OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2019.XX.XX

Research Article
Antioxidant Activities and Phenolics Contents of *Garcinia talbotii* Fruit Rind

[1,4]Azazahemad Kureshi, [2]Amit Mirgal, [3]Siddhesh Salvi, [1]Premlata Kumari, [4]Raghuraj Singh and [4]Satyanshu Kumar

[1]Department of Applied Chemistry, Sardar Vallabhbhai National Institute of Technology, 395007 Surat, Gujarat, India
[2]Gogate Joglekar College, 415612 Ratnagiri, Maharashtra, India
[3]Regional Fruit Research Station, 416516 Vengurla, Maharashtra, India
[4]Indian Council of Agricultural Research, Directorate of Medicinal and Aromatic Plants Research, 387310 Boriavi, Anand, Gujarat, India

Abstract

Background and Objective: There is growing interest in search for safer and more effective antioxidants from natural sources. Processing of *G. talbotti* fruit generates rinds as a waste. Aim of the present investigation was to determine phenolics content and antioxidant activities of different extracts prepared from fruit rinds of *G. talbotii*. **Materials and Methods:** Six solvents of different polarities (hexane, chloroform, ethyl acetate, methanol, water and hydro-alcohol (methanol: water, 2:8) were used for the extract preparation. Total phenolics content in extract samples was determined by Folin-Ciocalteu method. Three assays namely 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, 2-2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) free radical scavenging activity and reducing power were used to assess the antioxidant activities of extracts *in vitro*. Trolox and gallic acid were used as positive control for comparing the antioxidant activities of the extracts. **Results:** The results demonstrated that total phenolics content was the highest in hexane extract and lowest in water extract. Moreover, extracts prepared with non-polar/medium polar solvent such as hexane, chloroform and ethyl acetate showed significantly higher antioxidant activities. Extracts prepared with polar solvents such as water and hydro-alcohol mixture showed moderate antioxidant activities in all three *in vitro* antioxidant assays. **Conclusion:** Extraction yield, total phenolics content and antioxidant activities varied on the polarity of the solvent used for extract preparation. Based on the results of present investigation, suitable solvent could be selected for preparation of extract with high phenolics and antioxidant activity.

Key words: *Garcinia*, plant extraction, total phenolic content, antioxidant, phytochemical, xanthones, polyisoprenylated benzophenone

Received: Accepted: Published:

Citation: Azazahemad Kureshi, Amit Mirgal, Siddhesh Salvi, Premlata Kumari, Raghuraj Singh and Satyanshu Kumar, 2019. Antioxidant activities and phenolics contents of *Garcinia talbotii* fruit rind. Res. J. Med. Plants, 13: XX-XX.

Corresponding Author: Satyanshu Kumar, Indian Council of Agricultural Research, Directorate of Medicinal and Aromatic Plants Research, 387 310 Boriavi, Anand, Gujarat, India Tel: +91 2692 271 602

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus *Garcinia* (Clusiaceae) comprises about 250 species distributed in pantropical region and South-East Asia has high species richness[1-4]. This genus consists of many species, which are widely used as a source of edible fruits, timber, resin and various other natural products[5]. From India, 43 species and 5 varieties of *Garcinia* are reported and 37 species and 4 varieties of *Garcinia* occur naturally in the wild[6]. Seven species and 2 varieties are endemic to Western Ghats of India. Also, 15 species of *Garcinia* were reported from North-eastern states of India[7]. Fruits of many *Garcinia* species are edible and serve as a substitute for tamarinds in curries. *G. talbotii* is an unexplored species as it is restricted to the geographical place of their availability and not explored properly for utility and scientific prominence[8]. *G. talbotii* is a dioecious medium sized tree distributed in the semi evergreen to evergreen forests of Western Ghats[9]. It is locally called as Undal, Tavir, Phansada, Chivar[10]. Like other species from this genus, *G. talbotii* is also an economic plant and produces fruit during rainy season. It produces fruit with sweet pulp and abundant yellow latex. Fruits of *G. talbotii* are globose berry, greenish, yellow colour turns to greenish yellow on maturity[9]. *Garcinia talbotii* is used as dye stuff, timber and food like tamarind in curries[11]. Each fruit weight is 46.3±9.03 g, 4.3±0.3 cm length, 4.1±0.3 cm breadth and contains 1-3 seeds[9]. *Garcinia talbotii* has now become rare in their natural habitat because of high predation and over exploitation for its use as a firewood[9]. *Garcinia talbotii* is often misidentified as *G. spicata* in the regional Floras of Northern Western Ghats[12].

Characterization of the medicinal plants is the first step in development of pharmaceutical products. In most of the Indian Floras, *G. talbotii* has been misidentified as *G. spicata*, which is not naturally occurring in India. Palkar *et al.*[12] delimited *G. talbotii* and *G. spicata* with unambiguous morphological and anatomical characters for authentic identification. Bansude *et al.*[10] studied the seed source variation in seed traits and germination in *G. talbotii*. Sabu *et al.*[9] reported seeds longevity of *G. talbotti*. A relationship between the seed viability with respect to different moisture content and storage temperature were analysed by the authors.

Limited research work has been carried out for *G. talbotii* and scanty reports are available about phytochemical investigation and assessment of antioxidant activities of fruit rinds of *G. talbotii*. Joshi *et al.*[13] isolated two biflavones namely talbotaflavone (Ia) and morelloflavone (IIa) from the roots of *G. talboti*. Guru *et al.*[14] reported that *G. talbotii* extracts possessed moderate to considerable leishmanicidal activity.

Oxidative stress results from an imbalance between the formation and neutralization of pro-oxidants. Free radicals initiate oxidative stress in body and they seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes lead to the occurrence of various diseases such as cardiovascular diseases, inflammatory diseases, atherosclerosis and cancer[15-17]. Diverse natural mechanisms to protect cellular molecules against damages induced by reactive oxygen species (ROS) is insufficient to overcome the continuous oxidative stress contributed by environmental factors including UV light, pollutants and non-equilibrated food. Consumption of antioxidants helps in balancing the levels of ROS in the human body[18].

In continuation to our earlier research work for bio-prospecting of Indian *Garcinia* species, the present investigation was carried out for assessment of total phenolics content (TPC) and *in vitro* antioxidant assay of fruit rind extracts of an unexplored *Garcinia* species *G. talbotii.* To the best of information available to us from the literature, this is the first report of TPC and antioxidant activities of *G. talbotii* fruit rind extracts.

MATERIALS AND METHODS

All experiments were carried out in laboratories of Indian Council of Agricultural Research-Directorate of Medicinal and Aromatic Plants Research, 387 310 Boriavi, Anand, Gujarat, India during July 2018-March 2019.

Collection and identification of plant materials: Mature fresh fruits of *G. talbotii* were collected from Tamhini, Pune, Maharashtra, India (latitude: N 18°23'52.36", longitude: E 73°23'56.18"). Taxonomical identification of collected fruits was confirmed at Department of Botany, The MS University of Baroda, Vadodara, Gujarat, India. The fruits were sorted and infected and mechanically damaged fruits were discarded. From fruits pulp, seeds were removed manually and fruit rind was collected. Fruit rind was dried under shade for a week and it was followed by oven drying (50°C, 12 h). The dried fruit rind pieces were then pulverised to a coarse powder and stored in airtight container free from moisture.

Chemical and solvents: Analytical grade organic solvents and chemicals were used throughout the analysis. Chloroform, ethyl acetate, hexane and methanol were purchased from Sisco Research Laboratory (SRL), Mumbai, India. Reagents and standards such as Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical, 2-2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and Trolox were purchased from Sigma-Aldrich, Mumbai, India. Analytical grade potassium ferricyanides, trichloroacetic acid and ferric chloride from SRL, Mumbai were used.

Preparation of extracts: The extracts were prepared by refluxing of dried fruit rinds powder with solvents of varying polarity (non-polar: Hexane, chloroform, medium polar: Ethyl acetate, polar: Methanol, water and methanol-water mixture, 2:8) on a water bath, individually[19]. Sample and solvent ratio was 1:20 and extraction time was 8h. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator (Heizbad Hei-VAP, Heidolph, Schwabach, Germany, temperature = 40±5°C) to yield dark gummy residue. All the extracts were stored in glass vials and away from direct sunlight.

Determination of total phenolic content: The dried extracts were dissolved in distilled water (1 mg mL^{-1}) and were sonicated before use. TPC in extracts was determined by a colorimetric method using Folin-Ciocalteu reagent[20]. Briefly, extract solution (0.5 mL), Folin-Ciocalteu reagent (0.5 mL) and distilled water (7.5 mL) were mixed in a test tube and further mixed vigorously by using a Vortex mixer. Test tubes were kept at room temperature for 10 min and thereafter sodium carbonate (20%, 1.5 mL) was added to test tube mixture. The resultant mixture was allowed to incubate in a water bath at 40°C for 20 min. The intensity of the blue colour developed was measured by recording the absorbance at 755 nm using a UV-visible spectrophotometer (UV-5704SS, Electronic Corporation of India). A blank sample with no added extract was also prepared using distilled water. For quantification of TPC in the extracts, a standard calibration curve was prepared using gallic acid. TPC of the extract samples was expressed as gallic acid equivalent (GAE) milligram per gram of the extract and was calculated using the following linear equation[20] on the calibration curve:

$$A = 0.0044\ C + 0.0028\ (R^2 = 0.9997)$$

where, A is the absorbance and C is the concentration (mg GAE g^{-1}).

Determination of DPPH free radical scavenging activity: Free radical scavenging activity of *G. talbotii* extracts was evaluated using DPPH free radical scavenging assay. Different concentrations of the extracts were taken in test tubes. The total volume was adjusted to 8.5 mL by the addition of methanol. Then 5.0 mL of methanolic solution of DPPH (0.1 mM) was added to these tubes and mixed thoroughly using a Vortex mixer. Thereafter, tubes were kept at room temperature for 20 min. The blank was prepared in the same way as described above but without the extract and methanol was used for the baseline correction. Changes in the absorbance of the extract samples were measured at 517 nm using UV-visible spectrophotometer.

Radical scavenging activity (RSA) was expressed as the inhibition percentage and was calculated using the following formula[19]:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Determination of ABTS free radical scavenging activity: Free radical scavenging activity was determined by ABTS radical cation decolorization assay[21]. ABTS was dissolved in water to get a 7 mM concentration and radical cation ($ABTS^{+}$) was produced by reacting ABTS solution with 2.45 mM potassium persulfate at room temperature in dark (12-16 h) before use. For assay, $ABTS^{\bullet+}$ solution was diluted with water to an absorbance value of 0.700±0.02 at 734 nm. After addition of 3.0 mL of diluted $ABTS^{\bullet+}$ solution to 100 µL of extract solutions, absorbance was recorded[19] after 6 min:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

where, absorbance of blank is the absorbance of $ABTS^{\bullet+}$ radical+methanol and absorbance of sample is the absorbance of $ABTS^{\bullet+}$ radical +extract/standard antioxidant.

Determination of reducing power: The total reducing power of standard antioxidants and extracts were determined as described by Oyaizu[22]. Different concentrations of extracts

were mixed with distilled water (2.5 mL), phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The resulting mixture was incubated at 50°C for 20 min in a water bath. After cooling, trichloroacetic acid (2.5 mL, 10%) was added to the mixture. The upper layer of solution (2.5 mL) was taken and mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%). The absorbance was recorded using a UV-visible spectrophotometer at 700 nm. The increasing absorbance value was interpreted as increased reducing activity[23].

Calculation of IC_{50} concentration: The extract concentration corresponding to 50% inhibition (IC_{50}) was calculated from the curve[19]. Trolox and gallic acid were used as standards. Each sample was assayed in triplicate for each concentration.

Statistical analysis: All experiments were performed in triplicate and the results were expressed as Mean±SD for three replications of each sample. Linear regression was carried out using Microsoft Excel 2010 to establish a correlation of antioxidant activities and extract concentration.

RESULTS

Extract yield and TPC affected solvent polarity: The extract yield and TPC of *G. talbotii* fruit rinds are described in Table 1. Extract yield (%) varied widely. It was in the following sequence: Water>methanol>hydro-alcohol>ethyl acetate>hexane>chloroform.

In *Garcinia*, xanthones are reported to be the major phenolic compounds. Xanthones show a wide range of biological activities such as antimicrobial, antiviral, anti-inflammatory, anti-cancer and antioxidant activities. TPC (%) also showed wide variation. It was maximum for hexane extract followed by ethyl acetate and chloroform extracts. Its value was minimum for water extract.

Antioxidant activity: The free radical scavenging activity of *G. talbotii* extracts were evaluated by DPPH method. Trolox and gallic acid were used as control (Table 1). The percentage of DPPH free radical scavenged was plotted against the concentration of extracts of *G. talbotii*. Concentration (μg mL^{-1}) corresponding to 50% inhibition (IC_{50}) was calculated. Hexane extract exhibited high antioxidant activity and it was comparable to the reference antioxidants selected in the present investigation. For reference antioxidants trolox and gallic acid IC_{50} (μg mL^{-1}) was 6.22±0.11 and 3.45±0.09, respectively. For extracts of *G. talbotii*, IC_{50} (μg mL^{-1}) value varied in the following sequence: Water>Hydro-alcohol>methanol>ethyl acetate>chloroform>hexane.

In ABTS assay of *G. talbotii* extracts, hexane extract had lowest IC_{50} (μg mL^{-1}) value followed by chloroform, ethyl acetate and methanol extracts. Water and hydro-alcohol extracts had comparatively high IC_{50} values. Trolox and gallic acid had IC_{50} value 6.96±0.08 and 4.33±0.12 μg mL^{-1}, respectively.

For measuring the reducing power of extract, the capability of the extract to convert ferric ion (Fe^{+3}) into ferrous ion (Fe^{+2}) was measured. In this assay, yellow colour of the solution changes to blue colour as the ferric ion (red) is converted into ferrous ion (blue). Absorbance was measured at 700 nm. Higher absorbance indicated that the extract had more antioxidant property. Here only hexane extract showed low IC_{50} value. It was followed by chloroform and ethyl acetate extracts. Methanol, water and hydro-alcohol (83.85±0.23) extracts had comparatively high IC_{50} values. Trolox and gallic acid had IC_{50} value 5.03±0.13 and 4.62±0.11 μg mL^{-1}, respectively.

Table 1: Extract yield (%), total phenolic content (TPC, mg g^{-1} GAE) and IC_{50} (μg mL^{-1}) values of *G. talbotii* fruit rind extracts (n = 3)

			IC_{50} (μg mL^{-1})		
Solvent	Extract yield (mg/100 mg)	TPC (mg g^{-1} GAE)	DPPH	ABTS	Reducing power
Hexane	08.10	7.09±0.16	04.65±0.20	08.97±0.24	07.42±0.19
Chloroform	05.94	3.94±0.09	08.95±0.15	11.25±0.29	09.72±0.13
Ethyl acetate	17.11	4.44±0.08	12.85±0.17	17.65±0.14	15.48±0.16
Methanol	30.88	2.81±0.10	36.99±0.12	40.59±0.18	42.52±0.15
Water	31.20	0.94±0.02	78.96±0.13	94.35±0.21	41.76±0.11
Hydro-alcohol	24.90	1.99±0.04	75.99±0.26	98.75±0.25	83.85±0.23
Trolox	-	-	06.22±0.11	06.96±0.08	05.03±0.13
Gallic acid	-	-	03.45±0.09	04.33±0.12	04.62±0.11

DPPH: 2, 2-diphenyl-1-picrylhydrazyl free radical, ABTS: 2-2' -Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

DISCUSSION

Extraction yield was found to be dependent on the polarity of solvent. Variation in the yields of various extract is attributed to compounds present in fruit rinds. Such differences have been reported in the literature[24,25]. In *Garcinia* species, xanthones are the major class of compounds followed by benzophenones and biflavonoids[26]. See *et al.*[27] reported that among solvents of various polarities (hexane, chloroform, ethyl acetate and methanol), ethyl acetate was most efficient in extracting the phenolic compounds from *G. benthamiana*, followed by methanol. In the present study, higher polarity solvents particularly water showed much lower ability to extract phenolic compounds compared to lower polarity solvent. Results of the present study are also in agreement of the results of See *et al.*[27]. TPC in *G. talbotii* fruit rinds extracts ranged from 0.94±0.02 to 7.09±0.16 mg g^{-1} GAE (Table 1). Earlier, Surinut *et al.*[28] reported TPC in 12 Thai fruits namely *Vitis vinifera* (grape), *Morus alba* (mulberry), *Mangifera indica* (mango), *Averrhoa carambola* (carambola), *Psidium guajava* (guava), *Litchi chinensis* (lichee), *Garcinia mangostana* (mangosteen), *Citrus aurantium* (orange), *Citrus maxima* (pomelo), *Carica papaya* (papaya), *Eugenia javanica* (rose apple) and *Artocarpus heterophylla* (jackfruit). TPC value in these fruits ranged from 6.0±0.21 to 236.4±16.58 mg GAE g^{-1} wet weight. Parthsarathy and Nandakishore[29] reported TPC (%) in fruit extracts of eight *Garcinia* species. TPC values for fruit extracts were as follows: *G. gummi-gutta* (3.26), *G. indica* (5.01), *G. mangostana* (2.33), *G. xanthochymus* (4.43), *G. subelliptica* (3.14), *G. kydia* (4.32), *G. lanceaefolia* (3.03) and *G. pedunculata* (2.43). Our result of TPC was also in agreement with Parthsarathy and Nandakishore[29].

Because different antioxidant compounds have different mechanisms of action, several methods have been used to assess the antioxidant efficacy of extracts. In the present study, various extract showed varying degrees of antioxidant activity in different assay. Hexane extract of *G. talbotii* fruit rinds exhibited high antioxidant activities measured in terms of IC_{50} (μg mL^{-1}) values for DPPH free radical scavenging activity (4.65±0.20), ABTS free radical scavenging activity (8.97±0.24) and reducing power assay (7.42±0.19). Similarly, IC_{50} (μg mL^{-1}) values of DPPH assay for fruit extracts of eight *Garcinia* species reported by Parthsarathy and Nandakishore[29] ranged from 35.75-48.12 μg mL^{-1}. It was minimum for *G. xanthochymus* followed *G. gummi-gutta*, *G. mangostana*, *G. kydia, G. indica, G. lanceaefolia*, *G. pedunculata* and *G. subelliptica.* Aravind *et al.*[30] reported antioxidant activities of methanolic extract of leaves of 9 *Garcinia* species from the Western Ghats. DPPH radical scavenging activity of *G. talbotii* was higher (IC_{50}: 2.8±0.6 μg mL^{-1}) compared to standard compound ascorbic acid (IC_{50}: 3.2±0.5 μg mL^{-1}), while *G. pushpangadaniana* showed the highest superoxide radical scavenging activity (IC_{50}:16.75±0.99 μg mL^{-1}) and reducing activity. Patil and Potdar[31] reported TPC, total flavonoids and DPPH radical scavenging activity of *G. talbotii* leaves extract. Solvent such as acetone, alcohol, ethanol, methanol and distilled water were used for extract preparation. TPC was 2.609±0.130% and the total flavonoid content was 5.043±0.252 mg 100 g^{-1} in the methanolic extract and DPPH radical scavenging activity was 73.51±3.68%. Antioxidant activities of *G. talbotii* fruit rinds from the present study as described above was better than antioxidant activities of fruit extracts of eight *Garcinia* species reported by Parthsarathy and Nandakishore[29]. Also, the results showed that the degree of antioxidant activity of the extract is in accordance with the amount of phenolics present in that extract.

CONCLUSION

TPC of natural products and its related antioxidant activity have health protective effects. *G. talbotii* has not yet explored for its chemical constituents or bioactivities. In the present study effect of polarity of solvents on extraction yield, concentration of TPC and antioxidant activities of *G. talbotii* fruit rinds extracts was examined. It was found that non polar solvents were more effective in extracting phenolic compounds compared to more polar solvents. *In vitro* antioxidant assay also established that hexane extract of *G. talbotii* had high antioxidant activity which was comparable to standard antioxidants trolox and gallic acid in DPPH, ABTS free radical scavenging and reducing power assay. Other extracts exhibited low to moderate antioxidant activity. Therefore, hexane could be selected as extraction solvent for *G. talbotii*. TPC of *G. talbotii* fruit rinds could be considered as an intrinsic characteristics of this *Garcinia* species for comparison among other species from the same genus. Also, the high content of phenolics and strong antioxidant activity of hexane extract of *G. talbotii* indicated that this extract may be used for imparting health benefits in functional food products.

SIGNIFICANCE STATEMENT

For the first time, TPC and antioxidant activity of fruit rinds of *G. talbotii* extracted with different solvents was studied.

Also, antioxidant activity of each extract was determined by using three complimentary assay procedures because various test systems differ among themselves and result of a single method could provide only a limited assessment of antioxidant properties of *G. talbotii* fruit rind extract. Results of our study established that the amount of phenolics and the antioxidants in *G. talbotii* fruit rind extracts is relatively high. Furthermore, hexane extract of *G. talbotii* showed comparatively higher antioxidant activity, therefore, this extract could be used as an antioxidant/preservative ingredient in the food and pharmaceutical products provided that resulting organoleptic effects are acceptable. However, more research work is needed before use of present study could be proposed with confidence.

ACKNOWLEDGMENTS

The present research work was funded by Indian Council of Agricultural Research (ICAR), New Delhi in the form of ICAR-Network Project on High Value Compounds/Phytochemicals. One of the authors Azazahemad A. Kureshi (Senior Research Fellow, UGC-MANF) gratefully acknowledges the University Grants Commission (UGC) and Ministry of Minority Affairs, New Delhi, Govt. of India for providing financial assistance (Award No: 2015-16/MANF-2015-17-GUJ-49309).

Authors are thankful to the Research Journal of Medicinal Plants for publishing this article FREE of cost and to Karim Foundation for bearing the cost of article production, hosting as well as liaison with abstracting and indexing services and customer services.

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