extracts from *F. talboti* fruit. Values are mean of three replicate determinations ± Standard deviation. Values are amount of sample necessary to decrease initial concentration of DPPH by 50% (IC₅₀). BHA - Butylated hydroxyanisole. BHT - Butylated hydroxytoluene

hydrogen donating ability. The antioxidants are believed to intercept the free radical chain of oxidation and donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end-product which does not initiate or propagate further oxidation of lipid (Jayaprakasha et al. 2004). The data obtained from this antioxidant assay revealed that the extracts are free radical inhibitors and act as primary antioxidants that react with free radicals.

Trolox equivalent antioxidant capacity (TEAC)

ABTS^{•+} assay is an excellent tool to determine the antioxidant activity of hydrogen-donating antioxidants (scavenging aqueous phase radicals) and of chain breaking antioxidants (scavenging lipid peroxyl radicals). The results of the TEAC analysis has been seen in Table 2. The antioxidant capacity obtained for *F. talboti* fruit extracts ranged from 587.2 to 5173.8 μmol Trolox/g extract. The extract with the lowest antioxidant activity was shown by petroleum ether and chloroform (TEAC values of 587.2 and 2889.1 μmol /g extract, respectively). The extracts with statistically significant antioxidant capacities in this assay were acetone (5173.8) and methanol (4866.7). However the TEAC of *F. talboti* fruit extracts were lower than those of positive controls BHT (10645.3) and BHA (8052.3). The low polar solvents showed lowest value of TEAC, which also had low phenolic contents in their extracts. The tannins and flavonoids were also not reported in this extract. The total antioxidant activity (TAA) of *F. talboti* fruit seems to be sufficient for functioning as potential nutraceuticals when they are ingested along with nutrients. The extracts tested with high antioxidant capacity in the DPPH model, also showed a high antioxidant capacity in ABTS^{•+} model ($R^2=0.932$). The high correlation may partly result from a similar mechanism and also both antioxidants are soluble in aqueous/ethanol systems. Further, a significant correlation also exists between the total antioxidant capacity and total phenolics ($R^2=0.884$). Hagerman et al. (1998) have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS^{•+}) and that effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group substitution than the specific functional groups.

Ferric reducing antioxidant power (FRAP)

The FRAP is a simple inexpensive assay and may offer putative index of antioxidant activity. The FRAP assay measures the ability of antioxidants to reduce the ferric 2, 4, 6-tripyridyl-s-triazine complex $[\text{Fe (III)-(TPTZ)}_2]^{2+}$ to intensely blue colored ferrous complex $[\text{Fe (II)-(TPTZ)}_2]^{2+}$ in acidic medium. FRAP values are calculated by measuring the absorbance increase at 593 nm and relating it to a ferrous

ions standard. The reduction capacity of a plant extract may serve as a significant indicator of its potential antioxidant activity. Reluctant phenolics and flavonoids have strong electron donating capacity which can induce the formation of a blue coloured Fe (II)-TPTZ from the colourless oxidized Fe (III) form. In the assay, the ability of *F. talboti* fruit extracts to reduce Fe^{3+} to Fe^{2+} where in the range of 121.3 to 687.3 mmol Fe (II)/g extract. Table 2 shows the reducing power of the extracts in the order of: acetone > methanol > chloroform > petroleum ether. The FRAP values of acetone and methanol extracts from *F. talboti* fruit were significantly higher than that of positive control BHA. The exception was petroleum ether extract which had the lowest reducing activity. Since, the concentration of phenolics and flavonoids is often higher in acetone and methanol extracts, these antioxidants play a protective role in extracts. In linear regression analysis, the FRAP values were positively correlated with the total phenolics and flavonoids. The corresponding correlation coefficient was 0.9822. Similar observations between the polyphenolic constituents and reducing power activity have also been reported for several plant extracts (Amarowicz et al. 2004).

Metal chelating activity

Ferrozine can form complexes with Fe^{2+} but in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore, allows estimation of chelating activity of the co-existing chelator. In this assay, extracts of *F. talboti* fruit interfered with the formation of ferrous-ferrozine complex, suggesting that they have chelating activity and can capture ferrous ion before ferrozine. As shown in Table 2, the chloroform extract exhibited sustainable iron chelating activity (20.2 mg EDTA/ g extract) over BHA (16.5 mg EDTA/ g extract) and BHT (11.5 mg EDTA/ g extract). The extracts of petroleum ether (7.0 mg EDTA/ g extract), acetone (6.8 mg EDTA/ g extract) and methanol (8.5 mg EDTA/ g extract) were found to have lower but comparable iron chelating values.

Several striking observations were noted in metal chelating activity. Acetone extract has potent primary antioxidant property but its function as secondary or preventive antioxidant is poor. Primary antioxidants scavenge radicals to inhibit chain initiation and break chain propagation. Secondary antioxidants suppress the formation of free radicals and powerful against oxidative damage. Though low polar extracts does not have high total phenolic content, flavonoids and antioxidant capacity values, they are the effective secondary antioxidants which contain active components that bind to metal ions strongly. On the whole, *F. talboti* fruit have strong primary and secondary antioxidant property.

Superoxide radical scavenging activity

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. The superoxide radical is known to be produced in vivo and results in the formation of H_2O_2 via dismutation reaction. Moreover, the conversion of superoxide and H_2O_2 into more reactive species, viz., the hydroxyl radical, has been thought to be one of the unfavourable effects caused by superoxide radicals (Halliwell 1991). $\text{O}_2^{\bullet-}$ -mediated oxidative stress is believed to be involved in the pathogenesis of cardiovascular disorders like Alzheimer's and Parkinson's diseases. The extracts were found to be efficient scavengers of superoxide radical generated in riboflavin-NBT-light system in vitro and their activities increased with increasing concentration. Photochemical reduction of flavins generates $\text{O}_2^{\bullet-}$, which reduces NBT, resulting in the formation of blue formazan. IC_{50} value, the concentration of extract that is able to scavenge half of the free radicals present in test solution was calculated and presented in Table 2. The lower IC_{50} value means higher in antioxidant activity. Interestingly, acetone and methanol extracts exhibited significant activity with IC_{50} of 652.0 and 702.5 $\mu\text{g/mL}$, respectively, but compared to BHA (297.2 $\mu\text{g/mL}$) and BHT (312.6 $\mu\text{g/mL}$), the activity is much lower. However, petroleum ether and chloroform extracts exhibited lower activity (1236.4 and 961.3 $\mu\text{g/mL}$ respectively). This may be explained by the interaction of different phenolics and flavonoids in these extracts (Zhishen et al. 1999). It was reported that the superoxide anion scavenging activity could be due to the action of a free hydroxyl group of phenolic compounds. Furthermore, flavonoid molecule with polyhydroxylated substitution on ring A or B and free 3-hydroxyl substitution could possess high superoxide scavenging activity (Siddhuraju et al. 2002). The results showed that *F. talboti* fruit extract exhibiting scavenging activity, is of potential health interest as it may be effective in reducing the level of $\text{O}_2^{\bullet-}$ which is generated during oxidative stress in the body.

Nitric oxide scavenging activity

Nitric oxide or reactive nitrogen species such as NO_2 , N_2O_4 , N_3O_4 , NO_3^- and NO_2^- formed during the reactions of nitrogen with oxygen or with superoxides under oxidative stress are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components. Nitric oxide radical, which is produced in vivo by a variety of cell types, is an important bio regulatory molecule with a number of physiological functions. Therefore, the scavenging effect of *F. talboti* fruit extracts were assessed against this radical presented in Table 2. The extracts were capable of scavenging NO radical and did so in concentration dependant manner. Based on

the IC_{50} values the order of the activity is as follows: BHA (621.3 $\mu\text{g/mL}$) > acetone (652.1 $\mu\text{g/mL}$) > methanol (707.6 $\mu\text{g/mL}$) > BHT (726.9 $\mu\text{g/mL}$) > chloroform (795.7 $\mu\text{g/mL}$) > petroleum ether (1241.4 $\mu\text{g/mL}$). As in previous assays, the acetone and methanol extracts exhibited higher and significant NO radical scavenging activity than BHT. Based on the IC_{50} values, the extracts were less active than the positive control BHA. Phenolic compounds of this plant including flavonoids and tannins might have probably involved in radical scavenging activity of the extracts. The NO scavenging activity also showed a linear correlation with the total phenolic content ($R^2=0.8224$). The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body.

Hydroxyl radical scavenging activity

Hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, this species is considered to be one of the quick initiator of lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids. In the present study hydroxyl radical scavenging activity is estimated by generating the hydroxyl radicals using ascorbic acid - iron-EDTA. The hydroxyl radical is formed by the oxidation reaction with dimethyl sulfoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent (Singh et al. 2002). The efficacy of the different extracts to quench the hydroxyl radicals is shown in Table 2. Among the extracts tested acetone and methanol extracts registered the highest hydroxyl scavenging potential. The petroleum ether and chloroform extracts exhibited moderate hydroxyl radical scavenging activity compared with more polar extracts. The activity were in the order: acetone (252.1 $\mu\text{g/mL}$) > methanol (267.8 $\mu\text{g/mL}$) > BHT (278.6 $\mu\text{g/mL}$) > BHA (312.3 $\mu\text{g/mL}$) > petroleum ether (415.9 $\mu\text{g/mL}$) > chloroform (436.2 $\mu\text{g/mL}$). From the foregoing investigation, it is obvious that all the sample extracts had good hydroxyl radical scavenging activity and may serve as good hydroxyl radical scavengers.

Conclusions

F. talboti exhibited a strong antioxidant activity in the following assays studied, including DPPH radical, ABTS⁺, phosphomolybdenum, FRAP, metal ion chelating, superoxide,

hydrogen peroxide, nitric oxide and hydroxyl radical activity. The antioxidant activity of *F. talboti* might be attributed to effective hydrogen donating ability and metal ion chelating capability. Moreover the nutritional, fatty acid and amino acid parameters results were expressed the *F. talboti* fruit may be used as a natural dietary food supplement. Finally, based on the safety profile of fig fruit products, pleasant taste and its antioxidant constituents, the plant can be used for further pharmacological studies. Because the antioxidant action is also a mean of lowering chronic anti-inflammatory action and insulin resistance, fig fruits hold potential scope in functional food approaches aimed at normalizing metabolic syndrome and boosting wellness beyond the widely accepted role of figs in the diet for improving bowel performance, and as a source of naturally sweet, readily available, quick energy. Further detailed investigation is underway to determine the extract phytoconstituents, which are responsible for antioxidant.

References

- Ajila CM, Naidu KA, Bhat UJS, Rao P (2007) Bioactive compounds and antioxidant potential of mango peel extract. *Food Chem* 105:982–988
- Amarowicz R, Pegg R, Rahimi MP, Barl B, Weil J (2004) Free-radical scavenging capacity antioxidant activity of selected plant species from the Canadian prairies. *Food Chem* 84:551–562
- AOAC (1990a) Official methods of analysis, 15th edn. Method 930. Association of Official Analytical Chemists, Arlington
- AOAC (1990b) Official Methods of Analysis, 15th edn. Method 969. Association of Official Analytical Chemists, Arlington
- Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 44:276–287
- Blios MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature* 26:1199–1200
- Dinis TCP, Madeira VMC, Almeida LM (1994) Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxyl radical scavenger's. *Arch Biochem Biophys* 315:161–169
- FAO (1990) Food and agriculture year book, statistic series no. 94. Food and Agriculture Organisation, Rome
- FAO/WHO/UNU (1985) Energy and protein requirements. WHO Technical Report Series No. 724. Geneva
- Field CJ, Angel A, Clandinin MT (1985) Relationship of diet to fatty acid composition of human adipose tissue structural and stored lipids. *Amer J Clin Nutr* 42:1206–1220
- Hagerman AE, Rice ME, Ritchard NT (1998) Mechanisms of protein precipitation for two tannins, pentagalloyl glucose and epicatechin 16(4→8) catechin (procyanidin). *J Agric Food Chem* 46:2590–2595
- Halliwell B (1991) Reactive oxygen species in living systems: source, biochemistry and role in human disease. *Amer J Med* 91:14–22
- Ishida Y, Fugita T, Asai K (1981) New detection and separation method for amino acid by high performance liquid chromatography. *J Chromatogr* 204:143–148
- Jayaprakasha GK, Jaganmohan RL, Sakariah KK (2004) Antioxidant activities of flavinidin in different in-vitro model systems. *Bioorg Med Chem* 12:5141–5146
- Khare CP (2007) Indian medicinal plants. An illustrated dictionary. Springer, New Delhi, p 269
- Kislev ME, Hartmann A, Bar-Yosef O (2006) Early domesticated fig in the Jordan Valley. *Sci* 312:1372–1374
- Klein SM, Cohen G, Cederbaum AI (1991) Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. *Biochem* 20:6006–6012
- Luximon-Ramma R, Baharun T, Crozier A (2003) Antioxidant actions and phenolic and vitamin C contents of common Mauritius exotic fruits. *J Sci Food Agric* 83:496–502
- Makkar HPS, Siddhuraju P, Becker K (2007) Plant secondary metabolites-methods in molecular biology. Humana Press, New Jersey, p 393
- Naczki M, Shahidi F (2006) Phenolics in cereals fruits and vegetables: Occurrence, extraction, and analysis. *J Pharm Biochem Anal* 41:1523–1542
- Nadkarni KM (1994) Indian Materia medica. Vol (2): pp 39
- Pearson D, In Asibey-Berko E, Tayie FAK (1976) Proximate analysis of some underutilized Ghanaian Vegetables. *Ghana J Sci* 39:91–92
- Pulido R, Bravo L, Sauro-Calixto F (2000) Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J Agric Food Chem* 48:3396–3402
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26:1231–1237
- Shirin AP, Jamuna P (2001) Evaluation of nutritional composition and antioxidant activity of Borage (*Echium amoenum*) and Valerian (*Valerian officinalis*). *J Food Sci Technol*
- Siddhuraju P, Mohan PS, Becker K (2002) Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem* 79:61–67
- Singh D, Febbo PG, Ross K, Jackson DG, Manola J et al (2002) Gene expression correlates of clinical prostate cancer behavior. *Cancer Cell* 1:203–209
- Sreejayan N, Rao MNA (1997) Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol* 49:105–107
- Yen FH, Wu TH, Lin LT, Cham TM, Lin CC (2008) Concordance between antioxidant activities and flavonol contents in different extracts and fractions of *Cuscuta chinensis*. *Food Chem* 108:455–462
- Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I (1998) Study on the inhibitory effect of tannins and flavonoids against the 1,1-Diphenyl-2-picrylhydrazyl radical. *Biochem Pharmacol* 56:213–222
- Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 64:555–559