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## Flavonoid Fraction, Total Glycoside, Free Radical Scavenging Activities of *Ananas Comosus* Methanol Leaf Extract

Swati Vinod Jogdand<sup>1</sup>, S .Vasantha Lakshmi<sup>2</sup>, Vishnupriya Sethuraman<sup>3\*</sup>,  
Laxminarayan Patidar<sup>4</sup>, Santosh Kumar Ranajit<sup>5</sup>, Tasneem Mohammed<sup>6</sup>, A.Nanaji<sup>7</sup>,  
B. Madhu Harika<sup>8</sup>

<sup>1</sup>Department of Pharmacology, CAYMETs Siddhant College of Pharmacy, Sudumbare, Pune, India Pin- 412109.

<sup>2</sup>Department of Pharmacognosy, Avanthi Institute of Pharmaceutical Sciences, Vizianagaram Andhra Pradesh, India Pin- 535006.

<sup>3</sup>Department of Nutrition and Dietetics, Rabiammal Ahamed Maideen College for Women Tiruvarur, Tamil Nadu Pin Code:-610001.

<sup>4</sup>Department of Pharmacognosy. B.R. Nahata College of Pharmacy, Mandsaur University, Mandsaur (MP) – India Pin- 4583001.

<sup>5</sup>Department of Pharmacology, Centurion University of Technology and Management, Bhubaneswar, Pin-752050, Odisha.

<sup>6</sup>Department of General Science, Ibn Sina National College for Medical Studies, Jeddah, Saudi Arabia. Pin- 21418.

<sup>7</sup>Department of Pharmacognosy, Avanthi Institute of Pharmaceutical Sciences, Vizianagaram, Andhra Pradesh, India PIN: 535006.

<sup>8</sup>Department of Pharmaceutical Chemistry, Vikas Institute of Pharmaceutical Sciences , Near Airport, Rajahmundry, Andhra Pradesh, India Pin- 533105.

### Corresponding Author

Vishnupriya Sethuraman<sup>3\*</sup>

<sup>3</sup>Department of Nutrition and Dietetics, Rabiammal Ahamed Maideen College for Women Tiruvarur, Tamil Nadu Pin Code:-610001.

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doi: [10.33472/AFJBS.6.6.2024.2009-2022](https://doi.org/10.33472/AFJBS.6.6.2024.2009-2022)**ABSTRACT:**

This study aimed to evaluate the phytochemical composition and antioxidant potential of a plant extract through various assays. Phytochemical qualitative analysis revealed the presence of anthraquinones, tannins, saponins, flavonoids, glycosides, terpenoids, and steroids. Further quantitative assays determined the total flavonoid fraction and total glycoside content in the extract. Additionally, the DPPH radical scavenging activity assay was conducted to assess its antioxidant capacity. Results indicated significant levels of flavonoids and glycosides in the extract, with total concentrations of 2036.2 µg and 861.9 µg, respectively. The DPPH assay revealed concentration-dependent scavenging activity, with the extract demonstrating substantial inhibition of DPPH radicals, comparable to reference antioxidants Quercetin and Vitamin C at higher concentrations. These findings suggested the potential therapeutic value of the extract as a natural antioxidant agent.

**Keywords:** Flavonoid fraction, Total glycoside, Antioxidant, Free radical, DPPH radical.

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**1. INTRODUCTION**

Medicinal plants have played a significant role throughout human history in managing and treating various diseases. Across cultures and civilizations, traditional healers and herbalists have relied on the healing properties of plants to alleviate ailments and promote health. The therapeutic potential of medicinal plants stems from their rich phytochemical composition, which includes a diverse array of bioactive compounds such as alkaloids, flavonoids, phenols, terpenoids, and polyphenols [1, 2]. These compounds possess a wide range of pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, analgesic, and immunomodulatory effects, making them valuable assets in disease management. One of the primary advantages of medicinal plants is their holistic approach to health, addressing the underlying causes of disease rather than merely alleviating symptoms. Unlike conventional pharmaceuticals that often target specific molecular pathways or symptoms, medicinal plants contain complex mixtures of bioactive compounds that act synergistically to exert therapeutic effects on multiple targets within the body [3-7]. This multifaceted approach not only enhances efficacy but also reduces the risk of adverse side effects and promotes overall well-being. Furthermore, medicinal plants offer a sustainable and environmentally friendly approach to healthcare. Many pharmaceutical drugs are derived from plant sources or inspired by natural compounds found in plants. By harnessing the healing power of nature, herbal medicine can

provide accessible, affordable, and culturally relevant healthcare solutions, particularly in resource-limited settings where access to conventional medical services may be limited. Numerous studies have demonstrated the efficacy of medicinal plants in managing and treating various diseases, including chronic conditions such as diabetes, hypertension, cardiovascular diseases, and cancer, as well as infectious diseases such as malaria, tuberculosis, and viral infections. For example, plants such as bitter melon, cinnamon, and fenugreek have been shown to lower blood sugar levels and improve insulin sensitivity in diabetes management [8-12]. Similarly, herbs like garlic, hawthorn, and ginger have been used to support cardiovascular health by reducing cholesterol levels, lowering blood pressure, and improving circulation. In addition to their therapeutic benefits, medicinal plants also offer cultural and socioeconomic value, preserving traditional knowledge and practices passed down through generations. Integrating traditional herbal remedies with modern healthcare systems can enhance healthcare access, promote cultural diversity, and empower local communities to take charge of their health. Overall, medicinal plants play a crucial role in managing and treating diseases, offering a holistic, sustainable, and culturally relevant approach to healthcare. By leveraging the therapeutic potential of nature's pharmacy, we can unlock new possibilities for health promotion, disease prevention, and personalized medicine, ultimately improving the quality of life for individuals and communities worldwide [13-18].

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defence mechanisms, is implicated in the pathogenesis of various diseases. ROS, including free radicals like superoxide anion ( $O_2^-$ ), hydroxyl radical ( $\bullet OH$ ), and non-radical species like hydrogen peroxide ( $H_2O_2$ ), are generated during normal cellular metabolism and play essential roles in cell signalling and immune defence. However, excessive ROS production or inadequate antioxidant defences can lead to oxidative damage to biomolecules such as lipids, proteins, and DNA, contributing to the development and progression of numerous diseases. Medicinal plants have garnered significant attention for their potent antioxidant properties, which make them valuable resources in combating oxidative stress-related diseases [19-25]. Plants produce a wide array of bioactive compounds, including phenolic compounds, flavonoids, carotenoids, and vitamins, which possess strong antioxidant activity and can scavenge free radicals, neutralize ROS, and restore redox balance within cells. These phytochemicals act through various mechanisms, including direct radical scavenging, metal chelation, and upregulation of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The importance of medicinal plants in managing oxidative stress-related diseases is evident across a spectrum of conditions, including cardiovascular diseases, neurodegenerative disorders, diabetes, cancer, and aging-related conditions. For instance, in cardiovascular diseases, oxidative stress contributes to endothelial dysfunction, inflammation, and atherosclerosis [19-25]. Medicinal plants like green tea (*Camellia sinensis*), garlic (*Allium sativum*), and hawthorn (*Crataegus* spp.) have been shown to improve endothelial function, reduce oxidative stress markers, and lower cardiovascular risk factors. Similarly, oxidative stress plays a central role in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). Medicinal plants rich in antioxidants, such as curcumin from turmeric (*Curcuma longa*), resveratrol from grapes (*Vitis vinifera*), and *Ginkgo biloba* (*Ginkgo biloba*), have shown neuroprotective effects by attenuating oxidative damage, reducing neuroinflammation, and promoting neuronal survival and plasticity. In diabetes, oxidative stress contributes to pancreatic  $\beta$ -cell dysfunction, insulin resistance, and diabetic complications such as nephropathy, retinopathy, and neuropathy. Medicinal plants like bitter melon (*Momordica charantia*), cinnamon (*Cinnamomum verum*), and fenugreek (*Trigonella foenum-graecum*) exhibit antidiabetic properties by enhancing insulin sensitivity, improving glucose metabolism, and reducing oxidative stress-induced

damage [19-25]. Overall, medicinal plants represent a rich source of bioactive compounds with potent antioxidant activity, offering promising avenues for preventing, managing, and treating oxidative stress-related diseases. Incorporating plant-based therapies into conventional treatment regimens can provide holistic approaches to healthcare, addressing the multifactorial nature of oxidative stress and promoting overall well-being and longevity. Continued research into the mechanisms of action, efficacy, and safety of medicinal plants will further enhance their therapeutic potential and contribute to the development of novel antioxidant-based interventions for improving human health.

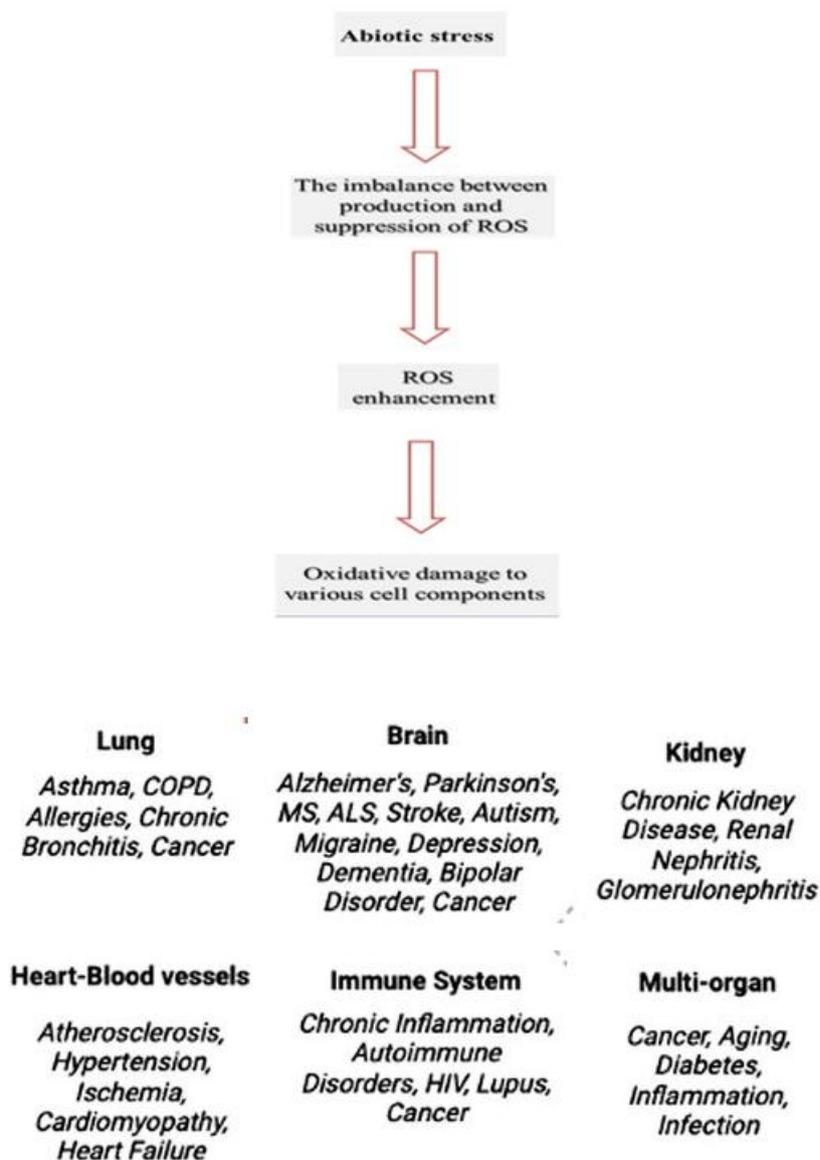


Figure 1. Oxidative stress and damages

*Ananas comosus*, commonly known as pineapple, is a tropical fruit belonging to the Bromeliaceae family. Native to South America, particularly Brazil and Paraguay, pineapples are now cultivated in tropical and subtropical regions worldwide for their sweet, juicy fruit and culinary versatility. The pineapple plant is a herbaceous perennial with long, sword-shaped

leaves arranged in a rosette formation [26, 27]. The plant typically reaches a height of 1 to 1.5 meters and produces a single fruit on top of a central stem. The fruit itself is a composite of many individual berries, known as "eyes," fused together around a central core. Each eye corresponds to a separate flower and is surrounded by a scaly, hexagonal pattern on the fruit's surface. The flesh of the pineapple is yellow to golden-orange in colour, with a sweet, tangy flavour and fibrous texture. Pineapples are not only enjoyed for their delicious taste but also prized for their nutritional value and health benefits. Rich in vitamins, minerals, and antioxidants, pineapples are a good source of vitamin C, manganese, and bromelain, a mixture of proteolytic enzymes with anti-inflammatory properties [28]. These nutrients contribute to the fruit's potential health benefits, including immune support, digestion aid, and anti-inflammatory effects. In traditional medicine, various parts of the pineapple plant, including the fruit, stem, and leaves, have been used to treat a range of ailments. Pineapple juice has been consumed as a digestive aid and natural remedy for indigestion and bloating due to its bromelain content, which helps break down proteins and improve digestion. Additionally, bromelain has been investigated for its potential anti-inflammatory effects and its role in reducing swelling and pain associated with conditions such as osteoarthritis and sports injuries. Beyond its medicinal uses, pineapples are widely used in culinary applications, adding flavor and sweetness to dishes such as fruit salads, smoothies, desserts, and savory dishes like stir-fries and marinades. Pineapple juice is also a popular beverage choice, either enjoyed on its own or used as a base for cocktails and mixed drinks [28]. Overall, *Ananas comosus* holds a special place in both culinary and traditional medicine practices, offering a combination of delightful flavour and potential health benefits. Whether enjoyed fresh, juiced, or incorporated into various dishes, pineapples continue to be a beloved fruit with a wide range of uses and applications. Therefore, this present research was designed to investigate the flavonoid fraction, total glycoside, free radical scavenging activities of *Ananas comosus* methanol leaf extract.

## 2. MATERIAL AND METHODS

### Chemicals, reagents, and drugs

Quercetin glycoside was procured from Sigma Aldrich, India. Vitamin C was received as gift sample from Herbal Trend, Ambala, India. DPPH radical was purchased from Himedia, India. All other chemical and reagents were arranged from reputed and pre-approved validated suppliers.

### Collection, authentication, and extraction

The plant material, *Ananas comosus* leaves were collected from local market and identified by a botanist. The leaves were shade dried and then grinded to a coarse powder by a mechanical grinder. The powdered coarse leaves were subjected to cold maceration using methanol as solvent for consecutive 7 days stirring occasionally in between at a temperature of 25°C. After the material was filtered, the extract was finally obtained and dried in an oven. After extraction, the resultant material was concentrated using a rotary evaporator running at 45°C with lowered pressure. The extraction procedure was found to be effective in isolating the required chemicals from the plant material, as evidenced by the extracted yield of 8.4%. Up until its next usage, the finished extract was stored in a vacuum desiccator.

### Phytochemical Qualitative Analysis

The plant extracts and methanolic and ethanolic aqueous solutions were assessed for the existence of the phytochemical analysis by using the following standard methods.

### Test for Anthraquinones

10 ml of benzene was added in 6 g of the Ephedra powder sample in a conical flask and soaked for 10 minutes and then filtered. Further 10 ml of 10% ammonia solution was added to the

filtrate and shaken vigorously for 30 seconds and pink, violet, or red colour indicated the presence of anthraquinones in the ammonia phase.

#### **Test for Tannins**

10 ml of bromine water was added to the 0.5 g aqueous extract. Decoloration of bromine water showed the presence of tannins.

#### **Test for Saponins**

5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously, and the foam appearance showed the presence of saponins.

#### **Tests for Flavonoids**

- **Shinoda Test:** Pieces of magnesium ribbon and HCl concentrated were mixed with aqueous crude plant extract after few minutes and pink color showed the presence of flavonoid.
- **Alkaline Reagent Test:** 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow colour was produced, which became colourless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

#### **Tests for Glycosides**

- **Liebermann's Test:** We added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled, and we added H<sub>2</sub>SO<sub>4</sub> concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.
- **Keller-Kiliani Test:** A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl<sub>3</sub> mixture was mixed with the 10 ml aqueous plant extract and 1 ml H<sub>2</sub>SO<sub>4</sub> concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.
- **Salkowski's Test:** We added 2 ml H<sub>2</sub>SO<sub>4</sub> concentrated to the whole aqueous plant crude extract. A reddish-brown colour formed which indicated the presence of steroidal aglycone part of the glycoside.

#### **Test for Terpenoids**

2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water bath and then boiled with 3 ml of H<sub>2</sub>SO<sub>4</sub> concentrated. A grey color formed which showed the entity of terpenoids.

#### **Test for Steroids**

2 ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

#### **Total Flavonoid Fraction Assay**

The Total Flavonoid Fraction Assay is a widely used spectrophotometric method designed to determine the concentration of flavonoids within plant extracts. Flavonoids are a class of secondary metabolites known for their antioxidant properties and potential health benefits. In this assay, the plant extract or a standard solution, often quercetin, is mixed with a solution of aluminum chloride and sodium acetate buffer. Aluminum chloride forms complexes with flavonoids in the sample, resulting in a color change. This color change is directly proportional to the concentration of flavonoids present. After incubation, the absorbance of the colored complex is measured at a specific wavelength, typically around 263 nm (Gallic acid). By comparing the absorbance of the sample to that of known concentrations of the standard solution of Gallic acid, the total flavonoid content of the extract can be quantified as gallic acid equivalent. This assay not only provides insights into the antioxidant potential of plant extracts but also aids in understanding their pharmacological properties and potential therapeutic applications.

### **Total glycoside content assay**

The spectrophotometric method for determining total glycoside content involved several steps. Initially, a standard solution of a known glycoside compound, typically using a compound like quercetin glycoside, was prepared, and a series of dilutions of the standard solution were made to create a calibration curve. To analyze the plant extract, it was dissolved in an appropriate solvent, such as methanol or ethanol, to extract the glycosides. The extract was then filtered to remove any particulate matter. An aliquot of the filtered extract was mixed with a specific reagent that reacted with the glycosides to form a coloured complex. This reagent could be Folin-Ciocalteu reagent or a specific reagent tailored for glycosides. After allowing the reaction to proceed for a predetermined time, the absorbance of the resulting colored complex was measured using a spectrophotometer at a 360 nm. The absorbance readings of the plant extract samples were then compared to the calibration curve generated from the standard solutions to determine the concentration of glycosides in the extract. Finally, the total glycoside content in the plant extract was calculated and expressed as milligrams of quercetin glycoside equivalents per gram of dry weight of the extract. This method provided a quantitative assessment of the glycoside content in the plant extract, allowing for comparisons between different samples and monitoring of extraction processes.

### **DPPH Free Radical Scavenging Assay**

To determine antioxidant activity 2,2-diphenyl-1-picryl-hydrezy (DPPH) was used as free radical. 100 µM concentration of DPPH was used in methanol. Serial dilutions were made to check the IC<sub>50</sub>. In 96-well micro plate total volume was 100 µl which was consisting of 90 µl of DPPH solution and 10 µl of the test solution. The contents were mixed and incubated for 30 minutes at 37°C. To determine the absorbance at 517 nm synergy HT BioTek USA micro plate reader was used. Ascorbic acid was used as standard antioxidant [27]. All readings were taken in triplicate. Ez-fit-5, Perrella Scientific Inc., Amherst, USA, software was used to calculate the IC<sub>50</sub>. Decrease in absorbance indicated increased radical scavenging activity which was determined by the following formula:

$$\% \text{ DPPH radical inhibition} = \frac{A_{\text{Control}} - A_{\text{Test}}}{A_{\text{Control}}} \times 100$$

where absorbance of control = total radical activity without inhibitor and absorbance of test = activity in the presence of test compounds.

### **Statistical analysis**

All the experimental measurements were performed in triplicate and expressed as the average of the three analyses ± standard deviations (SD). The correlation coefficient between variables, means, standard deviations, standard errors, standard curve, IC 50 values and one-way ANOVA followed by Bonferroni's post hoc test was calculated by using GraphPad Prism Software (V 8.0, USA). Figures are prepared in GraphPad Prism Software (USA). A P-value <0.05 was considered as statistically significant.

## **3. RESULTS AND DISCUSSION**

### **Preliminary Phytochemical screening**

The phytochemical qualitative analysis of the plant extracts revealed the presence of various bioactive compounds, as indicated by positive test results across different test methods. Anthraquinones, detected through the Ammonia Test, exhibited characteristic pink, violet, or red colours in the ammonia phase. Tannins, identified via the Bromine Water Test, caused decolouration of the bromine water, suggesting their presence. The Frothing Test confirmed the presence of saponins, as foam formed upon mixing with olive oil. Flavonoids were detected through both the Shinoda Test, showing a pink coloration upon mixing with magnesium ribbon and HCl, and the Alkaline Reagent Test, where a yellow colour turned colourless with diluted

acid. Moreover, glycosides were identified through various tests: Liebermann's Test exhibited a green colour indicating aglycone presence, the Keller-Kiliani Test showed a brown ring formation between layers, and Salkowski's Test resulted in a reddish-brown colour formation. Terpenoids, indicated by the Chloroform Evaporation test, led to the formation of a grey colour after boiling with concentrated H<sub>2</sub>SO<sub>4</sub>, while steroids, identified through the Chloroform Test, exhibited a red colour in the lower chloroform layer. The presence of these phytochemicals in the plant extracts suggests the potential bioactivity of the tested samples. Anthraquinones are known for their laxative properties, while tannins possess antioxidant and antimicrobial activities. Saponins exhibit diverse pharmacological effects, including anti-inflammatory and anticancer properties. Flavonoids are renowned for their antioxidant and anti-inflammatory properties, while glycosides often possess cardiovascular and anticancer activities. Terpenoids and steroids have varied biological activities, including anti-inflammatory, anticancer, and antidiabetic effects. These findings underscore the pharmacological potential of the tested plant extracts and warrant further investigation into their therapeutic applications.

Table 1. Results of the Preliminary Phytochemical screening for the plant extract

Phytochemical	Test Method	Observation	Inference (Extract)
Anthraquinones	Ammonia Test	Pink, violet, or red color in ammonia phase	+
Tannins	Bromine Water Test	Decoloration of bromine water	+
Saponins	Frothing Test	Formation of foam upon mixing with olive oil	+
Flavonoids	Shinoda Test	Pink color after mixing with magnesium ribbon and HCl	+
	Alkaline Reagent Test	Yellow color turning colorless with diluted acid	+
Glycosides	Liebermann's Test	Green color indicating aglycone	+
	Keller-Kiliani Test	Formation of brown ring between layers	+
	Salkowski's Test	Reddish-brown color formation	+
Terpenoids	Chloroform Evaporation	Grey color formation after boiling with H <sub>2</sub> SO <sub>4</sub>	+
Steroids	Chloroform Test	Red color in lower chloroform layer	+

#### Total flavonoid fraction assay

The results of the total flavonoid fraction assay indicated a linear relationship between the concentration of flavonoids and the absorbance measured at a specific wavelength. The equation of the line obtained from the assay is  $y = 0.0021x + 0.0294$ , where 'y' represents the absorbance and 'x' represents the concentration of flavonoids. The coefficient of determination ( $R^2$ ) value of 0.9882 suggested a strong correlation between the absorbance values and the concentration of flavonoids. Based on the calibration curve generated, the concentration of flavonoids in the sample solution was calculated to be 203.62  $\mu\text{g/ml}$ . This value represents the total flavonoid content present in the solution. The extract solution used in the assay was prepared by dissolving 10 mg of the plant extract in 10 ml of solution. Therefore, the total flavonoid content in the sample can be calculated using the concentration obtained from the

assay. Since the concentration of flavonoids in the extract solution was 203.62  $\mu\text{g/ml}$ , and the volume of the solution was 10 ml, the total amount of flavonoids present in the sample was calculated as follows: Total amount of flavonoids = Concentration  $\times$  Volume, Total amount of flavonoids = 203.62  $\mu\text{g/ml} \times 10 \text{ ml}$ , Total amount of flavonoids = 2036.2  $\mu\text{g}$ . Therefore, the total flavonoid content in the 10 mg extract is 2036.2  $\mu\text{g}$  Gallic acid equivalent. These results provided valuable information about the flavonoid content of the plant extract expressed as Gallic acid equivalent. Flavonoids are known for their antioxidant properties and have been associated with various health benefits, including anti-inflammatory, anticancer, and cardiovascular protective effects. Therefore, the high flavonoid content observed in the extract suggested its potential therapeutic value and highlights the importance of further investigation into its pharmacological activities. Additionally, the linear relationship established in the assay allowed for accurate quantification of flavonoids in similar plant extracts, aiding in quality control and standardization processes in the pharmaceutical and nutraceutical industries.

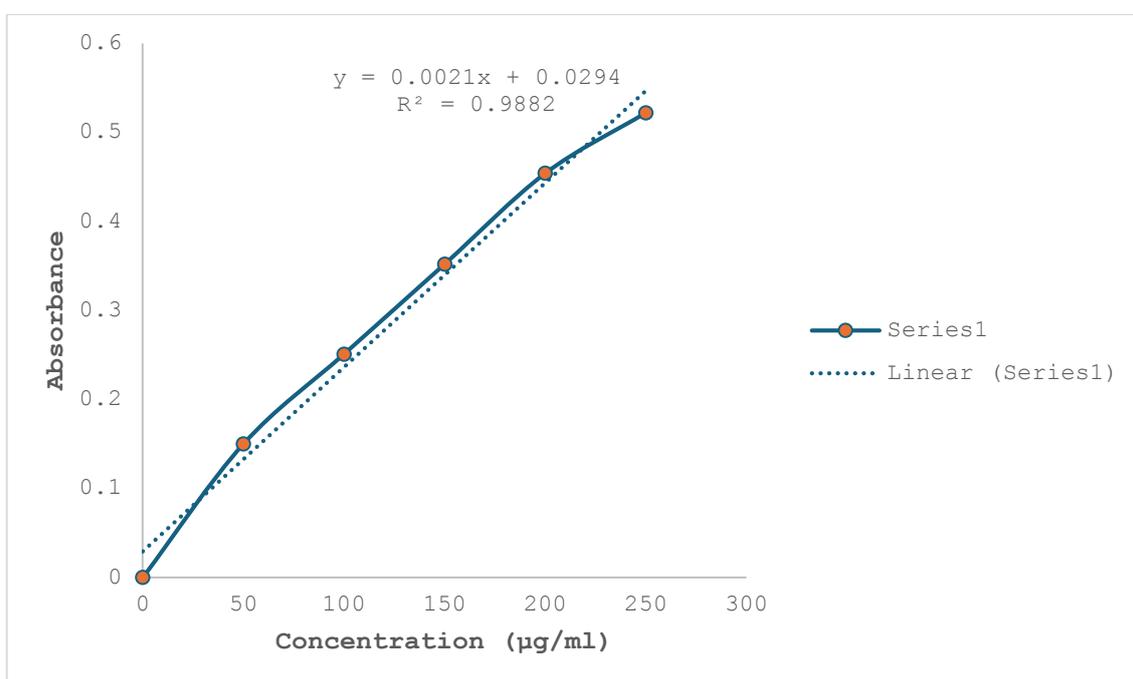


Figure 2. Total Flavonoid Fraction Assay of the extract

### Total glycoside content assay

The total glycoside content assay provided valuable insights into the quantitative analysis of glycosides within the tested sample of extract. The obtained data revealed a linear relationship between the absorbance measured at a specific wavelength of 360 nm and the concentration of glycosides. The equation of the calibration curve derived from the assay is  $y = 0.0026x + 0.0999$ , where 'y' represents the absorbance and 'x' represents the concentration of glycosides. The coefficient of determination ( $R^2$ ) value of 0.9321 indicates a strong correlation between the absorbance values and the concentration of glycosides, although slightly lower than optimal. The concentration of glycosides in the sample extract solution was calculated to be 86.19  $\mu\text{g/ml}$  based on the calibration curve of Quercetin glycoside. This value signified the total glycoside content present in the solution. Considering the preparation of the sample extract solution involved dissolving 10 ml of the extract in 10 ml of solution, the total amount of glycosides in the sample was determined: Total amount of glycosides = Concentration  $\times$  Volume, Total amount of glycosides = 86.19  $\mu\text{g/ml} \times 10 \text{ ml}$ , Total amount of glycosides = 861.9  $\mu\text{g}$ . Hence, the total glycoside content in the 10 ml extract was found to be 861.9  $\mu\text{g}$  Quercetin

equivalent. It was noteworthy that the standard used for comparison in this assay is quercetin glycoside, which was a well-known flavonoid glycoside. By employing a standard compound, the assay ensured the accuracy and reliability of the results by providing a reference point for comparison and expression of the glycoside content as Quercetin equivalent. Glycosides were a diverse group of compounds with various pharmacological activities, including antioxidant, antimicrobial, and anticancer properties. Therefore, the quantification of glycosides in the tested sample was a crucial step for assessing its potential therapeutic efficacy. However, further identification and characterization of specific glycosides present in the extract would be beneficial for understanding their individual contributions to the observed biological activities. In inference, the total glycoside content assay yielded significant information about the glycoside composition of the tested extract sample. The quantification of glycosides provided valuable data for quality control and standardization processes, ensuring the consistency and potency of herbal preparations in pharmaceutical and nutraceutical applications.

#### **DPPH Free Radical Scavenging Assay**

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay is a widely used method to evaluate the antioxidant potential of natural compounds. In this assay, the ability of the tested extract sample to scavenge the stable free radical DPPH was assessed, and the percentage inhibition of DPPH radical by the samples was measured. The results of the DPPH radical scavenging activity assay demonstrate the antioxidant capacity of the tested extract, along with reference standard compounds Quercetin and Vitamin C. The percentage inhibition of DPPH radical by the extract, Quercetin, and Vitamin C at various concentrations ( $\mu\text{g/ml}$ ) was presented in the figure 3. At lower concentrations (50  $\mu\text{g/ml}$ ), the extract exhibited moderate DPPH radical scavenging activity with an inhibition percentage of  $26.11 \pm 0.74\%$ . In comparison, quercetin and vitamin C showed higher inhibition percentages of  $44.76 \pm 0.94\%$  and  $35.89 \pm 0.85\%$ , respectively, indicating their stronger antioxidant potential. As the concentration of the extract increased, its DPPH scavenging activity also increased significantly. At 250  $\mu\text{g/ml}$ , the extract demonstrated a high percentage inhibition of  $93.21 \pm 0.30\%$ , indicating potent antioxidant activity comparable to quercetin ( $95.11 \pm 0.50\%$ ) and vitamin C ( $93.02 \pm 0.72\%$ ) at the same concentration. These results suggested that the extract possessed significant antioxidant properties, effectively scavenging DPPH radicals in a concentration-dependent manner. The observed antioxidant activity of the extract might be attributed to the presence of phytochemicals such as flavonoids, phenolic compounds, and other bioactive constituents. The comparison with Quercetin and Vitamin C, well-known standard antioxidants, provided valuable insights into the relative antioxidant potency of the extract. Although the extract showed slightly lower activity compared to the reference compounds at lower concentrations, it exhibited comparable or even superior activity at higher concentrations. Overall, the findings of the DPPH radical scavenging activity assay highlighted the potential of the plant extract as a natural antioxidant agent.

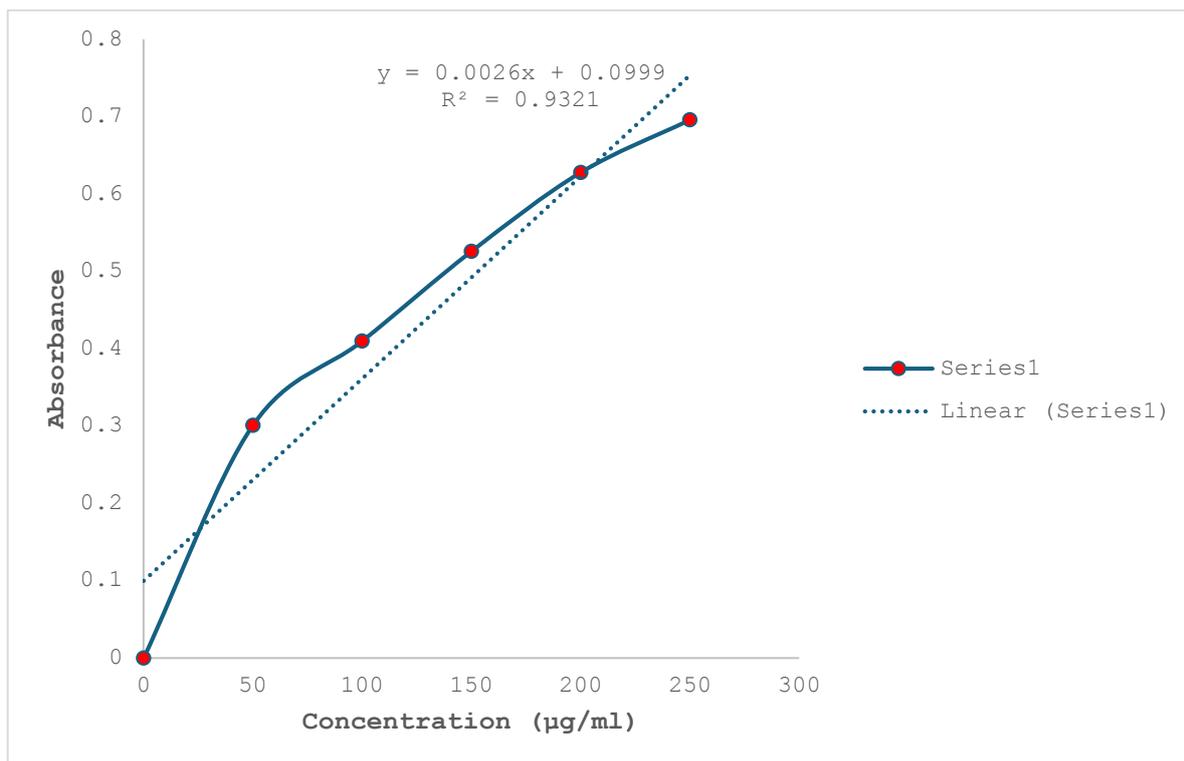


Figure 3. Total glycoside content assay of the extract

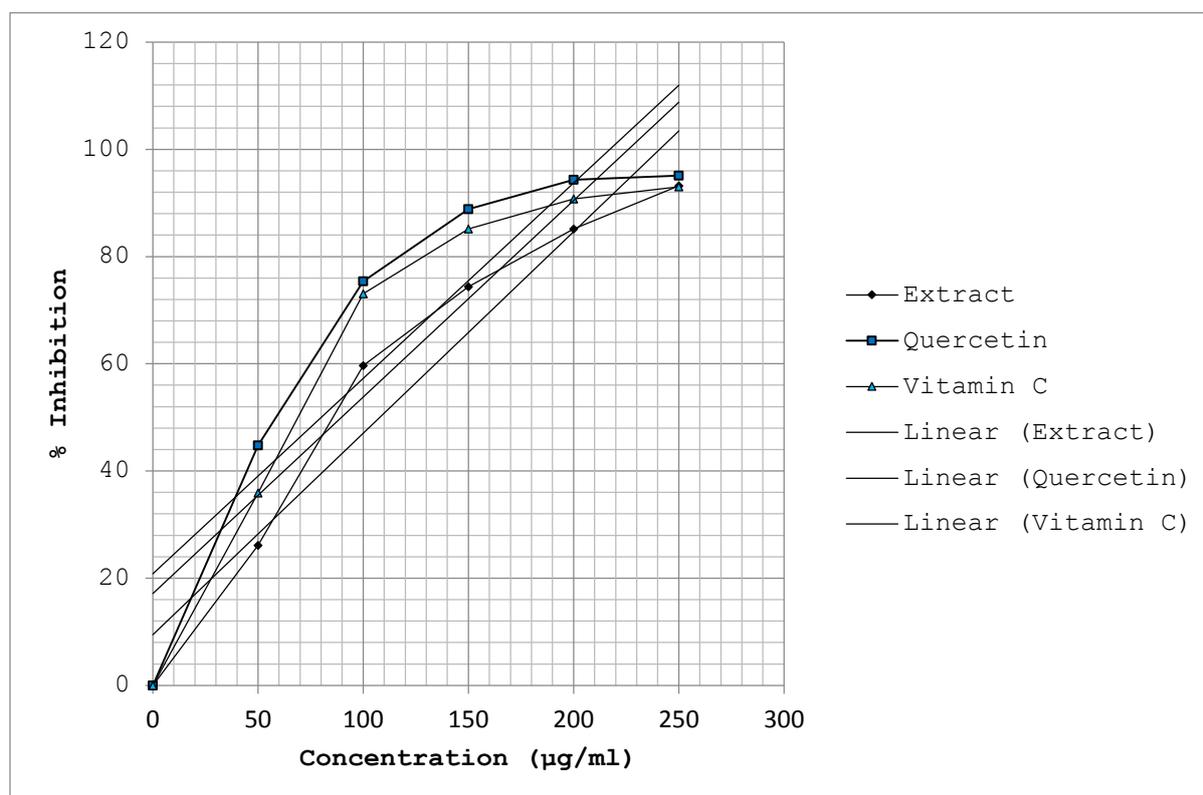


Figure 4. DPPH Free Radical Scavenging Assay of the plant extract

#### 4. CONCLUSIONS

In conclusion, the findings of this study demonstrated the diverse phytochemical profile and notable antioxidant potential of the tested plant extract. The presence of anthraquinones,

tannins, saponins, flavonoids, glycosides, terpenoids, and steroids indicated its rich phytochemical composition, which might contribute to its biological activities. The quantification of flavonoids and glycosides provided valuable insights into the extract's pharmacological properties, highlighting its potential as a natural source of antioxidants. Moreover, the significant DPPH radical scavenging activity suggested its ability to neutralize free radicals and mitigate oxidative stress-related damage. These results underscored the importance of further research to explore the extract's therapeutic applications in managing oxidative stress-related diseases and developing novel antioxidant formulations. In a nutshell, the tested extract holds promise as a valuable resource for the development of antioxidant-based pharmaceuticals and nutraceuticals, warranting further investigation into its bioactive constituents and potential health benefits.

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