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Research Article

Evaluating The Therapeutic Potential Of *Garcinia Gummi-Gutta*

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ABSTRACT

The study examines the phytochemical content and therapeutic properties of methanolic extracts from the rind and fruit of G. gummi-gutta. Both qualitative and quantitative analyses revealed the presence of flavonoids, alkaloids, tannins, and phenols in the rind, and flavonoids and tannins in the fruit. The therapeutic properties like anti-oxidant, anti-diabetic, anti-cancer, anti-inflammatory, wound healing, anti-ulcer, and anti-microbial were determined using DPPH assay, α-amylase inhibition assay, MTT assay, protein denaturation assay, wound scratch assay, acid neutralizing capacity method, and agar well diffusion method respectively. Results showed that anti-oxidant and anti-diabetic activities increased with extract concentration. The rind extract exhibited significant anti-cancer activity, with a 32.08% inhibition of MCF-7 cell lines at 100 and 200µg/mL. The anti-inflammatory effect was higher in the rind (79.35%) than in the fruit (58.04%) at 100µg/mL. The wound healing effect was greatest at 50 µg/mL with 87.45% closure. Anti-ulcer activity decreased with higher extract concentrations. Both extracts showed anti-microbial activity against bacteria, though not against fungi. The rind extract generally demonstrated greater therapeutic potential than the fruit extract.

INTRODUCTION

Garcinia is an indigenous tropical crop belonging to the Clusiaceae family, with around 390 species, making it the largest genus within the family [1]. Predominantly found in Southeast Asia [2]. Garcinia gummi-gutta, also known as Malabar tamarind, is highly valued for its medicinal properties [1, 2]. The tree reaches upto 12 meters in height, and its fruit, resembling a small pumpkin, changes color from yellow to green or red when ripe, containing a soft white pulp and six to eight seeds [1, 3]. The fruit has been used in cooking for its unique flavor and in various traditional remedies such as curing seafood, condiment in curry cuisines [4, 5]. Additionally, the fruit extracts are utilized as astringents, demulcents, rheumatism relievers, bowel problem relievers, and purgatives [6]. Garcinia fruit rinds, dark brown or black when dried, are used for

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storage, as fish seasoning, and as a spice [3, 4]. Garcinia's rind, rich in hydroxycitric acid (HCA), is known for its weight-loss properties and is widely used as a spice [4, 7]. The fruit's sun-dried rind is also utilized as a purgative, anti-bacterial, and astringent [8].Various phytochemicals are extracted from different parts of the tree. Phytochemicals like garcinol, isogarcinol, and rheediaxanthone A are extracted from Garcinia bark using solvents like benzene, methanol, and acetone under reflux [9]. The fruit contains benzophenones (garcinol, guttiferone I, J, K, M, N), xanthone (oxyguttiferone K), and organic acids (hydroxycitric acid, garcinia acid) extracted with ethanol under reduced pressure or methanol using soxhlet extraction [10, 11]. Fruit rind yields camboginol, isoxanthochymol, and hydroxycitric acid extracted using methanol under ultrasonication or ethanol [10, 12]. From the latex, camboginol and cambogin (isogarcinol) are isolated. These are extracted by a simple crystallization method using petroleum ether (solvent) [13]. Leaf extracts include a wide array of phytochemicals such as garcinol, mangostin, gambogic acid, hydroxycitric acid lactone (garcinia acid), protocatechuic acid, caffeic acid, ferulic acid, vanillic acid, epicatechin, isoorientin, orientin, isovitexin, vitexin, kaempferol-3-O-rutinoside, luteolin, quercetin, apigenin, kaempferol, fukugiside, GB-1, amentoflavone, ursolic acid, and betunilic acid obtained via soxhlet extraction with methanol [11]. Root extracts predominantly contain garbogiol using reflux extraction with benzene, methanol, and acetone as the solvents [9]. These phytochemicals exhibit various medicinal properties, including anti-diabetic, anti-inflammatory, antioxidant, anti-cancer, anti-microbial, anti-ulcer, and wound healing effects [1, 2, 14, 15]. Oxidative stress and antioxidant imbalance serve as critical factors in the initiation of various diseases like diabetes, cancer, cardiovascular disease, inflammation, etc [15]. Flavonoids present in the plant extract demonstrated anti-oxidant properties [11, 16]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated during cellular processes can oxidize vital components, damaging proteins, lipids, and DNA [15, 17]. Anti-oxidants like superoxide dismutase, catalase, and methionine sulfoxide reductase scavenge these reactive species and repair damage [17]. Flavonoids from G. gummi-gutta, such as epicatechin, isoorientin, isovitexin, exhibit anti-oxidant properties [11, 16]. Another application of G. gummi-gutta extract is the anti-diabetic property. Diabetes mellitus is characterized by elevated blood glucose, leading to chronic hyperglycemia and lipid profile abnormalities [18, 19]. Garcinol and hydroxycitric acid from G. gummi-gutta have demonstrated anti-diabetic activity [1]. These phytochemicals are also studied for anti-cancer properties [1, 20, 21, 22]. Cancer involves uncontrolled cell growth, forming malignant tumors [23]. Phytochemicals from G. gummi-gutta, such as benzophenones and xanthones, showed anti-cancer activity against lung, colorectal, prostate, and breast cancers [1, 20, 21, 22]. These compounds also have potential anti-inflammatory properties [24, 25]. Inflammation occurs due to infections, injury, cell death, cancer, ischemia, and degeneration [24]. Saponins and tannins exhibit anti-inflammatory activities [16]. Garcinol and hydroxycitric acid from G. gummi-gutta demonstrate anti-inflammatory properties, aiding wound healing [1, 14]. Cells involved in wound healing release growth factors and cytokines like IL-6 and TNF-α [14]. Wound healing properties of Garcinia species like Garcinia mangostana and Garcinia brasiliensis have been studied [14,26]. Phenolic compounds like xanthones, which promote fibroblast proliferation and activate wound healing pathways [14,27]. Delays in wound healing can lead to diseases like ulcers, with chronic wounds

often causing ulcers [28]. Ulcers are open sores on skin or mucous membranes due to inflamed dead tissue [29]. G. gummi-gutta fruit extract showed significant anti-ulcer activity against indomethacin- and HCl-ethanol-induced ulcers in rats [1]. It also exhibited anti-microbial properties [3, 30]. G. gummi-gutta extracts showed antibacterial activity against gram-negative bacteria (e.g., Acinetobacter baylii, Escherichia coli) and gram-positive bacteria (e.g., Bacillus cereus, Staphylococcus aureus) [3]. The study aimed to evaluate the phytochemical analysis, anti-oxidant, anti-diabetic, anti-cancer, anti-inflammation, wound healing, anti-ulcer, and antimicrobial activities of G. gummi-gutta rind and fruit.

MATERIALS AND METHODS

Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazyl (SRL), 3-(4,5-dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide (SRL), Acarbose (Glucobay), Aluminium chloride (NICE), Ascorbic acid (HIMEDIA), Bovine Serum Albumin (SRL), Bromo cresol green (MEDILISE), Chloroform (MEDILISE), Ciprofloxacin (HIMEDIA), Diclofenac (SIGMA-ALDRICH), Dimethyl sulfoxide (HIMEDIA), Dulbecco's Modified Eagle Medium (HIMEDIA), Dulbecco's Phosphate Buffer Saline (HIMEDIA), Ferric chloride (Kanton Laboratories), Fetal Bovine Serum (HIMEDIA), Folin-Ciocalteu reagent (MEDILISE), Gallic acid (NICE), Gentamicin (American Pharma Remedies), Hydrochloric acid (NICE), Methanol (NICE), Nutrient broth (HIMEDIA), Potato Dextrose Agar (HIMEDIA), Quercetin (SRL), Sodium carbonate (NICE), Sodium hydroxide (Kanton Laboratories), Sodium nitrate (Kanton Laboratories), Starch (SRL), Sulphuric acid (MEDILISE), Tannic acid (MEDILISE), Wagner's reagent (Kanton Laboratories), α-amylase (Kanton Laboratories)

Instruments

Autoclave (FOURTECH), CO2 incubator (FORMA SERIES II WATER JACKET), Incubator (FOURTECH), Inverted microscope (TCM 400), Inoculation hood (ROTEK), Bio safety cabinet Class II Type B II (ROTEK) Magnetic stirrer (Lab Companion), Visible spectrophotometer (Thermo SCIENTIFIC), Vortex (Lab Companion), Water bath (ROTEK), Weighing machine (SHIMADZU)

Cell lines

L929 and MCF-7 cell lines were obtained from Bioroot Exploration India Pvt Ltd.

Preparation and extraction of the sample

Dried rind and fresh fruit of G. gummi-gutta were sourced from Kerala, India. 15 g of finely cut rind pieces were transferred into a thimble and 40 mL of methanol (solvent) was added to the round bottom flask. Soxhlet extraction process was carried out at 60°C for 4-6 hours. The fresh fruit was dried and powdered and extracted similarly. Both crude extracts were stored at 4°C for future use [31].

Qualitative screening of phytochemicals

Terpenoids

To 1 mL sample, 2 mL chloroform was added. Then a few drops of concentrated sulphuric acid were added. The appearance of an interface with a reddish-brown color indicated the presence of terpenoids. However, the solution exhibited a yellow color which indicated the absence of terpenoids [8].

Flavonoids

2 mL of 2% NaOH was added to 1 mL of the sample. The color of the solution turned into golden yellow. Upon adding a few drops of diluted HCl, the color of the solution changed to colorless, indicated the presence of flavonoids [32].

Alkaloids

A few drops of Wagner's reagent were added to 1 mL of the sample. The appearance of a brown or reddish precipitate indicated the presence of alkaloids [32].

Saponins

2 mL of distilled water was added into 1 mL of the extract, shaken vigorously, and allowed to stand for 10mins. There was a formation of foam on the surface of the mixture. Then shaken for 10mins, the appearance of foam confirmed the presence of saponins. However, there was no foam development in the solution. Hence, saponin presence was not detected [8].

Tannins

To 1 mL sample, 2 mL of 0.1% ferric chloride was added. The brownish-green or blue-black color indicated the presence of tannins [8].

Phenolic compounds

A few drops of 5% ferric chloride were added to 1 mL of the sample. The appearance of dark green or bluish-black color indicated the presence of phenolic compounds [32].

Steroids

To 1 mL sample, 10 mL chloroform was added. Then 10 mL of concentrated sulphuric acid was added slowly by the sides of the tube. The appearance of red color in the upper layer and yellow with green fluorescence in the sulphuric acid layer indicated the presence of steroids. However, a yellow color was displayed at the interface of the upper and lower layer which indicated the absence of steroids [6].

Quantitative estimation of phytochemicals

Phenols

To 0.5 mL of the sample, 2 mL of 1:10 diluted FC reagent and 4 mL of 7.5% Na2CO3 were added. The test tubes were covered with aluminium foil and incubated for 30mins with intermediate shaking. Gallic acid, with concentrations from 0.1 to 0.5 mg/mL, was used as the standard. All reagents except the sample were taken as blank. Absorbance was measured at 760 nm [33].

Tannins

Tannins were determined by the FC method. 0.1 mL of sample extract was added to a 10 mL volumetric flask with 7.5 mL of distilled water, 0.5 mL of FC reagent, and 1 mL of 35% sodium carbonate solution, then diluted to 10 mL with distilled water. The mixture was shaken and kept at room temperature for 30 mins. Tannic acid, with concentrations from 0.2 to 1 mg/mL, was used as standard. All reagents except the sample were taken as blank. Absorbance was measured at 700 nm [34].

Alkaloids

To 1 mL of the sample, 5 mL of phosphate buffer (pH 4.7) and 5 mL of bromocresol green solution were added. The mixture was transferred to a separating funnel and agitated. The complex was extracted with 1, 2, 3, and 4 mL chloroform by vigorous shaking and collected in a test tube. Quercetin, with different concentrations from 0.2 to 1 mg/mL, was used as the standard. All reagents except the sample were taken as blank. Absorbance was measured at 470 nm [35].

Flavonoids

2 mL of 5% NaNO3 was added to 1 mL of the sample. After 5 mins, 3 mL of 10% AlCl3 was added, followed by 2 mL 1M NaOH. The mixture was kept for 5 mins, then made up to 10 mL with distilled water. Quercetin, with concentrations ranging from 0.2 to 1 mg/mL, was used as the standard. All reagents except the sample were taken as blank. Absorbance was measured at 510 nm [36].

Anti-oxidant activity

DPPH Assay: 0.004% of DPPH was dissolved in methanol in a beaker covered with aluminium foil and incubated for 2 h. Samples at different concentrations (100, 200, 300, 400, 500 µg/mL) were prepared in methanol (solvent). Ascorbic acid, prepared at the same concentrations in water, was used as the standard. After 2 h, 4 mL DPPH solution was added to each test tube containing 1 mL sample or 1 mL ascorbic acid. DPPH solution without sample was taken as control, and methanol was used as blank. The tubes were incubated in the

dark for 30 mins, and absorbance was measured at 515 nm.

DPPH Scavenged (%) = [(Ac-As)/Ac] x 100

Where Ac is the absorbance of the control and As is the absorbance of the sample [37].

Anti-diabetic activity

α-amylase Inhibition Method: Different concentrations (50, 100, 150, 200, and 250 µg/mL) of the sample and standard (acarbose) were prepared. To both, 100µl of 20 mM phosphate buffer and 100µl of 0.5 mg/mL α-amylase solution were added, followed by incubation at 30°C for 10 mins. After adding 1 mL of 1% starch solution, the mixture was incubated again at 30°C for 10 mins. Then, 1 mL of DNS (3,5-Dinitrosalicylic acid) reagent was added and kept in a boiling water bath for 10 mins. After cooling, 1 mL of distilled water was added. All reagents except the sample/standard were used as control, and methanol was used as blank. Absorbance was measured at 540 nm.

% inhibition = [(AC-AS)/AC] x 100

where AC is the absorbance of the control and AS is the absorbance of the sample [38].

Anti-cancerous activity

MTT Assay: 0.1 x 10^6 cells/mL of MCF-7 cell lines were seeded in a 24-well plate for 24 h. The cells were cultured using complete media consisting of DMEM (Dulbecco's Modified Eagle Medium) with 5% FBS (Fetal Bovine Serum), 500 µl antibiotics, and 400µl gentamicin. After obtaining 80% confluency, the media was discarded. 1 mL of complete media was added to each well and samples with varying concentrations (10, 50, 100, 200, 300, 400, and 500 µg/mL) were added into it. Then, it was gently mixed and was kept in CO2 incubator at 37 °C for 24 h followed by a wash with 500µl DPBS (Dulbecco's Phosphate Buffer Saline). MTT (3-(4,5-dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide) reagent was prepared by dissolving MTT powder in DPBS. 500 µl of MTT reagent was added into each well and mixed gently. After 2 to 4 h of incubation, 1 mL DMSO (Dimethyl Sulfoxide) was added into each well and kept for 15 mins of incubation to solubilize purple-colored formazan in live cells. The experiment was carried out in Bio safety cabinet Class II Type B II. Absorbance was measured at 570 nm, with triplicate readings for each concentration [39].

% Cell viability = (Mean OD sample / Mean OD control) X 100

% Cell inhibition = (Mean OD control – Mean OD sample/ Mean OD control) X 100

Anti-inflammatory activity

Protein Denaturation Assay: To different concentrations (100, 200, 300, 400, and 500 µg/mL) of the sample and standard drug diclofenac, 1% w/v BSA (Bovine Serum Albumin) and PBS (Phosphate Buffered Saline, pH 6.4) were added. The solutions were kept at room temperature for 20 mins, then in a water bath for 5 mins at 70°C. Absorbance was measured at 660 nm after cooling. All reagents except the sample/standard were used as control, and methanol was used as blank.

% inhibition of BSA denaturation = [(AC-AS)/AC] x 100

where AC is the absorbance of the control and AS is the absorbance of the sample [40].

Wound healing activity

Scratch wound assay:

0.3 x 106 cells/mL of L929 cell lines were seeded in a 6-well plate for 24 h. The cells were cultured with complete media consisting of DMEM, 5% FBS, 500 µl antibiotics, and 400µl gentamicin. After obtaining 80% confluency, the spent media was discarded, and 1 mL complete media was added to each well. A scratch was made using a 200 µL tip, and the plates were kept in CO2 incubator at 37°C for 24 h. Samples of different concentrations (10, 50, 150, 300, and 500 µg/mL) were added, mixed, and incubated again at 37°C for 24 h. All reagents without sample were

taken as control. The experiment was carried out in Bio safety cabinet Class II Type B II. Wound healing activity was observed and photographed at 24-hour intervals.

% of wound closure = (A0h – A24h)/A0h x 100

where A0h and A24h is the area of wound at 0th hour and 24th hour [41].

Anti-ulcer activity

Acid Neutralizing Capacity:

Different concentrations (100, 200, 500, 1000 mg/mL) of the sample were prepared. 5 mL of the sample was made upto 70 mL with water, then 30 mL of 1N HCl was added and stirred for 15 mins. Phenolphthalein (2-3 drops) was added and the mixture was titrated with 0.5N NaOH until a pink color appeared. Gelusil (500 mg/mL) was used as a standard [42].

Moles of acid neutralized = (Volume of HCl x Normality of HCl) – (Volume of NaOH x Normality of NaOH)

Acid Neutralizing Capacity (ANC) per gram of antacid = Moles of HCl neutralized/ Gram of Antacid or Extract

Antimicrobial activity

Agar Well Diffusion Method: Nutrient broth and PDB (Potato Dextrose Broth) were prepared and sterilized in an autoclave. Gram-positive bacteria (Bacillus sphearicus, Staphylococcus aureus) and gram-negative bacteria (Klebsiella pneumonia, Pseudomonas aeruginosa) were inoculated into nutrient broth and fungi (Aspergillus niger) in PDB and incubated at 37°C for 24 h. Nutrient agar was prepared by dissolving 1.3g nutrient broth and 1.5g agar in 100 mL distilled water and PDA (Potato Dextrose Agar) was prepared by dissolving 0.975 g of PDA in 25 mL distilled water. Both media were autoclaved at 121°C, 15 lbs for 30 mins and were poured into petri plates to solidify. Plates were swabbed with specific organisms, and five wells were made in each plate using a pipette tip. Samples at 100, 200, and 300 mg/mL, positive control (PC) (0.2% w/v ciprofloxacin), and negative control (NC) (50% v/v methanol) were added (30 µl each). Plates were incubated at 37°C for 24 h, and the zones of inhibition were measured [43,44].

RESULTS

Extraction of G. gummi-gutta

The methanolic extract of G. gummi-gutta rind and fruit is shown in Figure 1 and Figure 2.

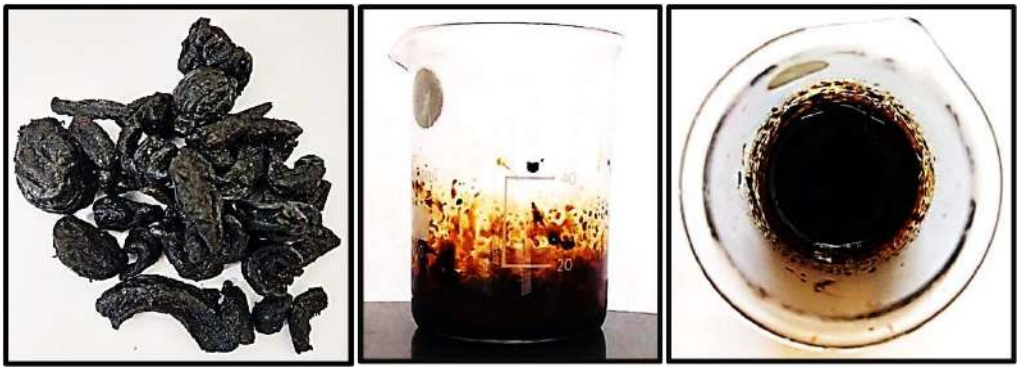


FIGURE 1: G. GUMMI-GUTTA RIND METHANOLIC EXTRACT



FIGURE 2: G. GUMMI-GUTTA FRUIT METHANOLIC EXTRACT

Phytochemical analysis of methanolic extract of G. gummi-gutta

Qualitative phytochemical analysis is performed on both G. gummi-gutta rind and fruit extract (Figure 3 and Table 1).

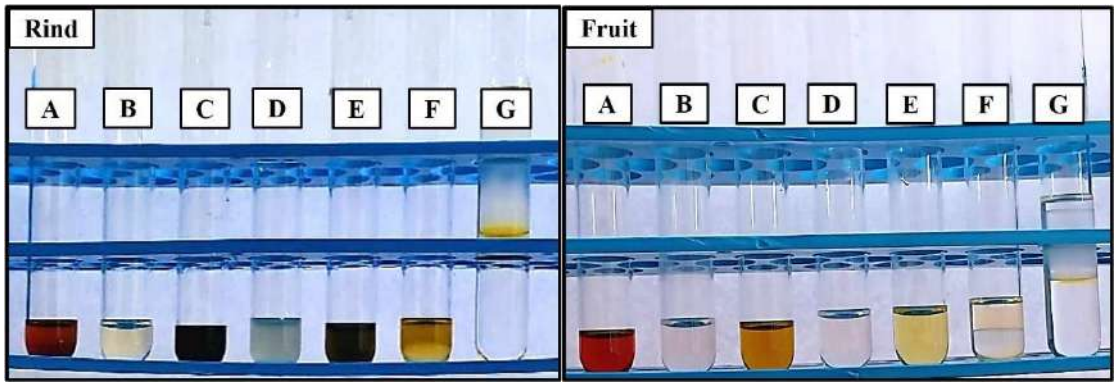


Figure 3: Qualitative Analysis Of Phytochemicals (A- Alkaloids, B- Saponins, C- Phenolic Compounds, D- Flavonoids, E- Tannins, F- Terpenoids, G- Steroids)

Table 1: Qualitative Analysis Of Phytochemicals ('+++' – High Expression, '++' - Moderate Expression, '+' - Low Expression, '-' – Absent)

Phytochemicals	Presence/Absence	
	Rind	Fruit
Alkaloids	+++	-
Saponins	-	-
Phenolic compounds	+++	-
Flavonoids	+	+
Tannins	+++	+
Terpenoids	-	-
Steroids	-	-

Quantitative estimation of phytochemicals

Phenols

Quantity of phenol from methanolic extract of G. gummi-gutta rind is calculated using the equation y = 0.408x + 1.0722 from the standard curve of gallic acid (Figure 4, Figure 5, Table 2, and Table 3).

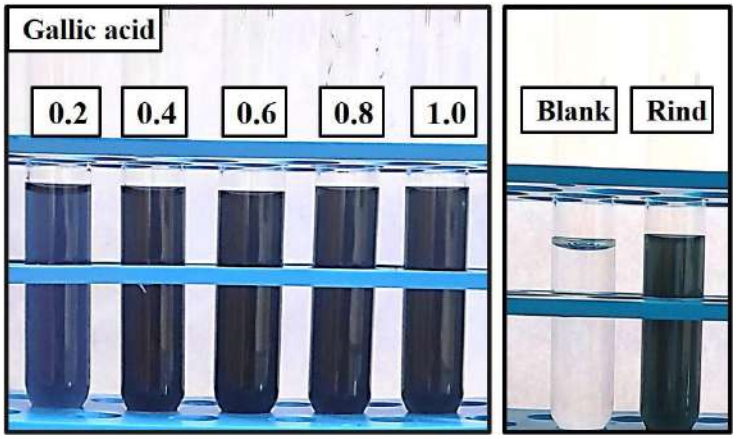


Figure 4: Quantitative Estimation Of Phenols

Table 2: Absorbance Value Of Gallic Acid And Rind Extract

Concentrations (mg/mL)	Absorbance at 760 nm
0.2	1.055
0.4	1.319
0.6	1.383
0.8	1.411
1	1.417
Rind	1.485

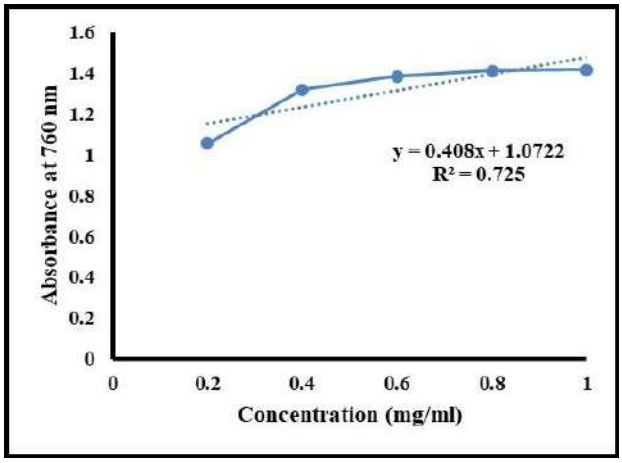


Figure 5: Standard Curve Of Gallic Acid

Table 3: Quantity Of Phenol In Rind Extract

Phytochemical	Quantity
Phenols	1.011 mg/mL

Tannins

Quantity of tannin from methanolic extract of G. gummi-gutta rind and fruit is calculated using the equation y = 1.0055x + 0.0267 from the standard curve of tannic acid (Figure 6, Figure 7, Table 4, and Table 5).

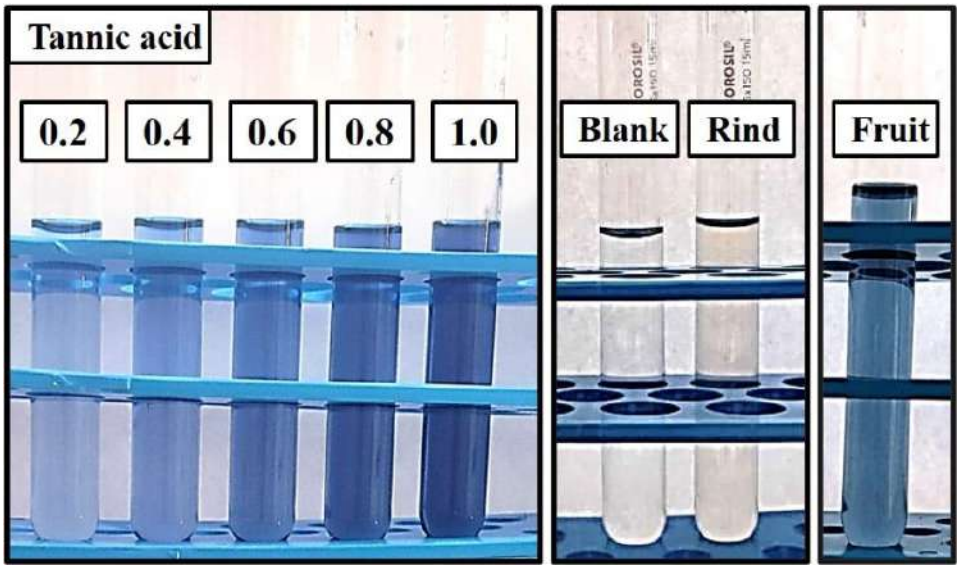


Figure 6: Quantitative Estimation Of Tannins

Table 4: Absorbance Value Of Tannic Acid, Rind And Fruit Extract

Concentrations (mg/mL)	Absorbance at 700 nm
0.2	0.223
0.4	0.431
0.6	0.638
0.8	0.828
1.0	1.030
Rind	0.119
Fruit	0.444

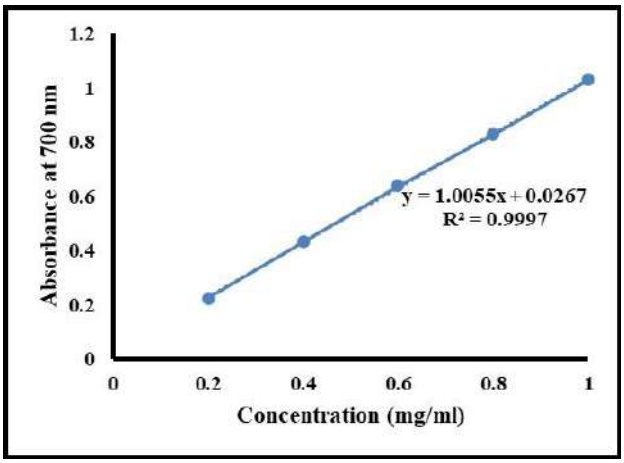


Figure 7: Standard Curve Of Tannic Acid

Table 5: Quantity Of Tannin In Rind And Fruit Extract

Sample	Tannin quantity
Rind	0.092 mg/mL
Fruit	0.415 mg/mL

Alkaloids

Quantity of alkaloid from methanolic extract of G. gummi-gutta rind is calculated using the equation

y = 0.024x + 0.0096 from the standard curve of quercetin (Figure 8, Figure 9, Table 6, and Table 7).

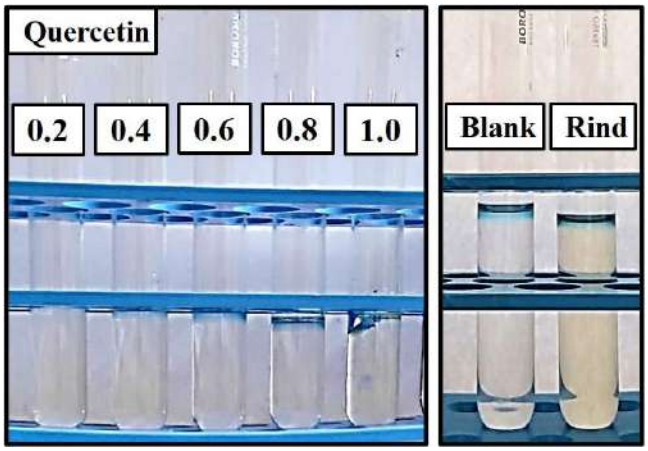


Figure 8: Quantitative Estimation Of Alkaloids

Table 6: Absorbance Value Of Quercetin And Rind Extract

Concentrations (mg/mL)	Absorbance at 470 nm
0.2	0.011
0.4	0.013
0.6	0.036
0.8	0.037
1.0	0.023
Rind	0.053

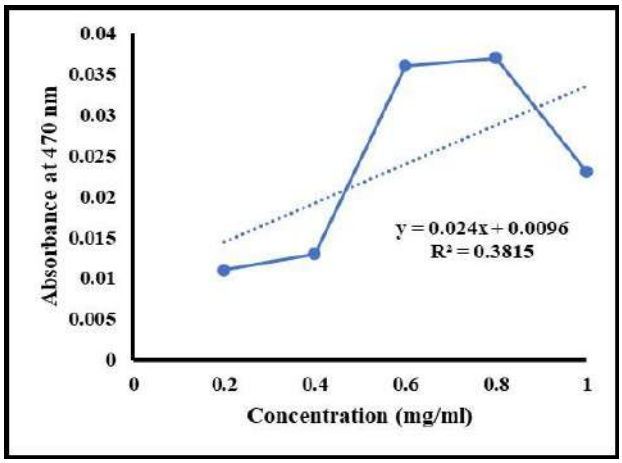


Figure 9: Standard Curve Of Quercetin

Table 7: Quantity Of Alkaloid In Rind Extract

Sample	Quantity
Rind	1.808 mg/mL

Flavonoids

Quantity of phenol from methanolic extract of G. gummi-gutta rind and fruit is calculated using the equation y = 2.25x + 0.226 from the standard curve of quercetin (Figure 10, Figure 11, Table 8, and Table 9).

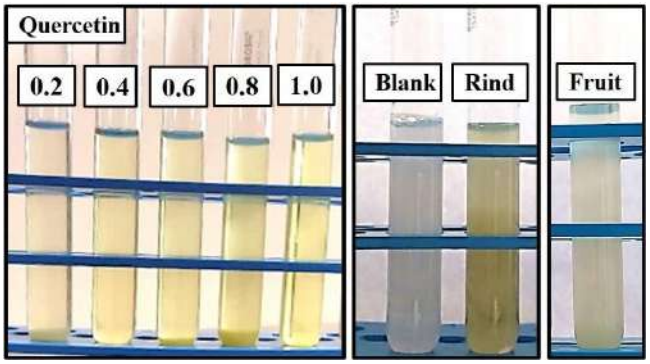


Figure 10: Quantitative Estimation Of Flavonoids

Table 8: Absorbance Value Of Quercetin, Rind And Fruit Extract

Concentrations (mg/mL)	Absorbance at 510 nm
0.2	0.718
0.4	1.158
0.6	1.501
0.8	1.912
1.0	2.591
Rind	0.867
Fruit	0.595

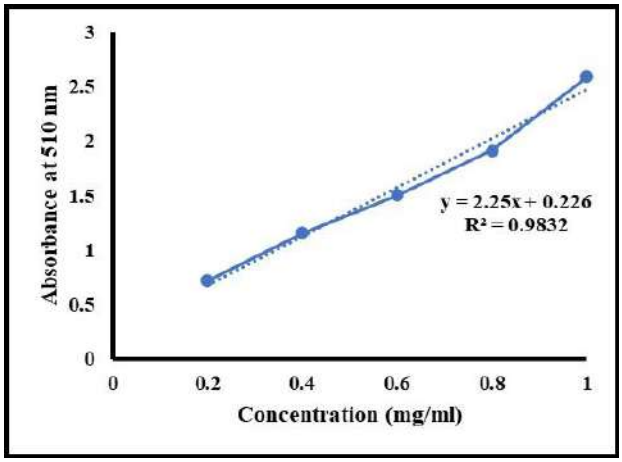


Figure 11: Standard Curve Of Quercetin

Table 9: Quantity Of Flavonoid In Rind And Fruit Extract

Sample	Flavonoid quantity
Rind	0.285 mg/mL
Fruit	0.164 mg/mL

Anti-oxidant activity

Anti-oxidant property was determined using DPPH assay with ascorbic acid as standard (Figure 12). The absorbance value of control at 515 nm is 1.836 (Table 10). The percentage of DPPH activity is displayed in Figure 13 and IC50 value of G. gummi-gutta rind is 117.42 µg/mL and fruit is 278.11 µg/mL.

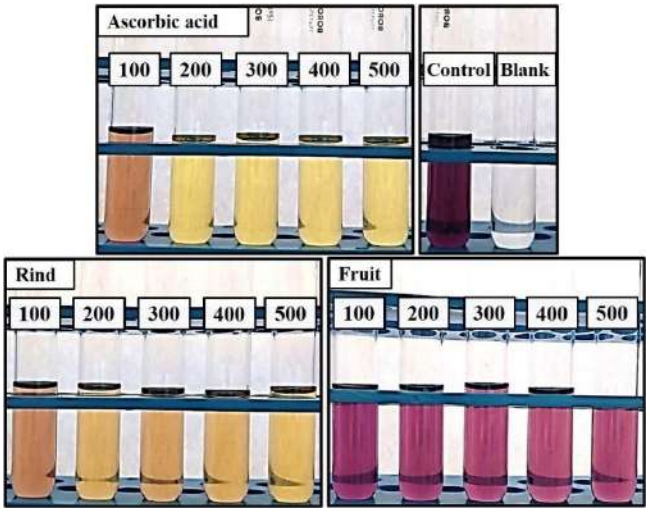


Figure 12: Dpph Assay- Anti-Oxidant Activity Of Ascorbic Acid, Rind And Fruit Extract

Table 10: Absorbance Value Of Ascorbic Acid, Rind And Fruit Extract

Concentrations (µg/mL)	Absorbance at 515 nm		
	Ascorbic acid	Rind	Fruit
100	0.471	0.846	0.954
200	0.083	0.288	1.090
300	0.078	0.330	0.583
400	0.077	0.273	0.858
500	0.077	0.204	0.537

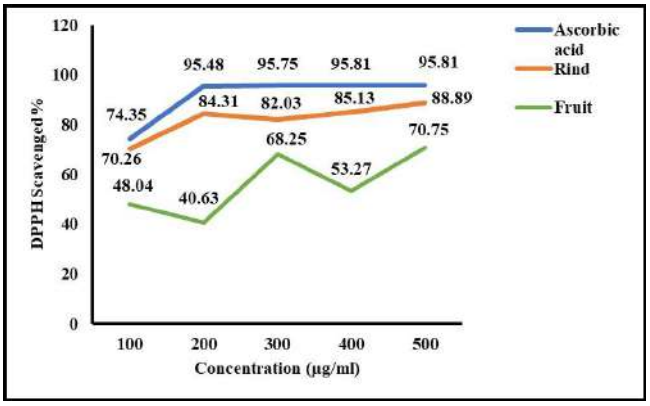


Figure 13: Dpph Scavenged % By Ascorbic Acid, Rind And Fruit Extract

Anti-diabetic activity

Anti-diabetic property is determined using α-amylase inhibition assay with acarbose as standard (Figure 14). The absorbance value of control at 540 nm is 2.150 (Table 11). The percentage of α-amylase inhibition is displayed in Figure 15 and IC50 value of G. gummi-gutta rind is 169.61 µg/mL and fruit is 970.57 µg/mL.

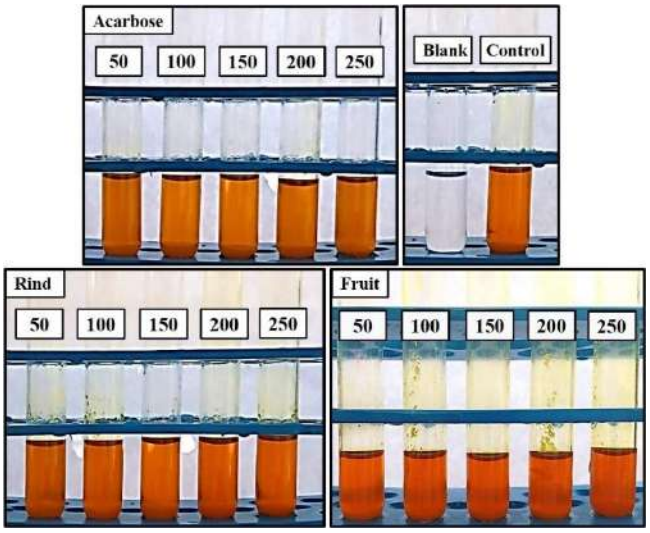


Figure 14: A-Amylase Inhibition Assay- Anti-Diabetic Activity Of Acarbose, Rind And Fruit Extract

Table 11: Absorbance Value Of Acarbose, Rind And Fruit

Concentrations (µg/mL)	Absorbance at 540 nm		
	Acarbose	Rind	Fruit
50	1.305	1.135	2.050
100	0.918	1.103	2.170
150	0.913	1.013	2.117
200	0.960	1.065	1.910
250	1.015	0.949	1.845

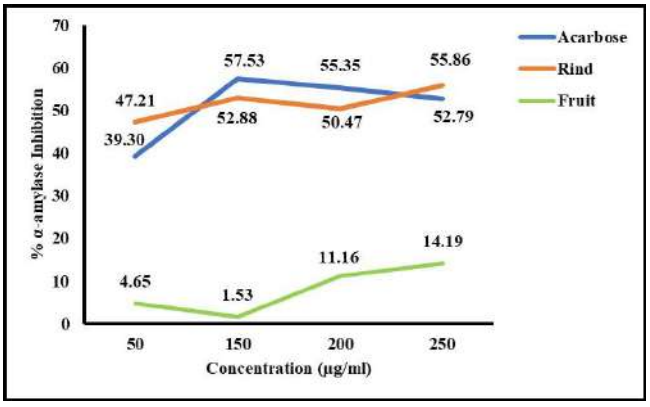


Figure 15: % A-Amylase Inhibition By Acarbose, Rind And Fruit Extract

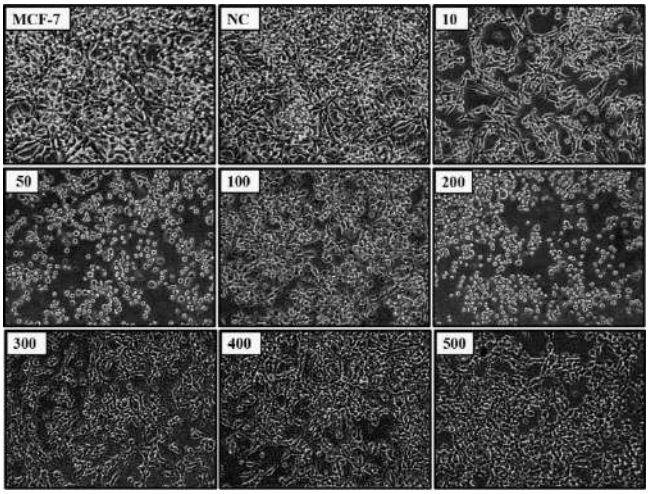


Figure 16: Mtt Assay- Mcf-7 Cancer Cell Lines After Treated With Different Concentrations Of Rind Extract For 24 H

Table 12: Mtt Assay- % Of Cell Viability And Inhibition Of Mcf-7 Cancer Cell Lines Against Rind Extract

Concentrations (µg/mL)	Absorbance at 570 nm					
	1	2	3	Mean	% of viability	% of inhibition
NC	0.157	0.158	0.162	0.159	100	0
10	0.119	0.121	0.122	0.121	76.10	23.90
50	0.100	0.107	0.127	0.111	69.81	30.19
100	0.103	0.111	0.109	0.108	67.92	32.08
200	0.110	0.104	0.109	0.108	67.92	32.08
300	0.105	0.107	0.115	0.109	68.55	31.45
400	0.106	0.109	0.114	0.110	69.18	30.82
500	0.104	0.110	0.117	0.110	69.18	30.82

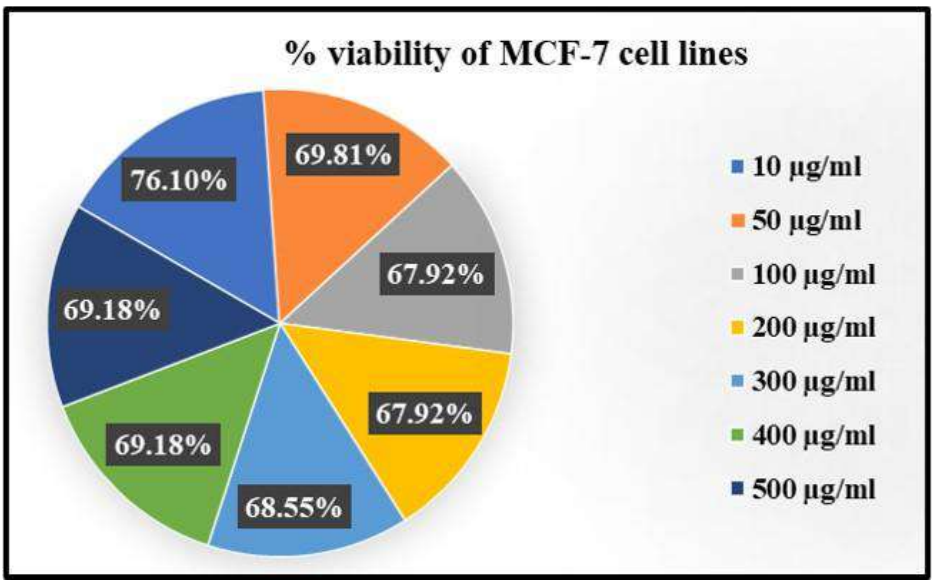


Figure 17: % Cell Viability Of Mcf-7 Cell Lines Against Rind Extract

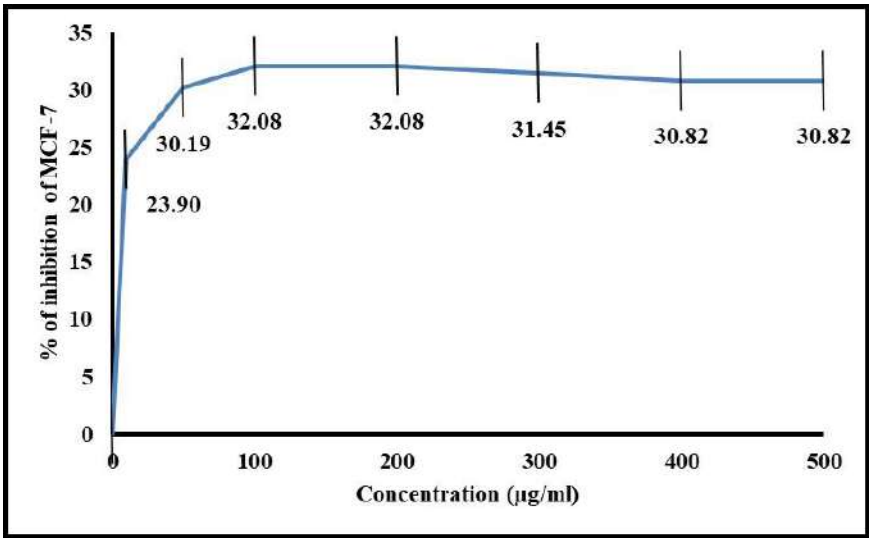


Figure 18: % Of Inhibition Of Mcf-7 Cell Lines Against Rind Extract

Anti-inflammatory activity

Anti-inflammatory property was determined using protein denaturation assay with diclofenac as standard (Figure 19). The absorbance value of control at 660 nm is 1.642 (Table 13). The percentage inhibition of BSA denaturation is showed in Figure 20 and IC50 value of G. gummi-gutta rind is 116.14 µg/mL and fruit is 86.50 µg/mL.

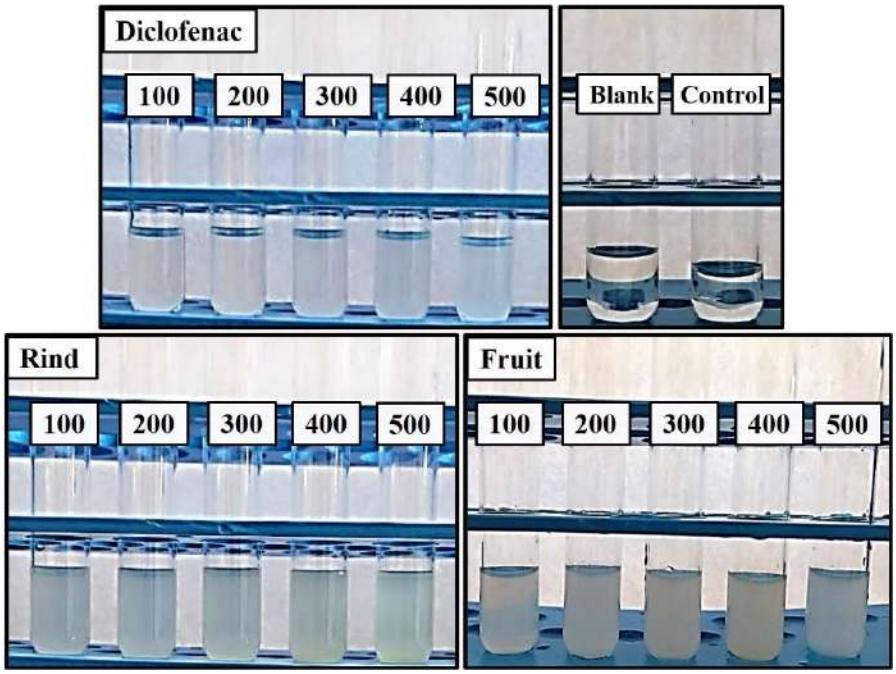


Figure 19: Protein Denaturation Assay- Anti-Inflammatory Activity Of Diclofenac, Rind And Fruit Extract

Table 13: Absorbance Value Of Diclofenac, Rind And Fruit

Concentrations (µg/mL)	Absorbance at 660 nm		
	Diclofenac	Rind	Fruit
100	0.055	0.339	0.689
200	0.053	0.413	1.500
300	0.060	0.334	1.638

400	0.049	0.385	1.397
500	0.046	0.387	1.468

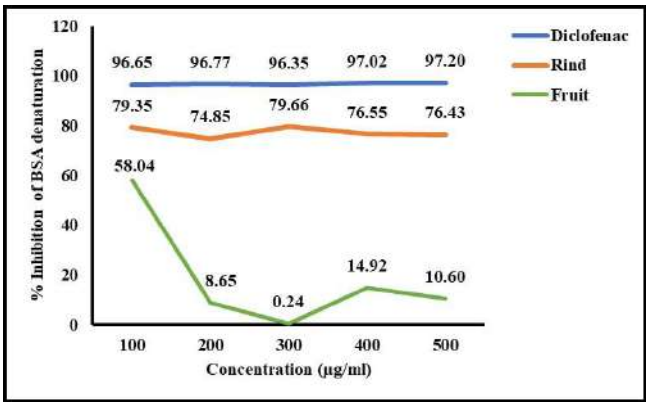


Figure 20: % Of Inhibition Of Bsa Denaturation By Diclofenac, Rind And Fruit Extract

Wound healing activity

Scratch wound assay is performed on L929 cell lines to determine the wound healing activity (Figure 21). % of wound closure is displayed in Table 14 and Figure 22. The IC50 value of G. gummi-gutta rind extract is 29.19µg/mL.

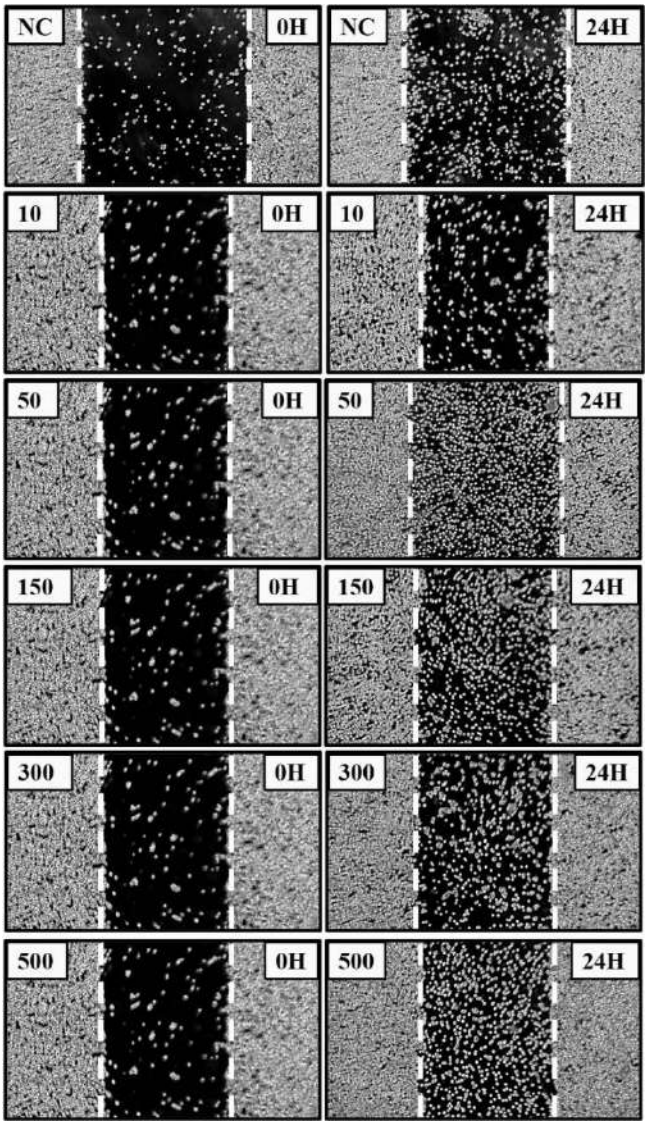


Figure 21: Wound Scratch Assay- Wound Healing Activity Of Rind Extract Against

L929 Cell Lines After 24 H Incubation

Table 14: % Of Wound Closure Of L929 Cell Lines Against Rind Extract

Concentrations (µg/mL)	% Wound closure $[(A_{0h} - A_{24h})/A_{0h} \times 100]$
NC	24.19
10	15.37
50	87.45
150	43.83
300	30.76
500	48.20

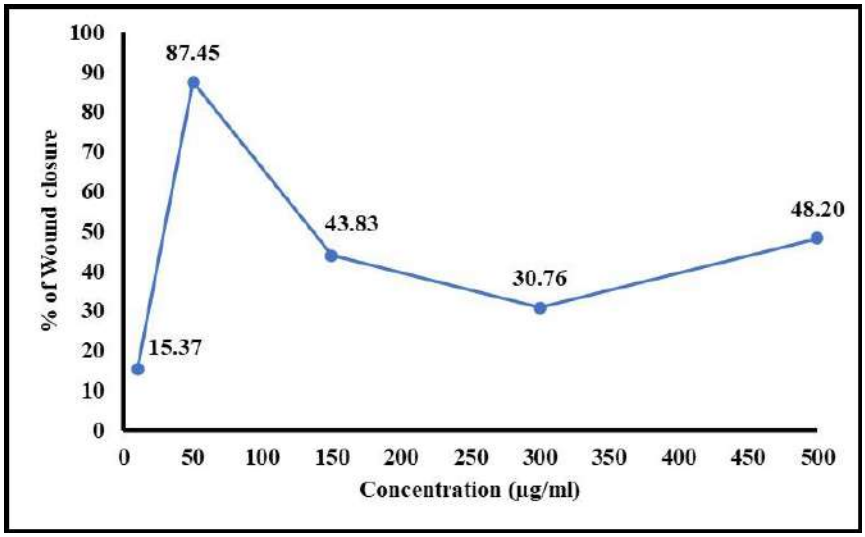


FIGURE 22: % OF WOUND CLOSURE OF L929 CELL LINES AGAINST RIND EXTRACT

Anti-ulcer activity

Anti-ulcer activity was determined using acid neutralizing capacity method (Figure 23 and Table 15).

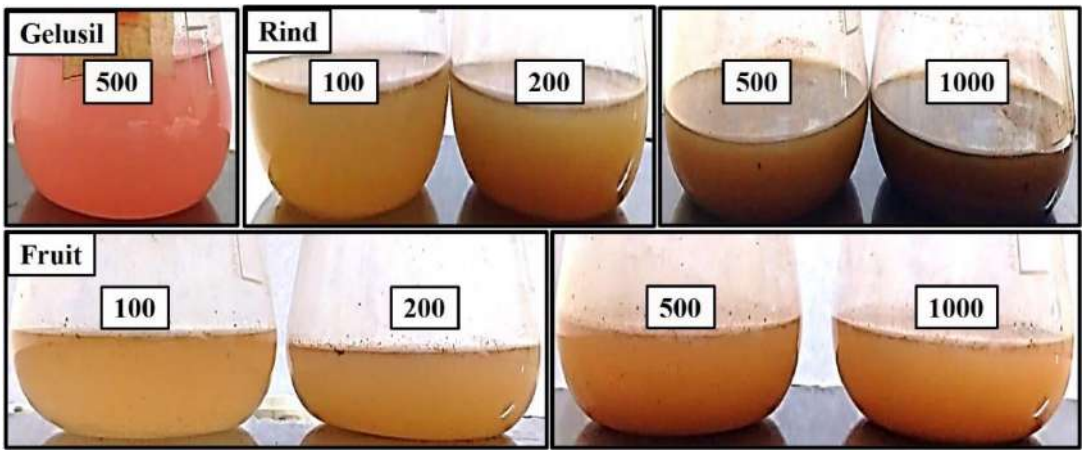


FIGURE 23: ACID NEUTRALIZING CAPACITY METHOD- ANTI-ULCER ACTIVITY OF GELUSIL, RIND AND FRUIT EXTRACT

TABLE 15: ACID NEUTRALIZING CAPACITY OF GELUSIL, RIND AND FRUIT EXTRACT

		Volume of NaOH consumed (mL)	mEq of acid consumed	Acid neutralizing capacity per gram of antacid
Gelusil	500	43.90	8.05	16.10
Rind	100	34.70	12.65	126.50
	200	40.50	9.75	48.75
	500	42.00	9.00	18.00
	1000	42.20	8.90	8.90
Fruit	100	36.00	12.00	120.00
	200	44.10	7.95	39.75
	500	38.00	11.00	22.00
	1000	39.10	10.45	10.45

Anti-microbial activity

Anti-microbial activity was assessed through agar well diffusion method. Both rind (Figure 24) and fruit extract (Figure 25) demonstrated significant anti-bacterial properties against both gram-positive bacteria (Bacillus sphaericus and Staphylococcus aureus) and gram-negative bacteria (Klebsiella pneumoniae and Pseudomonas aeruginosa). However, no activity was showed against fungi (Aspergillus niger). Zone of inhibition of rind and fruit is displayed in Table 16 and Table 17.

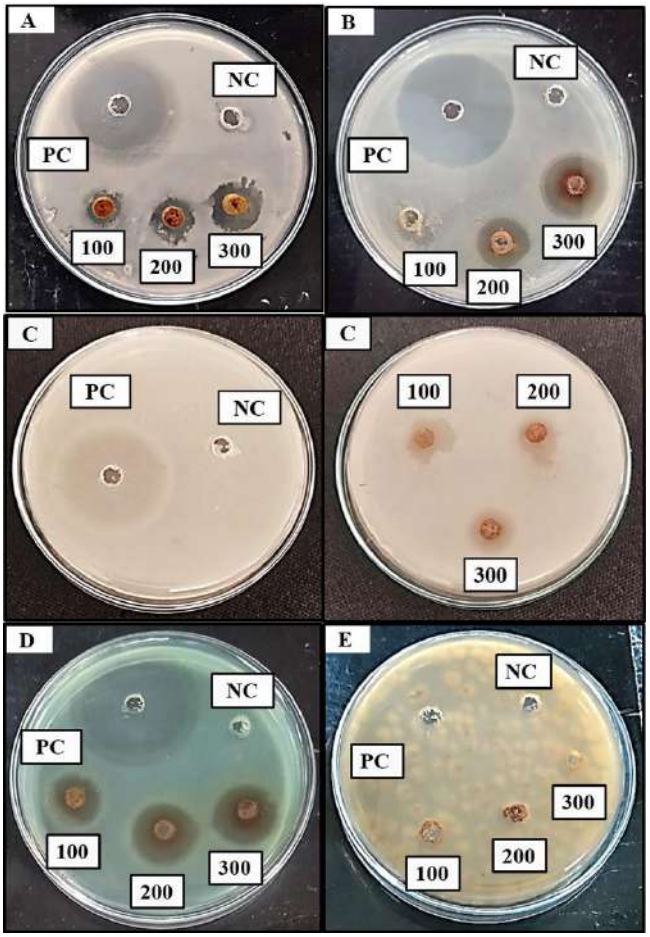


FIGURE 24: ANTI-MICROBIAL ACTIVITY OF RIND EXTRACT

AGAINST BACILLUS SPHAERICUS (A), STAPHYLOCOCCUS AUREUS (B), KLEBSIELLA PNEUMONIA (C), PSEUDOMONAS AERUGINOSA (D), AND ASPERGILLUS NIGER (E)

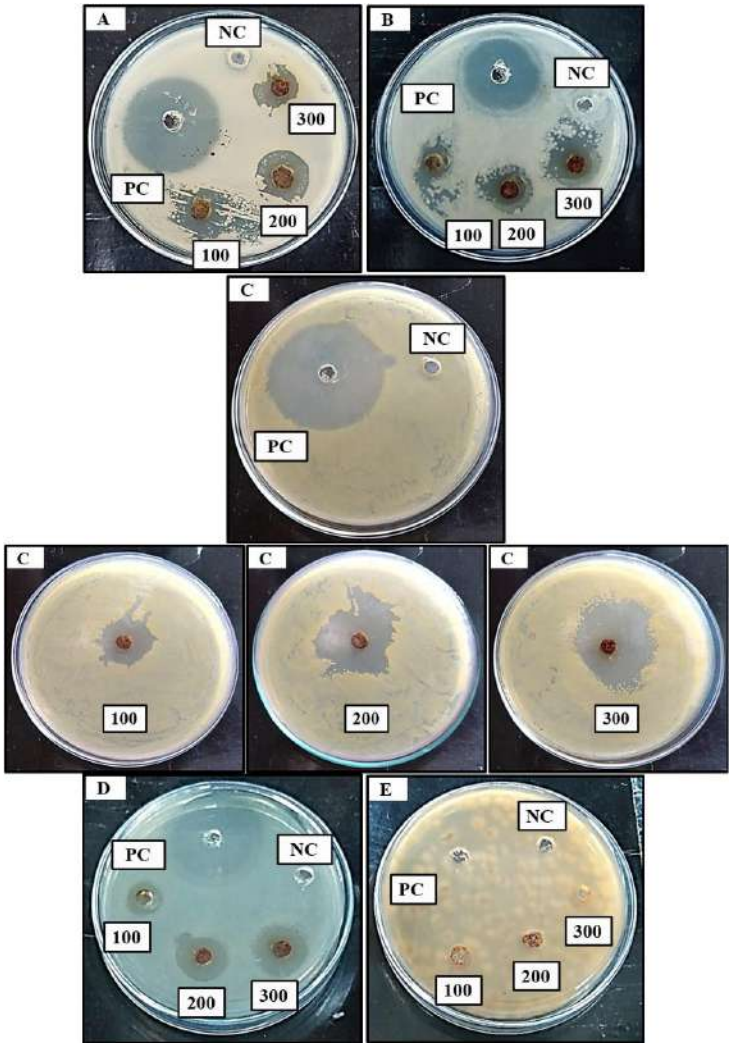


FIGURE 25: ANTI-MICROBIAL ACTIVITY OF FRUIT EXTRACT AGAINST BACILLUS SPHAERICUS (A), STAPHYLOCOCCUS AUREUS (B), KLEBSIELLA PNEUMONIA (C), PSEUDOMONAS AERUGINOSA (D), AND ASPERGILLUS NIGER (E)

TABLE 16: ZONE OF INHIBITION FOR DIFFERENT MICROORGANISM AGAINST RIND EXTRACT

Zone of Inhibition (mm)					
Concentration	**B. sphaericus**	**S. aureus**	**K. pneumonia**	**P. aeruginosa**	**A. niger**
PC (Ciprofloxacin) (0.2% w/v)	32	40	36	43	-
NC (Methanol) (50% v/v)	-	-	-	-	-
100 mg/mL	11	14	16	17	-
200 mg/mL	14	17	13	20	-
300 mg/mL	19	22	15	22	-

TABLE 17: ZONE OF INHIBITION FOR DIFFERENT MICROORGANISM AGAINST FRUIT EXTRACT

Zone of Inhibition (mm)					
Concentration	**B. sphaericus**	**S. aureus**	**K. pneumonia**	**P. aeruginosa**	**A. niger**
PC (Ciprofloxacin) (0.2% w/v)	35	30	45	41	-
NC (Methanol) (50% v/v)	-	-	-	-	-
100 mg/mL	16	11	25	14	-
200 mg/mL	19	16	40	18	-
300 mg/mL	18	18	45	20	-

DISCUSSION

This study emphasizes the phytochemical composition and therapeutic properties of G. gummi-gutta, highlighting its anti-oxidant, anti-diabetic, anti-cancerous, anti-inflammatory, wound healing, anti-ulcer, and antimicrobial activities. Qualitative screening of methanolic extract revealed the presence of flavonoids, alkaloids, tannins, and phenols in the rind, while only flavonoids and tannins were found in the fruit. Similar results were reported in a previous study [6]. Further quantification methods were incorporated to determine the quantities of the aforementioned phytochemicals. However, saponins, terpenoids, and steroids were absent in both extracts. The anti-oxidant activity of G. gummi-gutta rind extract is attributed to flavonoids, phenols, and tannins, while the fruit extract's activity is due to flavonoids and tannins. Studies have shown similar antioxidant effects of these compounds [4, 8]. The highest % DPPH scavenging was observed at 500µg/mL (88.89% for rind and 70.75% for fruit extract), indicating optimal anti-oxidant activity at this concentration. These findings suggest that G. gummi-gutta extracts can protect cells from oxidative stress, contributing to health benefits from antioxidant-rich diets [4]. The anti-diabetic activity of G. gummi-gutta is attributed to phenolic compounds and flavonoids [1, 45]. Rind extract consistently demonstrated high α-amylase inhibition across concentrations (47.21% to 55.86%), with the highest inhibition at 250µg/mL, indicating its effectiveness in enzyme inhibition even at low concentrations. The fruit extract showed a variable inhibition pattern, with minimal inhibition at lower concentrations (50µg/mL and 100µg/mL) due to insufficient bioactive compounds. Significant inhibition began at 150µg/mL (1.54%) and increased to 14.19% at 250µg/mL. This suggests that higher concentrations provide enough bioactive compounds, such as phenolic compounds and flavonoids, to effectively inhibit α-amylase [1, 45]. Overall, G. gummi-gutta rind and fruit extracts hold potential as anti-diabetic agents by inhibiting α-amylase and managing postprandial blood glucose levels by slowing down carbohydrate digestion [1]. The anti-cancer activity was due to the presence of phenols, flavonoids, alkaloids, and tannins [23, 46]. The rind extract showed cytotoxic activity against MCF-7 cells, with a dose-dependent increase in inhibition up to 100µg/mL, where it reaches 32.08%. Overall, the optimal concentration for the extract's efficacy was around 100-200 µg/mL, beyond which its effectiveness does not improve. A recent study also showed the anti-proliferative activity of G. gummi-gutta peel and seed extract against MCF-7 cancer cell lines [47]. The anti-inflammatory activity of G. gummi-gutta is

attributed to phenols and flavonoids [48]. The rind extract consistently showed high inhibition across all concentrations, indicating strong and stable anti-inflammatory potential. In contrast, the fruit extract exhibited significant variability, with sharp drops at 200 µg/mL and 300 µg/mL, suggesting limited efficacy at higher concentrations. The rind extract's consistent performance indicates stable anti-inflammatory compounds, beneficial for conditions like fevers, pain, migraines, and arthritis, while the fruit extract's efficacy is more complex [49]. The wound healing activity of G. gummi-gutta on L929 cell lines were previously unreported. The wound healing activity was attributed to flavonoids, phenols, and tannins and was evaluated on L929 cell lines [50]. The rind extract showed optimal efficacy at 50µg/mL, achieving 87.45% wound closure. This indicates that at this concentration, the bioactive compounds in the extract are highly effective in promoting wound healing by accelerating the repair of damaged tissues, making them useful in the treatment of cuts, burns, and other skin injuries, promoting faster recovery [14, 27]. At lower concentrations (10 µg/mL), the extract may not contain enough bioactive compounds to promote healing effectively. Most wound healing studies on G. gummi-gutta have focused on in-vivo models in mice, with limited in-vitro studies such as those on nasal epithelial cells [51]. The anti-ulcer activity of methanolic extract of G. gummi-gutta rind and fruit was due to the presence of flavonoids and tannins [52]. The data indicates that antacids with higher concentrations of active ingredients generally require less NaOH to neutralize acids, reflecting greater acid-neutralizing capacity per gram. The fruit and rind extracts exhibited the highest anti-ulcer activity at 100 mg/mL, with acid neutralizing capacities significantly higher than the commercial antacid Gelusil. This indicates an optimal concentration around 100 mg/mL for maximum effectiveness in prevention and healing of gastric ulcers, providing a natural alternative or complement to conventional ulcer treatments. The antimicrobial activity of fruit and rind extracts showed a concentration-dependent increase in inhibition zones, with higher concentrations generally providing more significant microbial inhibition [53]. The fruit extract tends to be more effective against K. pneumonia compared to rind extract. However, it was observed that the fungi A. niger showed resistance to methanol extract of the rind and fruit indicating limited antifungal properties, which supports the results reported in previous studies [54, 55]. The antibacterial properties of the extract were due to the presence of phytochemicals such as tannins, phenols, and flavonoids in G. gummi-gutta rind, as well as flavonoids and tannins in G. gummi-gutta fruit [8]. These findings highlight the potential of G. gummi-gutta extracts as antimicrobial agents, particularly against certain bacterial strains.

CONCLUSION:

The present study was aimed at the therapeutic efficacy of G. gummi-gutta rind and fruit, which showed prominent anti-oxidant, anti-diabetic, anti-cancer, anti-inflammatory, wound healing, anti-ulcer, and anti-microbial activities. Moreover, the phytochemicals phenols, tannins, flavonoids, and alkaloids were confirmed in the methanolic extract of G. gummi-gutta rind and fruit. Additionally, our study focused on in-vitro assays, which provided remarkable evidence of the therapeutic efficacy of G. gummi-gutta rind and fruit against various disease conditions such as cardiovascular disease, diabetes, inflammation, ulcer, cancer, wounds, bacterial infections, etc. Further studies on in-vivo models and clinical trials might generate more information about its therapeutic mechanism and translational potential.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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