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# QUANTITATIVE INVESTIGATION OF PHARMACOGNOSTIC PHYTOCHEMICALS AND *IN-VITRO* RADICAL SCAVENGING AND THERAPEUTIC POTENTIAL OF *PSIDIUM GUAJAVA LINN* FRUIT EXTRACT

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**Abstract :**
The aim of present study is quantitative investigation of pharmacognostic phytochemicals and in-vitro radical scavenging and therapeutic potential of *P. guajava.* Fruits of *P. guajava* have no of reported pharmacological activities such as anti-diarrheal, anti-hypertensive, anti-lipidemic, anti-cancer, etc. To know the actual fact about these actions this research has been conducted. Selected fruits undergone for evaluations of various pharmacognostic parameters. Standardization of fruit powder has done by loss on drying (6 %), total ash value (2.50%), acid insoluble ash (2.8 %), water insoluble ash (3.6 %) and extractive value for ethanol (80.42%). Preliminary phytochemical screening done on ethanolic extract shows the presence of active phytoconstituents like glycosides, phenols, flavonoids, tannins etc. The extract was subjected to quantitative determination for total phenolic content by Folin- Ciocalteau method (500ug/ml) which shows maximum concentration of phenols i.e. 51.06 mg/GAE/g; flavonoids content by Aluminum Chloride Colorimetric method (1mg/ml) gives 41.10 mg/QC/g which is maximum conc. of flavonoids. And *In-vitro* radical scavenging potential has checked by DPPH method, and Nitric Oxide method, (1mg/ml) sample concentration shows 16.49 % and 45.56 % radical scavenging activity respectively. The phenolic compound found rich in white guava. Different study shown that the phenolic compound Rosmarinic acid possesses more antioxidant potential than Vitamin E and helps to relief from stress, neurodegenerative diseases such as Alzheimer and Parkinson's, cancer, cardiac disease, inflammation, etc.

**Keywords**: *Psidium Guajava Linn*, Rosmarinic acid, Glycosides, Radical scavenging.

## Introduction:

Psidium guajava (common name-guava) is well known tropic tree which is abundantly grown for fruit. It belongs to phylum Magnoliophyta, class Magnoliopsida and Myrtaceae family [1]. It has about 133 genera and more than 3,800 species. Psidium guajava and it's all parts have an old history of medicinal value [2]. The plant is well known by a common name "Guava" in English, guayabo in Spanish, goyaveandgoyavier in French, guyabaorgoeajaab in Dutch, goiaba and goaibeira in Portuguese and jambubatu in Malaya. Pichi, posh and enandi are the names commonly used in

Mexico and America [3]. Guava plant grows widely in the tropic areas because it is a plant that can be grown on a big range of soils [4]. Ascorbic acid and citric acid are the major ingredients of guava that play important role in anti-mutagenic activity [5]. The chemical structures of quercetin and ascorbic acid. The skin of fruit contains ascorbic acid in very high amount; however, it may be destroyed by heat. The strong pleasant smell of fruit is credited to the carbonyl compounds [6]. Guava fruit contains terpenes, caryophyllene oxide and p-selinene in large quantity which produce relaxation effects [7]. The flavonoid content is higher in the methanolic extract of the guava [8]. There are 41 hydrocarbons 25 esters, 13 alcohols and 9 aromatic compounds in guava [9]. Titratable acidity and the total soluble solids are present in fruit, guajadial is also present in guava, phenolic compound like rosmarinic aicid, chlorgenic acid caffeic acid are also present in fruits[10].

This plant finds applications for the treatment of diarrhea, dysentery, gastroenteritis, hypertension, diabetes, caries and pain relief and for improvement in locomotors coordination. Fruits contains high content of organic and inorganic compounds like secondary metabolites e.g. antioxidants, polyphenols, antiviral compounds, anti-inflammatory compounds. The phenolic compounds in guava help to cure cancerous cells and prevent skin aging before time. The presence of terpenes, caryophyllene oxide and *p*-selinene produces relaxation effects. Guava leaves contain many compounds which act as fungistatic and bacteriostatic agents. Guava has a high content of important antioxidants and has radio-protective ability [21]

## PLANT MATERIAL

### Collections and Drying

Fruit of *Psidium Guajava Linn,* purchase from local market at Nanded Maharashtra, India, in the month of December - January.

### Authentication

The plants, *Psidium Guajava Linn*, was authenticated by Botanist of Botanical Survey of India, Pune by comparing morphological features. The herbarium of the plant specimen was deposited at Botanical Survey of India, Pune; with the Voucher specimen number 01-02 (Ref. No.BSI/WRC/Iden. /Cer. /2022/0502220000411. Dated 07/02/2022).

### Preparation of Plant Material

Fruits of *Psidium Guajava Linn,* dried under shade and & powdered. Powdered part was passed through 40# and stored in closed air tight container individually.

## PHARMACOGNOSTIC STUDY-

Fruits of *Helicteres isora linn* pharmacognostic study were carried as per below procedure and methods [11].

## MACROSCOPY

Organoleptic characters, extra feature and macroscopical details for all parts of plants were carried out.

## MICROSCOPY

Microscopical study was done according to method described by Khandelwal, (2008). Powder stained with phloroglucinol: Hydrochloric acid (1:1) and observed under microscope at 10X, 45X.

**Evaluation of Physical Constants**- physical parameters has been checked for determination of foreign organic matter, determination of moisture content, ash value, Determination of Acid - insoluble ash, Determination of Water- soluble ash, Extractive values, Determination of water-soluble extractive value, Determination of Alcohol-soluble extractive value [12,13].

## EXTRACTION OF *HELICTERES ISORA LINN* FRUIT EXTRACT:

### Maceration:

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

## PREPARATION OF ETHANOLIC EXTRACT FROM *Psidium guajava linn* FRUIT

The *Psidium guajava linn* fruit, washed well using clean water to remove adhering matter, dried under shade and powdered separately. 1kg of powder was transferred into conical flasks containing 100ml of methanol. The flasks were left for two days at room temperature with occasional stirrings. Later, the contents of the flasks were filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrates were evaporated to dryness and stored in refrigerator until use. The weight and colour of extracts was noted **[14].**

**PHYTOCHEMICAL SCREENING:** The phytochemical studies of the plant are an important step to identify the bioactive compounds present in the medicinal plants. These compounds have various use in medicinal industries to prepare novel drugs. Phytochemical screening of ethanolic fruit extracts has done by the procedure as given in the book of Dr. K.R. Khandelwal. Screening of extract has done for glycosides, flavonoids, tannins, saponins, proteins, sugars etc [15].

## THIN LAYER CHROMATOGRAPHY

**Principle:** Thin layer chromatography, like other chromatography techniques, is based on the idea of separation (TLC). The difference is based on the relative affinity of each phases' chemicals. During the mobile phase, the compounds travel over the surface of the stationary phase. The movement occurs in such a manner that compounds with a higher affinity travel slowly while others rush to the stationary process. Following that, the combination will be degraded. The various components of the combination show on the plates as spots at their respective phases once the separation procedure is completed. Appropriate identification techniques categories their personality and characteristics [16-18].

### *Steps involved in performing TLC of extracts*

- **Preparation of TLC plate:** Prepared the slurry of adsorbent media (silica gel-G) in distilled water and poured the slurry on the TLC glass plates to obtain a thin layer.
- **Activation of TLC plate:** TLC plate was activated by heating in oven for 30min at $105^{O}C$.
- **Sample application:** Dipping the capillary into the solution to be examined and applied the sample by capillary touched to the thin layer plate at a point about 2cm from the bottom. Air-dried the spot.
- **Chamber saturation:** The glass chamber for TLC should be saturated with mobile phase. Mobile phase was poured into the chamber and capped with lid. Allowed saturating about 30 min
- **Chromatogram development:** After the saturation of chamber and spotting of samples on plate, it was kept in chamber. The solvent level in the bottom of the chamber must not be above the spot that was applied to the plate, as the spotted material will dissolve in the pool of solvent instead of undergoing chromatography. Allowed the solvent to run around 10-15cm on the silica plate

**Visualization:** Plates were removed and were examined visually, under UV and suitable visualizing agent (ethanol, sulphuric acid, glacial acetic acid and anisaldhyde reagent (135:5:1:3.7)) after that $R_f$ was calculated by formula

## TOTAL PHENOLIC CONTENT DETERMINATION

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging.

### *Helicteres isora linn* fruit extract:

1. Total phenolic content was analysed by Folin –Ciacaltue Colorimetric method.
2. The gallic acid was used as standard, solution (5,10,15,20 &20 µg/ml) was prepared in methanol and 100 µg/ml stock solution of test sample were also prepared.
3. Test sample was prepared by adding 0.3ml of distilled water, 0.4ml of Folin – Ciacaltue reagent and sample were allowed to stand for 6min before adding 4ml of 7 % sodium carbonate ($Na_2CO_3$).
4. The water was used to adjust the volume up to 10ml. After incubation at 90min, the absorbance was recorded 765nm. Reference curve were prepared using (5-25 µg/ml) of gallic acid and results were prepared are presented at amount of phenolic content (Gallic acid Equivalent) per dry weight.
5. The blank solution was prepared using 0.4ml of Folin- Ciacaltue reagent, 4ml of 7 % of sodium carbonate and water was used to adjust the volume up to 10ml.

## TOTAL FLAVONOID CONTENT DETERMINATION:

1. Total Flavonoid Content was determined by Aluminum Chloride Colorimetric Method
2. The solution standard sample of quercetin was prepared using 50% methanol to produce concentration of 1000 µg/ml.
3. The total flavonoid contents were determined in the test samples a with minor modification.
4. Briefly, 40 of µl test samples and quercetin at various concentrations were separately mixed with 200 µl of AlCl3.6H2O (2%, w/v) in 96 well plate.
5. The resultants mixtures were incubated for 10 minutes at normal temperature.
6. The optical density was measured at 440 nm using microplate reader (EPOCH, BioTek-Agilent, USA).
7. The standard calibration curve for quercetin standard was prepared from stock solution and used for calculation of total flavonoid contents for test samples. The results are expressed as quercetin content in 1 g of test sample [19].

## *IN VITRO* ANTIOXIDANT ACTIVITY BY DPPH METHOD

**Principle**: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical and is commonly used to evaluate the radical scavenging activity of antioxidant agents. The principle of this method is based on the fact that, decrement of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H.

### Procedure:

1. The free radical scavenging activity of test sample was determined by DPPH scavenging method.
2. 0.1mM DPPH solution was prepared in methanol by adding 39.4 mg of DPPH in 1000 ml of methanol, and to 0.5 mL of this solution, 1.5 mL of test sample dissolved in DMSO was added at various concentrations (1000, 500, 250, 125, 62.5, 15.62, 1.95ug/ml).
3. The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes.
4. Then the absorbance was measured at 517nm using non-coated 96 well plate on microplate reader (EPOCH, Agilent BioTek, US). Ascorbic acid was used as standard compound.
5. Reduction in absorbance by test compounds indicates radical scavenging activity.

The scavenging activity by the DPPH radical was determined by

**DPPH scavenging effect (% inhibition) = {(A0 -A1)/A0) ×100}**

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance test compound or Ascorbic acid

## *IN VITRO* ANTIOXIDANT ACTIVITY BY NITRIC OXIDE METHOD

**Principle:**

Nitric oxide (NO) is a free radical that produced by interaction of NO with oxygen or reactive oxygen species. It is free radical due to its unpaired electron and exhibits similar properties like superoxide free radicals. This assay is based on the reduction of nitrate to nitrite by a reducing agent at 37 0C. Converted nitrite and endogenous nitrite are collectively converted by Griess reagent to a blue coloured azo compound. This compound can be measured spectroscopically between 580-630 nm and absorbance is directly proportional to the total nitric oxide concentration.

**Procedure:**

1. Nitric oxide (NO) radical scavenging activity of test extracts *Psidium guajava liin* were done, various concentrations of test compounds test extract *Psidium guajava liin*, such as 1000, 500, 250, 125, 62.5, 15.62, 1.95 ug/ml were dissolved in dimethylsulphoxide (DMSO).
2. To which, 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was added, then, 1 ml of various concentrations 1000, 500, 250, 125, 62.5, 15.62, 1.95 of test compounds were mixed, and to this equal volume of freshly prepared Griess reagent was added, solution was then incubated at 25°C for 3 hours.
3. From above solution, 100 µl of the reaction mixture was transferred to a noncoated 96-well plate, and the absorbance was read at 546 nm using a microplate reader (EPOCH, Agilent BioTek, US). Ascorbic acid was used as standard control.

The percentage of nitrite radical scavenging activity of test compounds was calculated by

$$\text{Nitric oxide scavenging activity} = \frac{\text{Absorbance of control-Absorbance of test compounds} \times 100}{\text{Absorbance of control}}$$

## PHARMACOGNOSTIC STUDY:

### Macroscopy

The fruits of *Psidium guajava linn* is green coloured when fresh, 6-7 cm oval and smooth in appearance.

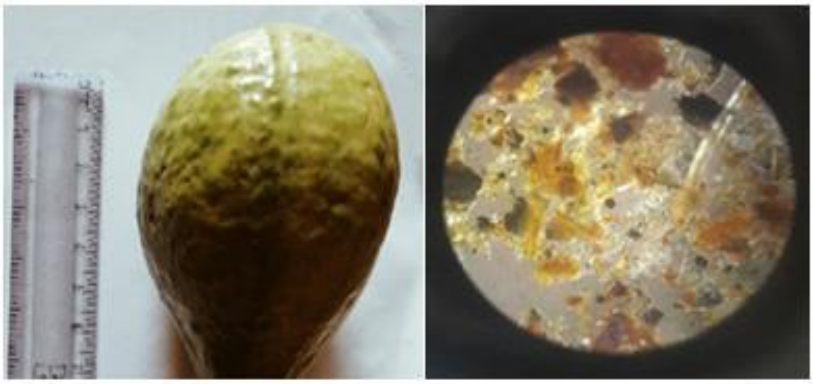

**Figure 1:** Macroscopy

## POWDER MICROSCOPY:

The powder is brownish in coloured with charecteristic odour and slightly bitter taste. Powder shows the character like cells of epicarp, Stellate trichomes, fragments of misocarp and endocarp

**EVALUATION OF PHYSICAL CONSTANTS:**

The drug material has been evaluated for various physical constants like determined of foreign organic matter, moisture content, ash value, Acid -insoluble ash, Water- soluble ash, Extractive values, water-soluble extractive value and Alcohol-soluble extractive value as procedure described in IP1996, shows the significant result as shown in table no. 1.

***Table 1*** *Evaluation of Physical Constants*

| **Parameters** | *Psidium guajava liin* |
|---|---|
| Chloroform Soluble Extractives Values % (w/w) | 17.29 + 0.38 |
| Methanol Soluble Extractives Values % (w/w) | 56.21+0.27 |
| Ethanol Soluble Extractives Values % (w/w) | 80.42+0.51 |
| Ethyl Acetate Soluble Extractives Values % (w/w) | 70.5 +0.61 |
| Water Soluble Extractives Values % (w/w) | 4.35 +0.52 |
| Total Ash Values % (w/w) | 2.4 +0.59 |
| Acid Insoluble Ash Values % (w/w) | 2.8 +0.44 |
| Water Soluble Ash Values % (w/w) | 3.6±0.66 |
| Moisture Content % (w/w) | 3.14±0.66 |

**PHYTOCHEMICAL SCREENING:** Phytochemical study helps to know the composition vital phytochemical present in the drug which will helps to design new drugs and development of formulation. Phytochemical screening of *Helicteres isora linn* Ethanolic fruit extract shows the presence of glycosides, flavonoids, saponin glycosides, proteins and tannins and phenols as shown in table no. 2

***Table 2:*** *Phytochemical screening of extract*

| **Sr. No.** | **Test For** | *Psidium guajava liin* **Ethanolic fruit extract** |
|---|---|---|
| **1.** | Alkaloids | - |
| **2.** | Carbohydrate | - |
| **3.** | Glycosides | + |
| **4.** | Flavonoids | + |
| **5.** | Proteins | - |
| **6** | Fats and Oils | + |
| **7** | Tannins & Phenols | + |
| **8** | Saponin Glycosides | + |

**THIN LAYER CHROMATOGRAPHY:**

Ethanolic fruit extract, was performed chromatographed on pre-coated silica gel plates with solvent mixture Acetic Acid: Methanol:Water (6.4:3.2:1.2:0.8). The spots were visualized by spraying the plates with a mixture of ethanol, sulphuric acid, glacial acetic acid and anis-aldehyde reagent (135:5:1:3.7) and heating them in an oven for 3–5 min at 90 °C. The Rf value of extracted residue was in agreement corresponding to standard diosgenin, (Rf 0.56) which confirmed the presence of diosgenin. The Rf value of extracted residue was in agreement corresponding to standard diosgenin, (Rf 0.5) which confirmed the presence of rosmarinic acid.

## TOTAL PHENOLIC CONTENT

Total phenolic content was analysed by Folin –Ciacaltue Colorimetric method. Gallic acid was used as standard by taking concentration (5, 10, 15, 20, & 25 ug/ml) and calibration curve has drawn concentration vs absorbance. HIET concentration (10, 20, 30, 40 & 50 ug/ml) shown significant concentration of phenols. Data expressed as (±) SD (n=3). (µg GAE/ µg DW denote Gallic acid equivalent per dry weight of extract.

***Table 3.*** *Total Phenolic Content*

| Concentration of Gallic acid (ug/ml) | Absorbance at 765nm | TPC of Gallic acid (µg Quercetin/ µg DW) | Concentration of (PGET) (ug/ml) | Absorbance 765nm (PGET) | TPC of (PGET) (µg GAE/ µg DW) |
|---|---|---|---|---|---|
| 5 | 0.250 | **45.09 ± 0.12** | 100 | 0.392 | **39.08 ± 0.00** |
| 10 | 0.414 | **51.03 ± 0.19** | 200 | 0.599 | **41.03 ± 0.3** |
| 15 | 0.690 | **53.05 ± 0.00** | 300 | 0.735 | **45.12 ± 0.10** |
| 20 | 0.852 | **56. 08 ± 0.22** | 400 | 0.898 | **47.07 ± 0.05** |
| 25 | 1.016 | **65.09 ± 0.60** | 500 | 0.102 | **51.06 ± 0.99** |

**Note: Data expressed as (±) SD (n=3). ( µg GAE/ µg DW denote Gallic acid equivalent per dry weight of extract**

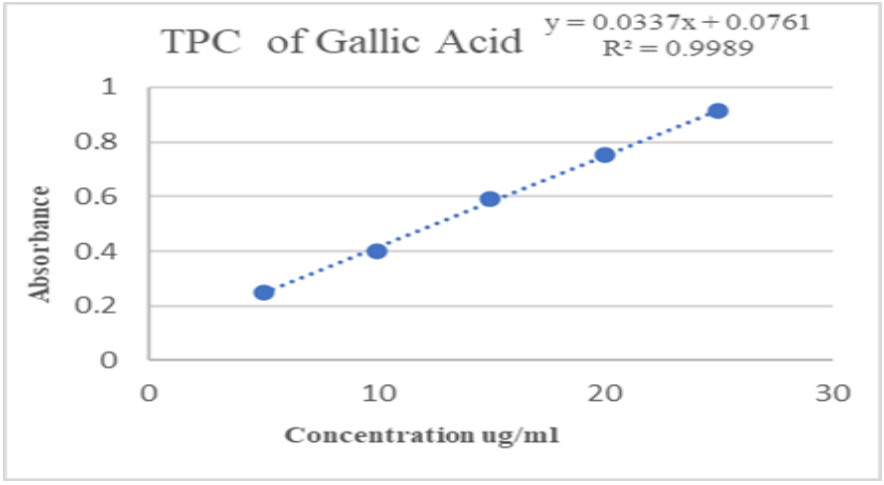


***Figure 4:*** *Calibration curve of Gallic Acid*

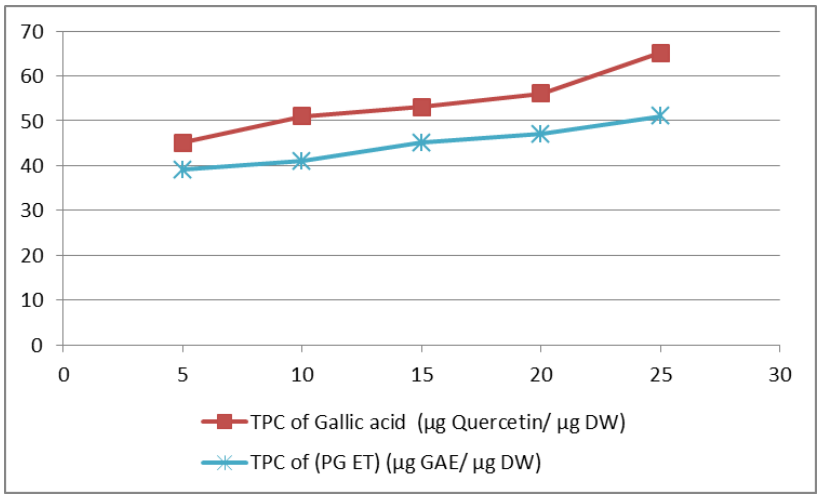


***Figure 5.*** *Total Phenolic Content*

**TOTAL FLAVONOID CONTENT**

Total Flavonoid Content was determined by Aluminium Chloride Colorimetric Method. Quercetin was taken as standard at the concentration (6.25, 15, 250, 500, & 1000 ug/ml). And the extract concentration was taken same as standard shows significant concentration of flavonoids present in the extract. Data expressed as (±) SD (n=3). (µg Quercetin/ µg) denote Quercetin equivalent per dry weight of quercetin.

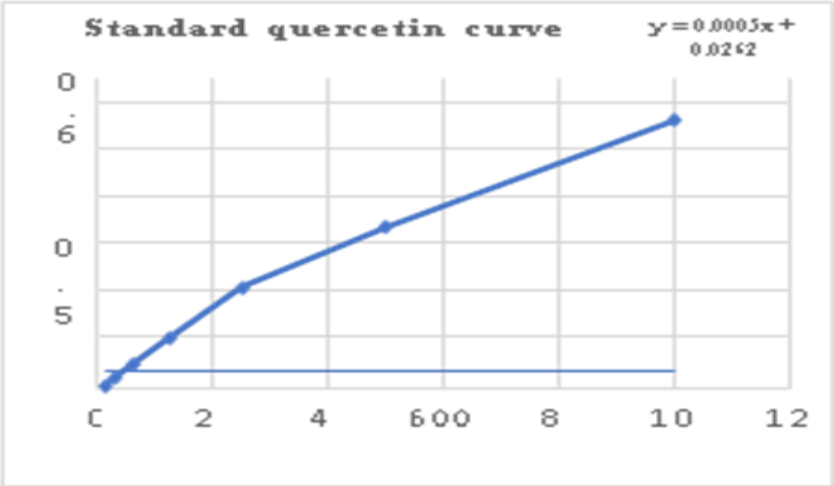


**Figure 6 :** Calibration curve for Quercetin

**Table 4:** Total Flavonoids Content Figure

| Conc. of Quercetin (µg /ml) | Abs. at 517nm | TFC (µg of Quercetin /µg DW) | Conc. of (PGET) (ug/ml) | Abs. at 517nm (PGET) | TFC (PGET) (µg of Quercetin/ µg of sample |
|---|---|---|---|---|---|
| 62.5 | 0.049 | 25.03 ± 0.55 | 62.5 | 0.008 | 15.49 ±0.01 |
| 125 | 0.099 | 27.01 ± 1.00 | 125 | 0.015 | 25.00 ± 0.80 |
| 250 | 0.197 | 31.50 ±0.90 | 250 | 0.049 | 33.07 ± 0.33 |
| 500 | 0.312 | 49.03 ± 0.33 | 500 | 0.058 | 38.00 ± 0.50 |
| 1000 | 0.519 | 60.00 ± 0.65 | 1000 | 0.063 | 41.10 ± 0.20 |

**Note: Data expressed as (±) SD (n=3). ( µg Quercetin / µg DW denote quercetin equivalent per dry weight of extract**

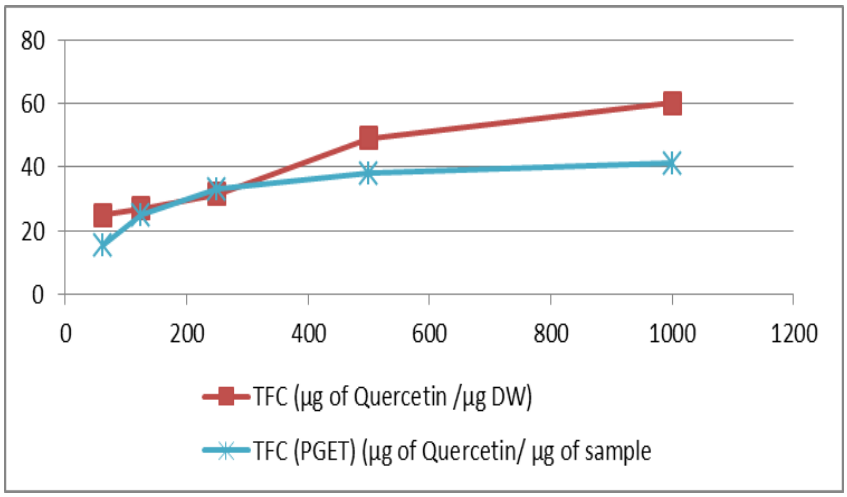


**Figure 7:** Total Flavonoids Content Figure

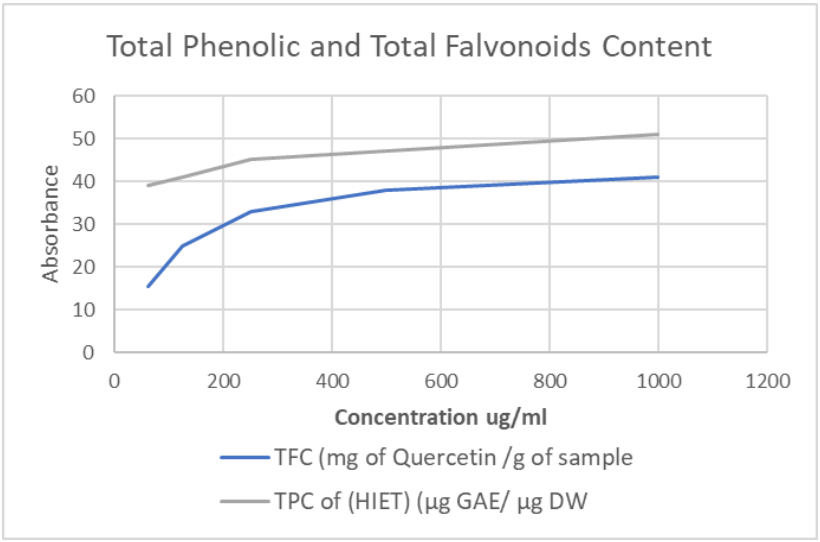


**Figure 8:** Chart for Total Phenolic and Total Flavonoids Content

## *IN VITRO* ANTIOXIDANT ACTIVITY BY DPPH METHOD

• The free radical scavenging activity of test sample was determined by DPPH scavenging method, Ascorbic acid was used as standard compound at concentration (1.92, 15.62, 62.5, 125, 250, 500, & 1000 ug/ml) and test concentrations has also been taken same as standard. Test sample shows significant radical scavenging potential. Data expressed as mean ± SD (n=3). µg Vit.C/ µg DW per dry weight.

## *IN VITRO* ANTIOXIDANT ACTIVITY BY NITRIC OXIDE METHOD

• Nitric oxide (NO) radical scavenging activity of test extracts *Psidium guajava liin* were done, various concentrations of test compounds test extract *Psidium guajava liin*, such as 1000, 500, 250, 125, 62.5, 15.62, 1.95 ug/ml were dissolved in dimethylsulphoxide (DMSO). The standard was used same Vitamin C.

$$\textbf{Nitric oxide scavenging activity} = \frac{\textbf{Absorbance of control} - \textbf{Absorbance of test sample}}{Absorbance\ of\ control} \textbf{x100}$$

***Table 5:*** *Anti-oxidant activity by DPPH method & Nitric oxide*

| Conc. of Vit. C (ug/ml) | Abs. 517 nm | (%) Scavenging activity DPPH | Conc. of PGET (µg/ml) | Abs. of PGET at 517 nm | (%) Scavenging activity of (HIET)by DPPH | Conc. (ug/ml) | Abs. at 596 nm | (%) Scavenging activity, of (PGET) by NO |
|---|---|---|---|---|---|---|---|---|
| 1.95 | 0.059 | 40.22 ± 0.03 | 1.95 | 0.013 | 1.09 ±0.01 | 1.95 | 0.112 | 1.03 ± 0.03 |
| 15.62 | 0.045 | 55.43 ± 0.05 | 15.62 | 0.075 | 3.088 ±0.22 | 15.62 | 0.102 | 2.150 ± 0.50 |
| 62.5 | 0.029 | 77.17 ± 0.30 | 62.5 | 0.065 | 8.055 ± 0.33 | 62.5 | 0.097 | 2.99 ± 0.65 |
| 125 | 0.019 | 81.52 ± 0.50 | 125 | 0.059 | 10 .05±0.00 | 125 | 0.094 | 3.09 ± 0.54 |
| 250 | 0.016 | 88.04 ± 0.23 | 250 | 0.054 | 13.05 ± 0.36 | 250 | 0.09 | 7.22 ± 0.56 |
| 500 | 0.010 | 90.22 | 500 | 0.049 | 25.26 ± 0.25 | 500 | 0.082 | 15.46 ±0.20 |
| 1000 | 0.009 | 91.3 | 1000 | 0.042 | 45.56 ± 0.56 | 1000 | 0.081 | 16.49 ± 0.32 |

**Note: Data expressed as mean ± SD (n=3). µg Vit.c µg DW per dry weigh**

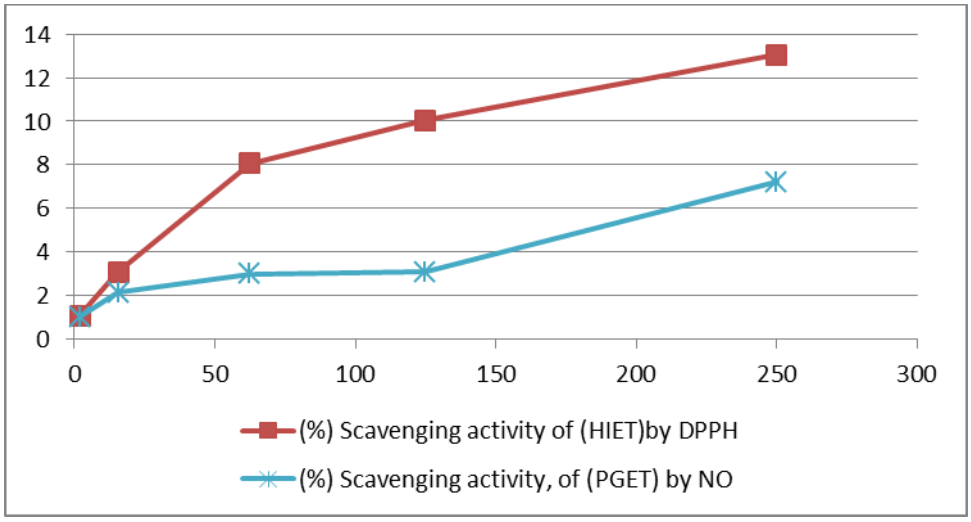


***Figure 9:*** *Anti-oxidant activity by DPPH method & Nitric oxide*

## CONCLUSION:

From the research investigation conducted, it could be said that the fruit extracts possess potent medicinal values and shows the presence of vital phytoconstituents like glycosides, alkaloids, tannins, phenols, proteins etc. of pharmacological significance. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened can yield compounds of pharmaceutical importance. The biochemical analysis revealed that fruit has diosgenin and rosmarinic acid in good amount. Further research can be carried out to isolate, purify and characterize the detail chemical constituents in the fruit with a view to utilise these bioactive compounds in drug development. This study is an attempt to validate the tribal claims of medicinal value of *Psidium guajava linn* through experimental observation.

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