

In-Vitro Antioxidant Activities of Leaves and Fruit Rind Extracts of *Garcinia gummi-gutta*(*G.cambogia*)

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Abstract

Objective: The present study focused on the assessment of antioxidant activities, total phenolic content (TPC), and total flavonoid content (TFC) of *Garcinia gummi-gutta* leaves (GTL) and fruit rind extracts (GTF).

Materials & Methods: The leaves and fruit rind of the plant *Garcinia gummi-gutta* were assembled and authenticated. They were washed with distilled water, shade dried and powdered. The powdered drug was extracted with different solvents such as petroleum ether, chloroform, ethyl acetate, acetone and ethanol based on their polarity. Of the different extracts of *G.gummi-gutta*, the results showed that ethylacetate of leaves (IC50) and ethanol extract of fruit rind extract(IC50) has shown better in vitro antioxidant inhibitory activity using DPPH, ABTS and Nitric oxide radical scavenging assays when compared with Ascorbic acid (IC50) as standard.

Results: The studies performed to evaluate the antioxidant activity using DPPH, ABTS and Nitric oxide scavenging activity have revealed significant IC50 values indicating the ability of ethyl acetate leaf extract and ethanol extract of fruit rind of *Garcinia gummi-gutta* to inhibit oxidative stress.

Conclusion: This study illuminates the encouraging health advantages offered by these botanical extracts, indicating their capacity to serve as organic reservoir of antioxidant activity

Key words: Antioxidant, ABTS, DPPH, and Nitric oxide, *Garcinia gummi-gutta* and IC50 values.

INTRODUCTION

Free radical stand as the foremost instigators of numerous disorders. During typical metabolism, the body generates Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). These entities are renowned for their dual role, function of crucial component substances can lead to the functioning both as detrimental and beneficial agents. Excessive production of these substances can lead to the oxidation of crucial component within the cell, resulting in potential damage. Among the biological macromolecules susceptible to interaction with ROS are fats, proteins, polypeptides carbohydrates.^[1]

Oxidative stress occurs when oxygen-derived free radicals surpass the cell's ability to neutralize them. The excessive accumulation of these radicals leads to harm inflicted upon biological components and serves as a pivotal factor in the onset of diverse ailments, including diabetes, cancer, cardiovascular disease, inflammation, as well as hepatic, renal, and neurodegenerative conditions. The presence of antioxidant defense mechanisms in healthy organisms seeks to maintain a delicate equilibrium with the generation of these reactive species. Antioxidants play a crucial role in halting reactions mediated by free radicals, actively seeking them out and thus safeguarding individuals from their harmful consequences. Herbs serve as essential therapeutic aids in reducing human diseases. It is widely acknowledged that plants harbor abundant antioxidants, countering cellular damage resulting from oxidative stress.^[6,9]

Herbal drugs are widely prescribed even when their biologically active compounds are unknown due to minimal side effects in clinical experience, perceived effectiveness and relatively low cost. Hence plants are

utilised to develop drugs that always effectively used in Indian traditional system like Ayurveda for treatment of diabetes mellitus, hypertension, Alzheimers etc. The plant *Garcinia gummi-gutta* also known as Penampuli or Kudampuli belongs to the family Clusiaceae. The plant possess very good medicinal value and marketed as a hypolipidemic agent [9].

MATERIALS AND METHODS

Plant parts of Leaves and Fruit rind of *Garcinia gummi-gutta* were collected in the month April- May. The leaves were washed with distilled water, blotted with cotton cloth to remove water and dried under shade dry. The fruit rind were cut into pieces, dried under sunlight and properly preserved. The plant *Garcinia gummi-gutta* was identified by Taxonomist, Department of Botany, Kariyavattom, Thiruvananthapuram. Herbarium of voucher specimen were prepared and deposited in the University College Kariyavattom (NO.KUBH 10884) for future reference.

Preparation of leaf extract

The leaves of the plant were collected, shade dried, and made into coarse powder using a grinder and extracted using soxhlet apparatus for 48hours using suitable solvents like petroleum ether, chloroform, ethyl acetate, acetone and ethanol based on their polarity. The extracts were dried using rotary evaporator^[6].

Preparation of fruit rind extract

The dried fruit rind of the plant were extracted using soxhlet apparatus for 48hours using suitable solvents like petroleum ether, chloroform, ethyl acetate, acetone and ethanol based on their polarity. The extracts were dried using rotary evaporator.

Evaluation of in vitro antioxidant activity of extracts of *G.gummi-gutta*

Estimation of Total Phenolic Content (TPC):

The entire phenolic content of different extracts of GL and GF was examined with Folin–Ciocalteu technique according to method of Singleton and Rossi, 1965 with a minor alteration. 1 ml herb extract was mixed to 5 ml Folin–Ciocalteu's testing agent. After 5 minutes, 1 ml 7.5 %w/v Na₂CO₃ was supplemented, which was held at normal temperature for 30 min and absorbance was determined at 765 nm. Varied Gallic acid concentrations in ethanol were availed to make a standard curve. The estimation of the sample was done in triplicate. The TPC of the extract was stated as gallic acid equivalents (GAE) in mg/100ml.

Estimation of Total Flavonoid Content (TFC):

TFC in both plant extracts was evaluated by spectrophotometry, explained by Quittier et al 21. GL and GF extracts in 1 mg/ml were mixed with 1ml 2% AlCl₃ in ethyl alcohol and let to remain for 60 min at room temperature. Absorbance was computed by spectroscopy at 415 nm. For each test, samples were made in triplet, and the mean value of absorbance was attained. The same process was replicated for standard rutin solution, and calibration graph was made. Based on measured absorbance, flavonoid matter was interpreted from the graph. TFC was stated as mg of RU/g of extract^[10]

DPPH scavenging radical activity

The DPPH radical scavenging activity was assessed using the Blois method. Essentially, this technique involves a decolorization test, measuring the reduction in absorbance upon introducing an antioxidant to a DPPH sample dissolved in ethanol. The DPPH assay is considered reliable and straightforward for gauging antioxidant scavenging activity, given the stability of the radical molecule, which does not form under different radical conditions. Ascorbic acid was employed as a reference standard, with a stock solution prepared by dissolving 1mg/1000µl in distilled water. The ethyl acetate extract of GL AND GF or the standard solution at varying concentrations was mixed with 1ml of 0.3mM DPPH ethanolic solution.^[15]

Thorough mixing of the blend was followed by incubation at 20-25°C in a dark environment for 30 minutes, after which the absorbance was measured at 517 nm. A blank consisting of 3 ml ethanol was used, and a control comprised of 1 ml of 0.3 mM DPPH ethanolic solution without extract or standard was established. The experimental process was conducted in triplicate. Scavenging activity was expressed as the percentage of inhibition, calculated using the following formula

$$\% \text{Inhibition} = \frac{Ac - At}{Ac} \times 100$$

Where Ac = absorbance of control, At = absorbance of extract or standard

ABTS Radical Scavenging Assay

The ABTS radical scavenging assay, based on the methodology outlined by Re et al. is designed to evaluate the ability of test samples to counteract the radical cation ABTS⁺. This assay involves a chemical reaction between 2,2-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)

(ABTS) and potassium persulfate, leading to the formation of the ABTS radical cation. The process includes the oxidation of ABTS by potassium persulfate, resulting in the generation of a free radical that is subsequently reduced by hydrogen-donating radical scavengers present in the samples being tested. The capacity of each sample extract to quench the ABTS radical cation is measured to determine its antioxidant potential.^[20]

To initiate the reaction, an aqueous solution containing ABTS and potassium persulfate in a 1:1 ratio is prepared and left in the dark at a temperature of 20-25°C for a duration of 12-16 hours. This incubation period leads to the production of the ABTS radical cation. Subsequently, different concentrations of the sample extract are added to a 0.3 ml solution of ABTS, and the final volume is adjusted to 1 ml using ethanol. After an initial mixing period of 30 minutes, the absorbance is recorded. A corresponding blank solution is prepared, containing all reagents except the sample extract, to serve as a baseline reference.^[15]

This entire procedure is repeated in triplicate to ensure accuracy and reproducibility. The antioxidant activity of the sample is then compared to that of Ascorbic acid, which is utilized as a standard reference. The assessment of percentage inhibition is carried out by measuring the absorbance at a wavelength of 745 nm, and this inhibition is calculated using the following equation:

$$\% \text{Inhibition} = \frac{Ac - At}{Ac} \times 100$$

Where, Ac = absorbance of control, At = absorbance of extract or standard

Nitric Oxide Scavenging Assay

The nitric oxide scavenging assay was conducted following the procedure outlined by Madan et al. 23. Sodium nitroprusside, when dissolved in water at pH 7.2, spontaneously generates nitric oxide. This nitric oxide reacts with oxygen to produce nitrite ions, which can be quantified using the Griess reaction. The presence of nitric oxide scavengers leads to a reduction in nitrite ion production, as they compete with oxygen for nitric oxide.^[19]

Test samples of varying concentrations (10-100µL/ml) were mixed with sodium nitroprusside (10mM) dissolved in PBS (pH 7.2). The mixture was allowed to incubate for 5 hours at 30°C. Subsequently, 0.5ml of the solution was combined with Griess reagent (created by mixing equal amounts of 1% sulphanilamide in 2% H₃PO₄ and 0.1% NEDA in water). After thorough stirring, the mixture was left to undergo diazotization for 5 minutes. Then, 1ml of NEDA was added and the solution was kept in subdued light for 30 minutes. The optical density of the resulting rose-colored chromophore was measured at 540nm. A control sample was prepared with the same volume but without any test sample or standard, and Ascorbic acid was employed as the reference standard. The experiment was repeated three times, and the percentage of inhibition was calculated. The radical scavenging activity was determined using the formula provided below:

$$\% \text{Inhibition} = \frac{Ac - At}{Ac} \times 100$$

Where, Ac = absorbance of control, At = absorbance of extract or standard

RESULTS

Quantitative Determination of Phenolic Contents

The total phenolic content within the ethyl acetate extracts of GL and GT was quantified using gallic acid equivalents as the reference. The quantification of phenolic compounds ranged from 7.37 to 60.08 mg GAE/g across different GL extracts and from 7.96 to 61.08 mg GAE/g for various GF extracts. Notably, exhibited the highest phenolic content for leaf and fruit rind extracts of *Garcinia gummi-gutta*/cambogia among the extraction solvents-ethanol, acetone, chloroform, and pet ether (Table1). Polyphenolic compounds present in herbal extracts interact with a redox agent, resulting in the formation of a blue chromophore composed of a phospho-tungstic-phosphomolybdenum complex. This chromophore can be quantified using a UV-visible spectrometer, with the intensity of absorption correlating to the extent of phenolic complexes present.

Table-1 Total Phenolic content of *Garcinia gummi-gutta* leaves and fruit rind extract

Plant	Extract	TPC (μg of Gallic acid equivalent /mg)
GTL	GLPE	7.37 \pm 0.05
	GLCH	8.42 \pm 0.26
	GLEA	10.54 \pm 0.09
	GLAC	60.08 \pm 0.12
	GLET	20.72 \pm 0.07
GTF	GFPE	7.96 \pm 0.28
	GFCH	8.87 \pm 0.18
	GFEA	11.36 \pm 0.04
	GFAC	61.06 \pm 0.15
	GFET	20.44 \pm 0.09

Data are mean SEM (n=3), GLPE: *G. gummigutta* leaves pet ether extract; GLCF: *G. gummigutta* leaves chloroform extract; GLEA: *G. gummigutta* leaves ethyl acetate extract; GLAC: *G. gummigutta* leaves acetone extract; GLET: *G. gummigutta* leaves ethanol extract; GFPE: *G. gummigutta* fruits pet ether extract; GFCH: *G. gummigutta* fruits chloroform extract; GFEA: *G. gummigutta* fruits ethyl acetate extract; GFAC: *G. gummigutta* fruits acetone extract; GFET: *G. gummigutta* fruits ethanol extract.

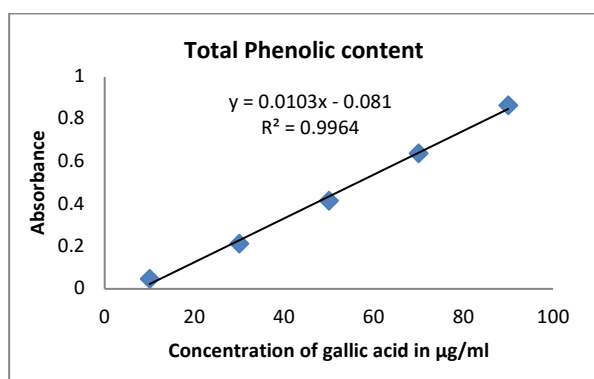


Fig-1 Total Phenolic content of *Garcinia gummi-gutta* leaves and fruit rind extract

Quantitative Determination of Flavanoid Content:

The content of flavonoids within GL and GT was determined using the AlCl_3 method. The quantity of flavonoids present in each extract was calculated and expressed in terms of rutin equivalent per gram of extract (RU/g). This quantitative assessment revealed a range of

flavonoid content from 18.33 to 96.66 RU/g for various GF extracts and from 16.22 to 106.67 RU/g for different GF extracts, as outlined in Table 2.

Table-2 Total Flavanoid content of *Garcinia gummi-gutta* leaves and fruit rind extract

Plant	Extract	TFC (μg of Rutin equivalent/mg)
GTL	GTLPE	18.33 \pm 0.13
	GTLCH	19.74 \pm 0.07
	GTLEA	96.66 \pm 0.39
	GTLAC	27.64 \pm 0.05
	GTLET	30.27 \pm 0.16
GTF	GTFPE	16.22 \pm 0.75
	GTFCH	18.74 \pm 0.18
	GTFEA	106.67 \pm 0.42
	GTFAC	25.24 \pm 0.07
	GTFET	30.82 \pm 0.63

Data are mean SEM (n=3), GLPE: *G. gummigutta* leaves pet ether extract; GLCF: *G. gummigutta* leaves chloroform extract; GLEA: *G. gummigutta* leaves ethyl acetate extract; GLAC: *G. gummigutta* leaves acetone extract; GLET: *G. gummigutta* leaves ethanol extract; GFPE: *G. gummigutta* fruits pet ether extract; GFCH: *G. gummigutta* fruits chloroform extract; GFEA: *G. gummigutta* fruits ethyl acetate extract; GFAC: *G. gummigutta* fruits acetone extract; GFET: *G. gummigutta* fruits ethanol extract

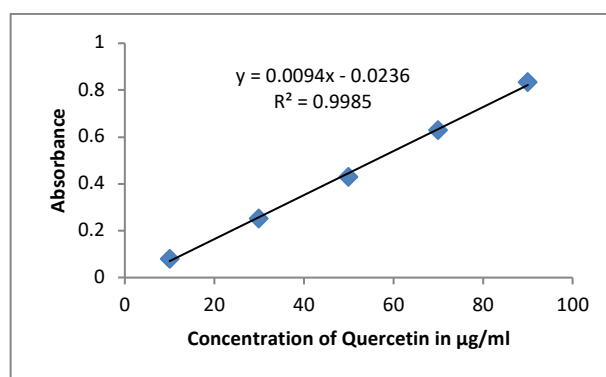


Fig-2 Total Flavanoid content of *Garcinia gummi-gutta* leaves and fruit rind extract

Determination of *In Vitro* Antioxidant Activity Assays

Anti-oxidants exerts very predominant component where by retained the capability for assured physique pertaining desecration originated alongside oxidative radicle induced oxidant stressfulness. The anti-oxidants prospective for *Garcinia gummi gutta* ensue dissected along research for novel biologically activated components pertaining mineral deposits. Herein, the anti-oxidative venture undergo appraised by innumerable approaches circumscribing nitric oxide, DPPH, ABTS along with radical rummaging.

DPPH scavenging radical activity

DPPH free scavenging radical is an accepted mechanism for screening the antioxidant activity of plant extracts. The DPPH scavenging radical activity of fruits along with leaflet extraction within various solvents were subjected and results are shown in Fig 1. All the extracts showed different DPPH radical scavenging potential; but in a dose dependent manner. The percentage DPPH radical scavenging activity of GLPE, GLCF, GLEA, GLAC and

GLET at concentration 100 µg/ml was found to be 73.00±2.52 %, 80.67 ± 2.81 %, 86.33 ±1.76%, 80.00± 4.58% and 82.00± 1.73% respectively. Under similar conditions, it is relevant to point out that among leaf extracts, ethyl acetate along with ethanol exhibited highest antioxidant levels with low IC₅₀ values 16.45±2.10 µg/ml and 32.76±3.04 µg/ml respectively. Smallest IC₅₀, best may be the antioxidant activity for extraction. IC₅₀ value of ascorbic acid was 11.10±0.29 µg/ml.

One way ANOVA test indicated that IC₅₀ of all the extract were significantly different (p<0.001) from IC₅₀ of Ascorbic acid, except GLEA. Though IC₅₀ values for whole extraction were higher than standard, results indicated that extracts may exhibited capability for scavenging free radicals as things go manifestations fore phenolic components. The IC₅₀ values proclaimed the radical rummaging capability ensue in the order: AA>GLEA>GLET>GLAC>GLCF>GLPE (Figure 1).

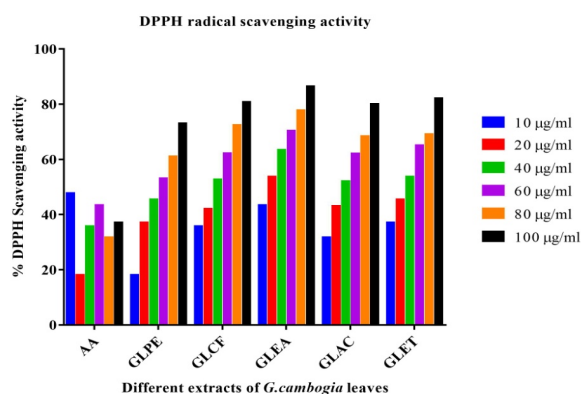


Figure. 3 The percentage inhibition on DPPH radical by different extracts of *G. gummi-gutta*(*G.cambogia*) leaves.

The average of % inhibition is presented. Statistics ensue mean±SEM (n=3). AA: Ascorbic acid; GLPE: *G. cambogia* leaves pet ether extract; GLCF: *G. cambogia* leaves chloroform extract; GLEA: *G. cambogia* leaves ethyl acetate extract; GLAC: *G. cambogia* leaves acetone extract; GLET: *G. cambogia* leaves ethanol extract.

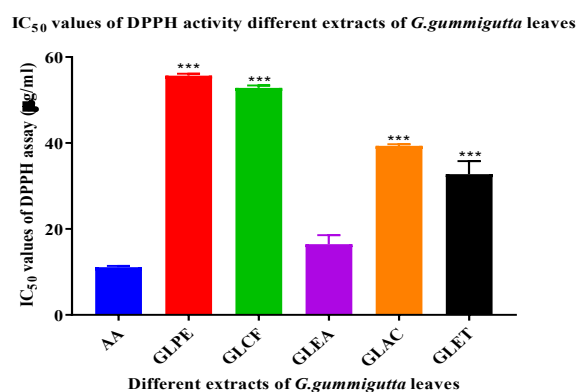


Figure 4: IC 50 values of difeerent extractsa of *G.gummi-gutta* leaves

The values are mean of three replicates ± SEM. Statistics ensue surveyed by ANOVA one-way accompanied fore Dunnett's multiplex method experimentation. * represents p<00.05, ** represents p<00.01 when compared to Ascorbic acid. AA: Ascorbic acid; GLPE: *G. gummi-gutta* leaves pet ether extract; GLCF: *G. gummi-gutta* leaves chloroform extract; GLEA: *G. gummi-gutta* leaves ethyl acetate extract; GLAC: *G. cambogia* leaves acetone extract; GLET: *G. gummi-gutta* leaves ethanol extract.

In the case of fruit extract, highest antioxidant level was exhibited by ethanol extract (IC₅₀ 47.20±1.25 µg/ml) followed by ethyl acetate (67.35±2.31 µg/ml) and acetone (71.49±0.64 µg/ml). The pet ether extract of fruit showed the percentage inhibition of 8.00±1.5 to 46.33±0.88 % at 10-100 µ/ml concentration whose IC₅₀value was 107.45±2.46 µg/ml. The chloroform extract of fruit showed its IC₅₀ value as 89.58±2.40 µg/ml.

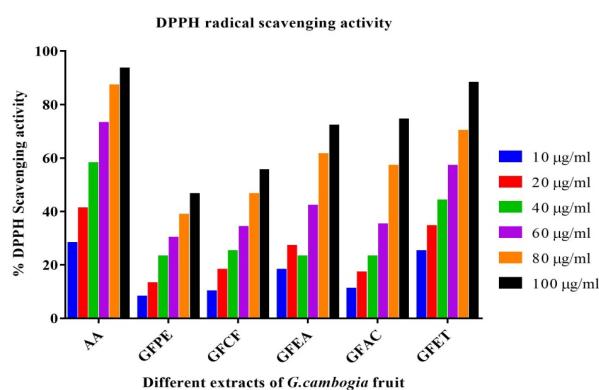


Figure 5 The percentage inhibition on DPPH radical by different extracts of *G. gummigutta* fruits.

The average of % inhibition is presented. Statistics ensue mean±SEM (n=3). AA: Ascorbic acid; GFPE: *G. gummigutta* fruits pet ether extract; GFCE: *G. gummigutta* fruits chloroform extract; GFEA: *G. gummigutta* fruits ethyl acetate extract; GFAC: *G.gummigutta* fruits acetone extract; GFET: *G. gummigutta* fruits ethanol extract.

The IC₅₀ value of fruit extracts were significantly different from ascorbic acid; AA vs GFPE (p<0.001), AA vs GFCE (p<0.001), AA vs GFEA (p<0.001), AA vs GFAC (p<0.001), AA vs GFET (p<0.01). The IC₅₀ values showed the radical scavenging ability was in the order: AA>GFET>GFEA>GFAC>GFCH>GFPE.

IC₅₀ value of DPPH activity of different extracts of *G.gummigutta* fruit

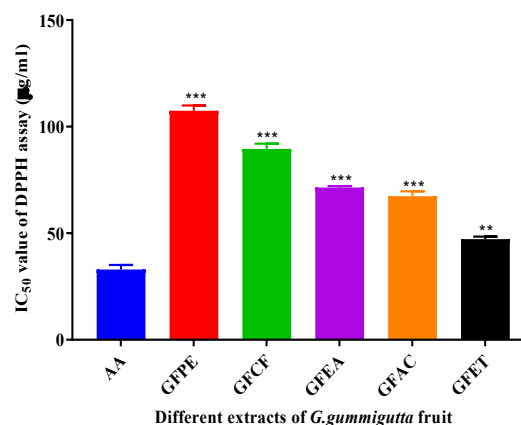


Figure 6 IC50 values of different extracts of *G.gummi-gutta* Fruits.

The values are mean of three replicates ± SEM. Statistics ensue surveyed by one-way ANOVA accompanied for Dunnett's method test. * represents p<00.05, ** represents p<00.01, *** represents p<00.001 when compared to Ascorbic acid. AA: Ascorbic acid; GFPE: *G. gummi-gutta* fruits pet ether extract; GFCE: *G. gummi-gutta* fruits chloroform extract; GFEA: *G. gummi-gutta* fruits ethyl acetate extract; GFAC: *G.gummi-gutta* fruits acetone extract; GFET: *G. gummi-gutta* fruits ethanol extract.

ABTS radical scavenging activity

The results of the ABTS radical scavenging assay were expressed as the percentage of inhibition. In the ABTS assay method, radical rummaging potentiality for the extraction ensue evaluation utilising ABTS++ modification solution with respect to reaction between ABTS along with potassium persulfate solution [2] along with the IC₅₀ of these extractives ensue evaluation. ABTS scavenging activity computed the prunning of the blue-green chromophore ABTS+ (2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to colourless ABTS by an antioxidant.

From, the graph 7 it is evident that the percentage inhibition of ABTS radical was in a dosage dependant manner in all the extracts of *G. gummi gutta* leaves. The pet ether extract of leaves of *Gracinia gummi gutta* showed the percentage inhibition of 17.33±3.52 % to 93.67±2.02 % at concentration 10 to 100 µg/ml and its IC₅₀ value was 40.23±2.89 µg/ml. At highest concentration, the chloroform extract exhibited 82.50±4.66 % of inhibition; ethyl acetate: 88.00±1.52%, acetone extract: 88.33±2.40 %; ethanol extract 90.00±2.06.

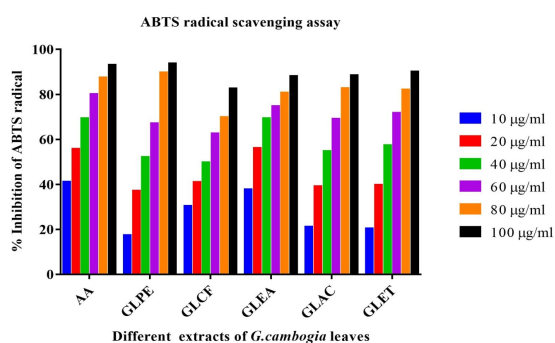


Figure 7 The percentage inhibition on ABTS radical scavenging activity by different extracts of *G. gummi gutta* leaves.

The average of % inhibition is presented. Statistics are mean±SEM (n=3). AA: Ascorbic acid; GLPE: *G. gummi gutta* leaves pet ether extract; GLCF: *G. gummi gutta* leaves chloroform extract; GLEA: *G. gummi gutta* leaves ethyl acetate extract; GLAC: *G. gummi gutta* leaves acetone extract; GLET: *G. gummi gutta* leaves ethanol extract.

The IC₅₀ value of ascorbic acid was found to be 13.44±1.89 µg/ml. The IC₅₀ values of leaf extracts were in the order: GLEA<GLET< GLAC< GLPE < GLCF. ANOVA One-way followed by Dunnett's method expressed that all the extractives (p<0.001) were significantly different from ascorbic acid, except GLEA.

However in fruit extract, the percentage ABTS radical scavenging activity of GFPE, GFCE, GFEA, GFAC and GFET at 100µg/ml was found to be 54.33±1.45 %, 62.00±4.16 %, 85.67 ±5.20 %, 79.00±1.00 % and 90.67±2.84 % respectively. Among different extracts of *G cambogia* fruit, the highest percentage inhibition was found in ethanol extract. Ascorbic acid showed its percentage inhibition of 86.67±1.76 % at higher concentration.

The IC₅₀ value of ascorbic acid was found to be 33.02±2.09 µg/ml. The IC₅₀ values of leaf extracts were in the order: GFET < GFEA < GFAC< GFCE < GFPE.

ANOVA One-way followed by Dunnett's multiple comparisons test indicated that all the extracts were significantly different from ascorbic acid.

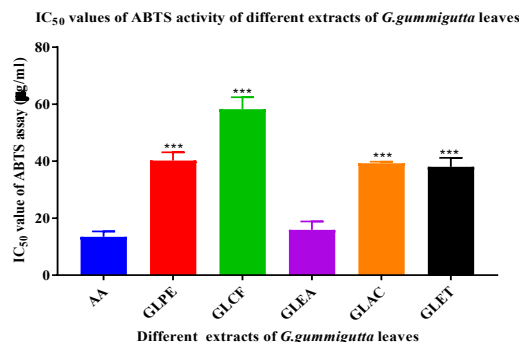


Fig :8 IC₅₀ values of different extracts of *G. gummi gutta* leaves

The values are mean of three replicates ± SEM. Statistics analyzed for ANOVA one-way regulated utilising Dunnett's collective analogy test. * represents p<0.05, ** represents p<0.01, *** represents p<0.001 when compared to Ascorbic acid. AA: Ascorbic acid; GLPE: *G. gummi gutta* leaves pet ether extract; GLCF: *G. gummi gutta* leaves chloroform extract; GLEA: *G. gummi gutta* leaves ethyl acetate extract; GLAC: *G. gummi gutta* leaves acetone extract; GLET: *G. gummi gutta* leaves ethanol extract.

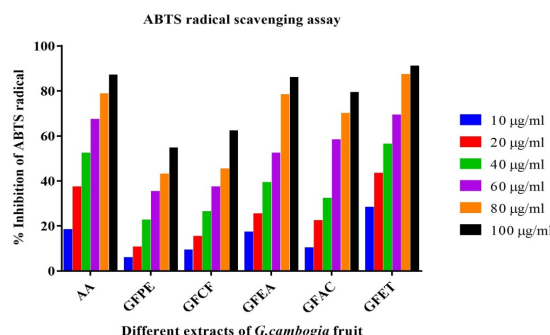


Figure:9 The percentage inhibition on ABTS radical by different extracts of *G. cambogia* fruits.

The average of % inhibition represented. Data are mean±SEM (n=3). AA: Ascorbic acid; GFPE: *G. gummi gutta* fruits pet ether extract; GFCE: *G. gummi gutta* fruits chloroform extract; GFEA: *G. gummi gutta* fruits ethyl acetate extract; GFAC: *G. gummi gutta* fruits acetone extract; GFET: *G. gummi gutta* fruits ethanol extract.

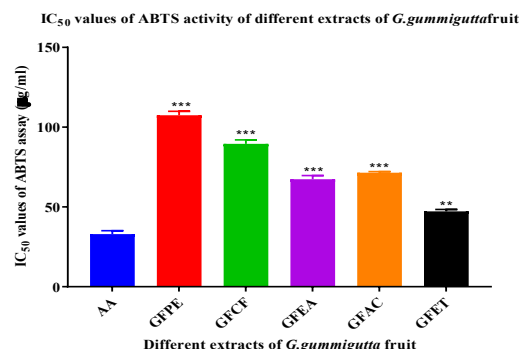


Fig10: The values are mean of three replicates ± SEM. Statistics analyzed for one-way ANOVA regulated utilising Dunnett's collective analogy test. * represents p<0.05, ** represents p<0.01, *** represents p<0.001 when compared to Ascorbic acid. AA: Ascorbic acid; GFPE: *G. gummi gutta* fruits pet ether extract; GFCE: *G. gummi gutta* fruits chloroform extract; GFEA: *G. gummi gutta* fruits ethyl acetate extract; GFAC: *G. gummi gutta* fruits acetone extract; GFET: *G. gummi gutta* fruits ethanol extract.

Nitric oxide radical scavenging activity

Results for the nitric oxide radical scavenging activity extract were evaluated by comparing within standard ascorbic acid. The reaction where nitric oxide produced between aqueous oxygen, sodium nitroprusside, and nitrite ions, under physiological pH conditions. Scavenging this free radical by Antioxidants and this property was compared within the standard.^[21]

The inhibition percentage by different extracts of *G. G. gummi-gutta* leaves of nitric oxide radicals were within dosage dependent pattern. The ascorbic acid percentage inhibition was 94.33±1.20 % at its highest concentration. Among various extracts of *G. G. gummi-gutta* leaves ethanol extract demonstrated highest inhibition percentage of 95.33±0.67%. The inhibition percentage for nitric oxide radical whereby GLPE, GLCH, GLEA and GLAC was found to be 83.67 ±1.45 %, 92.00 ±2.64 %, 92.00 ±1.15 % and 93.67 ±0.66 % respectively.

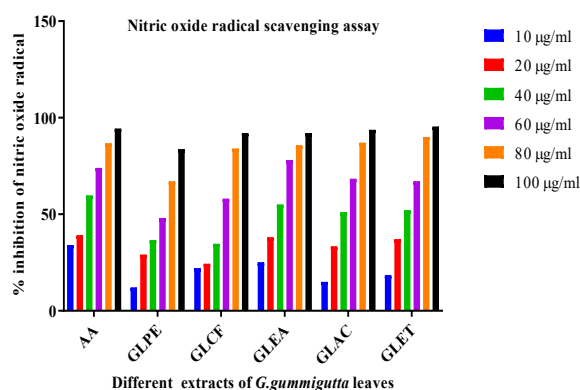


Figure. 11 The percentage inhibition on nitric oxide radical by different extracts of *G. cambogia* leaves.

The average of % inhibition is presented. Data are mean±SEM (n=3). AA: Ascorbic acid; GLPE: *G. gummigutta* leaves pet ether extract; GLCF: *G. gummi-gutta* leaves chloroform extract; GLEA: *G. gummi-gutta* leaves ethyl acetate extract; GLAC: *G. gummigutta* leaves acetone extract; GLET: *G. gummigutta* leaves ethanol extract.

The lowest IC₅₀ value was exhibited by GLEA which was 35.49±2.16 µg/ml. Moreover, GLEA was not statistically significantly different from ascorbic acid. The IC₅₀ values of ascorbic acid and different extracts of *G. cambogia* leaves were as follows: AA- 30.91±0.88 µg/ml; GLPE- 57.08±0.97 µg/ml; GLCF- 48.70±1.47 µg/ml; GLAC- 43.04±0.15 µg/ml and GLET- 40.02±1.01 µg/ml. Lower the IC₅₀ value, higher will be the antioxidant activity. All other extracts were significantly different from ascorbic acid.

The percentage inhibition of nitric oxide radical by different extracts of *G. cambogia* fruits were in a dose dependent pattern. The percentage inhibition by ascorbic acid was 92.00±1.252 % at its highest concentration. Among various extracts of *G. cambogia* fruit acetone extract demonstrated highest percentage inhibition of 94.67±1.45 %. The percentage inhibition of nitric oxide radical by GFPE, GFCH, GFEA and GFET was found to be 68.00 ±1.52 %, 67.00 ±3.60 %, 90.33 ±2.60 % and 93.67 ±1.85 % respectively.

IC₅₀ values of nitric oxide radical scavenging activity of different extracts of *G.gummigutta* leaves

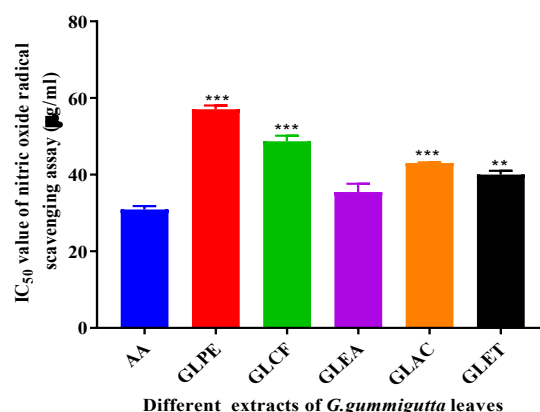


Fig12 IC₅₀ values of different extracts of *G.gummigutta* leaves.

The values are mean of three replicates ± SEM. Statistics analyzed for ANOVA one-way regulated utilising Dunnett's collective analogy test. * represents p<0.05, ** represents p<0.01, *** represents p<0.001 when compared to Ascorbic acid. AA: Ascorbic acid; GLPE: *G. gummigutta* leaves pet ether extract; GLCF: *G. G. gummigutta* leaves chloroform extract; GLEA: *G. gummigutta* leaves ethyl acetate extract; GLAC: *G. G. gummigutta* leaves acetone extract; GLET: *G. G. gummigutta* leaves ethanol extract.

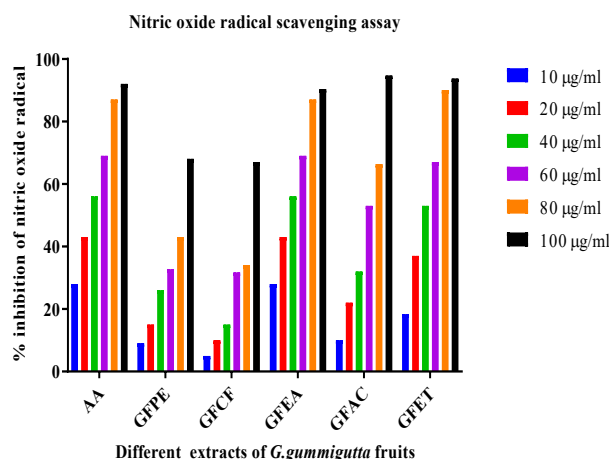


Figure. 13 The percentage inhibition on nitric oxide radical by different extracts of *G. gummigutta* fruits.

The average of % inhibition is presented. Data are mean±SEM (n=3). AA: Ascorbic acid; GFPE:*G. gummigutta* fruits pet ether extract; GFCH:*G. gummigutta* fruits chloroform extract; GFEA *G. Gummigutta* fruits ethyl acetate extract; GFAC: *G. gummigutta* fruits acetone extract; GFET:*G. gummigutta* fruits ethanol extract.

The lowest IC₅₀ value was exhibited by GFET which was 36.46±0.59 µg/ml. Moreover, GFEA and GFET were not statistically significantly different from ascorbic acid. The IC₅₀ values of ascorbic acid and different extracts of *G. cambogia* leaves were as follows: AA- 34.27±1.75 µg/ml; GFPE- 88.17±5.79 µg/ml; GFCH- 87.05±2.65 µg/ml; GFAC- 53.48±1.32 µg/ml and GFEA- 40.00±1.38 µg/ml. All other extracts were significantly different from ascorbic acid.

DISCUSSION

Herbal tissues are abundant reservoirs of phenolic compounds, which serve as potent antioxidants. These antioxidants exhibit remarkable efficacy in addressing certain ailments, owing to the presence of hydroxyl groups that play a pivotal role in their radical scavenging capability. Consequently, they can effectively neutralize active oxygen radicals such as hydroxyl radicals. Phenolic compounds possess aromatic characteristics, underscoring their substantial antioxidant potential. They are adept at quenching singlet oxygen, acting as hydrogen donors, acting as reductants, and exhibiting favourable metal chelation properties

The findings highlighted that the ethyl acetate extracts of leaves and fruit rind extracts of *G.gummi-gutta* exhibited the highest levels of flavonoids, while the pet ether extracts contained the lowest amounts. In specific terms, the total flavonoid content (TFC) in the ethyl acetate extracts of GL(GLEA) and GF(GFEA) was determined to be 96.66 RU/g and 106.67 RU/g of extract, respectively. Flavonoids represent a category of polyphenol compounds renowned for their potent radical scavenging activity, serving as effective safeguards against oxidative stress-induced damage within organisms.

In the DPPH analysis, colour violet DPPH solution ensue minimised for yellow coloured outcomes (diphenylpicryl hydrazine) towards admixture for extraction via concentration dependant methodology. Methodology ensures utilization widely fore predicting antioxidant activities by virtue of the relatively shortest period required for analysis. Our results revealed that both leaf along with fruit extraction have beneficial anti-oxidative venture.

The IC₅₀ values of leaf extracts were in the order: GLEA < GLET < GLAC < GLPE < GLCF. ANOVA One-way followed by Dunnett's method expressed that all the extractives (p < 0.001) were significantly different from ascorbic acid, expect GLEA.

In ABTS method also the results for leaf extracts were ethyl acetate extract and fruit rind extract were ethanol extract has shown comparable antioxidant activity with respect to ascorbic acid as standard as low IC₅₀ value high will be antioxidant activity. Similar results will be obtained for nitric oxide radicle scavenging activity.

Thus the study has shown that the leaves as well as the fruit rind extracts were the ethyl acetate fraction and ethanolic fractions are having higher antioxidant and free radical potential effects.

CONCLUSION

The research highlights the antioxidant abilities of *G. gummi-gutta* in a controlled environment, demonstrating its capacity to neutralize DPPH, ABTS, and nitric oxide, comparable to the established ascorbic acid standard. Additionally, the study measured the overall phenolic and flavonoid levels. An analysis of the plant's chemical composition revealed the existence of flavonoids such as quercetin and kaempferol in the leaf's ethyl acetate extract. Meanwhile, the primary component identified in the fruit rind extract was hydroxycitric acid. Consequently, further

investigations are necessary to explore its potential applications

Conflict of Interest

The authors collectively affirm that there are no conflicts of interest pertaining to the manuscript.

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