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# Estimation of Phytochemical Constituents and Evaluation of Antioxidant Potency of Piper betle Leaves

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## Abstract

Betel leaves (Piper betle) are a species of Piper genus which is rich in phytonutrients like steroids, phenols, flavonoids, alkaloids, steroids, saponins and terpenoids. Anti-cancer, antibacterial, anti-inflammatory, antioxidant, anti-mutagenic, antiplaque, and anti-diabetic properties are among the health benefits of this plant's leaf extract. This study aimed to quantify the phytochemical constituents and assess the antioxidant capacity of piper betle leaves. In this study, qualitative phytochemical screening were used done by using test for alkaloids, glycosides, phenols, flavonoids, steroids, terpenoids, saponins. Additionally, we calculated the betel leaves' total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity using DPPH, $H_2O_2$ scavenging activity after extraction by methanol using Soxhlet apparatus. The TPC and TFC was noted 51.5278 mg GAE/gm and 33.769 mg QE/gm respectively. There was a significant positive connection found between DPPH and Total Phenolic Content. ($R^2$=0.9939) (p value= 0.0002), H202 ($R^2$=0.9979) (p value<0.0001) respectively at a 95% confidence level which is consistent with the above literature reports. There is significant relationship between TFC and DPPH ($R^2$=0.9781) (p value= 0.0014) H202 ($R^2$=0.9591) (p value= 0.0036) respectively at a 95% confidence level. The leaves of Piper betle contain high amount of phenolic and flavonoid content, hence can be used as natural antioxidant. To fully comprehend antioxidants' potential to prevent diseases that have a major negative influence on life expectancy, more research must be done on the separation, identification, and mechanism of action of the relevant antioxidant components.

**Keywords:** Phenolic, Flavonoid, Antioxidant, Correlation, *Piper betle*

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## Introduction

Antioxidants are defined as those chemicals that considerably slows down or prevents the substrate's oxidation when it is present in smaller amounts than the substrate[1]. In terms of its physiological role, it avoids allowing chemical interactions involving free radicals to cause harm to biological components [2]. A wide variety of both endogenous and exogenous antioxidant defences are present to shield cellular components from damage caused by free radicals since radicals have the potential to react indiscriminately and cause harm to practically any cellular component. Numerous health problems, including rheumatoid arthritis, Alzheimer's disease, cardiovascular disease, cancer, and other neurological disorders, are mostly attributed to free radicals. [3]. Antioxidants that scavenge free radicals have been shown to be beneficial for various illnesses because they prevent damage to lipids, proteins, and carbohydrates. [4]. Oxidative damage by oxygen radicals to biomolecules such as lipids, proteins, carbohydrates, and DNA(5)[5]<sup>5</sup>(5)(5)<sup>5</sup>(5) [5] cause different illnesses and accelerated aging molecules. However, antioxidant chemicals that cleanse the body and scavenge free radicals can block this adverse action. Synthetic antioxidants suspect to cause adverse health effects; thus, their application has been confined and substituted with natural antioxidants. Investigators conduct research on many other plant species despite the widely used natural antioxidants from fruits, vegetables, tea, and many other plant species [6].

Present-day research has proven that antioxidant-rich foods show vital component in the prevention of diabetes, cancer, and cardiovascular disorders [7][8],respiratory distress syndrome as well as neurological conditions including Parkinson's and Alzheimer's [9] as well as inflammation[9,10]. Scientists

are focused on herbal remedies for its anti-inflammatory, anti-mutagenic, anti-cancer, anti-inflammatory, nutraceutical, antibacterial, antiplaque, and antidiabetic properties [11]. Piper betle L., frequently referred to as betel leaf, is one such significant herb [12] [13]. *Piper betle* (Piperaceae family) named " Betel nut", widely known as paan is an important medicinal and recreational plant [14] found in nations in South East Asia, including Malaysia, Indonesia, the Philippines, India, and Sri Lanka [15]. It is shade-loving, dioecious, glossy heart-shaped leaves containing perennial root climbers that climb as high as 10-15 feet. Leaves are simple, alternate possessing jointed stems with expanded nodes, although other species are monoecious or dioecious, making it challenging to categorise plants solely based on their physical traits. [16]. P. betle leaves are simply having large, blade ovate-oblong or rounded ovate-cordate, coriaceous leaves, acuminate apex, entire margin,5 to 7- 2 nerved, petiole 1.0-3.5cm long, base often unequal, male spikes 7.0-15.0cm long; female spike long peduncled, pendulous [17] [18]. Application of betel leaves (Piper betle L.) in Ayurveda for a number of purposes, including stimulant, antiseptic, antifungal, antibacterial, and anti-diabetic, antibacterial, oxidative stress and anti-allergic medications have been brought up [19]. The foliage combined with warmed mustard oil, then placed to the chest to relieve cough and respiratory difficulty [20].Therapeutically, It shows immunomodulatory, gastroprotective, and antidiabetic activity [21]. As an expectorant, to relieve respiratory tract irritation and infection, and to alleviate dyspnea [22].The main constituent of the leaf is betle oil, a volatile oil [23]. The Piper betle leaf extract includes active chemical components like such as saponins, eugenol, tannins, polyphenols, flavonoids, alkaloids, steroids, saponins, Hydroxychavicol, and Chavibetol [3], [15] [24]. Allylpyrocatechol is a phenolic constituent found in leaves that shows activity against obligate oral anaerobes accountable for halitosis[5].

During the literature review, we found that among the different solvents, methanol possess highest antioxidant properties inhibiting 50% of DPPH even a low concentration of 4.38 ug/mL [1]. Also, highest amount of phenolic compound yield was shown using methanol [2]. Thus, realizing its potential antioxidant activity and high phenolic content yield using methanol as solvent, we used methanol in our experiment.

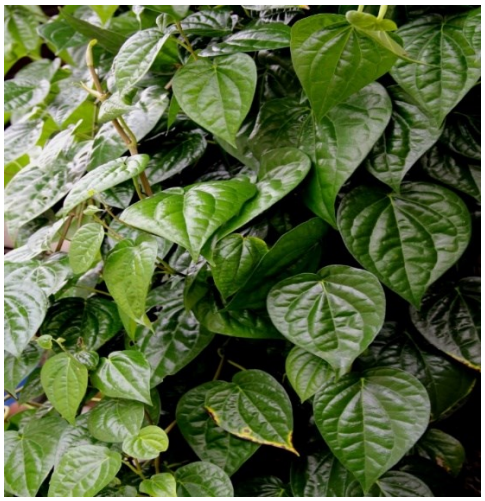


**Figure1.** Morphology of Piper betle

# Methods and methodology

## Chemicals

Quercetin, gallic acid, and DPPH were purchased from Sigma Aldrich in India. We bought ascorbic acid and Folin-Ciocalteu reagents from S. D. Fine Chem Limited in India. Likewise, ferric chloride, potassium ferricyanide, trichloroacetic acid, and aluminium chloride were obtained from Ranchem, India. There were no substandard chemicals or solvents utilised.

## Research Approval, Gathering and verifying plant material

The Universal College of Medical Sciences' Ethical Review Committee authorized the research. Piper betle leaves were gathered from various locations within Nepal's Rupandehi district. Identification of leaves and authentication was done from Department of Horticulture, Institute of Agriculture and Animal Sciences, Ranigaon, Bhairahawa, Rupandehi, Nepal, and deposited the voucher specimen.

## Processing and solvent extraction [30,31]

Piper betle leaves were washed to get rid of debris, then they were rinsed with water, allowed to air dry, and transformed into powder, kept at room temperature for further analyses. Methanol was used as the extraction solvent after 143 grams of powdered leaves had been defatted with petroleum ether through a soxhlet extractor. The extraction process was performed at a temperature of about 40℃, not exceeding the solvent's boiling point. The extraction was done for about 24 hours. After extraction, the extract was stored at 4°C in the refrigerator to be used later in phytochemical investigation. Phytochemical screening was next applied to the methanolic leaf extract.

## Phytochemical Examination

### 1. Evaluation for saponins

20 mL of distilled water was used to boil 2 g of the powdered sample. 5 mL of distilled water and 10 mL of filtrate were forcefully shaken. The frothy appearance suggested the presence of saponins. [25]

### 2. Evaluation for phenols:

A few drops of a 10% ferric chloride solution were added after 2 millilitres of extract had been diluted in 4 millilitres of distilled water. Phenols were present because they appeared blue or green. [26].

### 3. Evaluation for flavonoids

1 millilitre of 2N sodium hydroxide solution was added to 2 millilitres of extract. The presence of flavonoids was detected by the company of yellow color [27].

### 4. Evaluation for Steroids

One millilitre of fruit extract was mixed with an equal volume of chloroform, and a few drops of strong sulfuric acid were added. The emergence of a bluish-brown ring confirmed the presence of a steroid ring.[28].

### 5. Evaluation for terpenoids

A mixture of 0.2g of each sample, 2 mL chloroform, and 3 mL concentrated $H_2SO_4$ was prepared. Terpenoids were recognised by a reddish-brown colouring (Alamzeb et al., 2013).

### 6. Test for cardiac glycosides

To 3 drops of a robust solution of lead acetate, the extract's aqueous solution (2 mL) was added., then mixed thoroughly and filtered. 5 mL of chloroform and the filtrate were mixed together in a separating funnel. Then in a small evaporating dish, the chloroform layer was dried out from evaporation. The residue was dissolved in an ice cream acid solution containing trace amounts of ferric chloride. It was then transferred on the surface of a test tube with two millilitres of sulfuric acid. The upper layer showing the presence of bluish-green coloration shows the existence of glycosides [29].

### 7. Test for alkaloids (Dragendorff's Test)

After five minutes of stirring the extract in a water bath containing 1% HCl, it was filtered. To the filtrate, 1 millilitre of the potassium bismuth iodide solution (Dragendoff reagent) was added. The production of an orange-red precipitate served as proof that alkaloids were present. [30]

## Estimation of phytochemical content

### 1. Determination of Total Phenolics [31]

Plant extracts' total phenolic content was calculated by combining 1 millilitre of each sample with 2 millilitres of the Phenol Folin-Ciocalteau mixture . Next, 9 millilitres of pure deionized water were added to a volumetric bottle with a 25 millilitre maximum capacity and shaken well. After adding 10 mL of 7% Na2CO3, the mixture was vigorously stirred and the final volume was increased to 25 mL by immediately diluting the liquid with pure deionized water. The final mix was kept at 23 °C for at least 90 min. Concentrations were reported as milligrammes of gallic acid equivalents (GAE) per gramme of extract, and total phenolics were computed using standard gallic acid solutions used under the same circumstances. All analyses were completed in duplicate. The assay was determined by the formula:

T equals (C*V)/M

Where T = Total Phenolic Content

C = Concentration derived from the calibration curve that is equal to gallic acid

V stands for extract volume and M for plant extract weight in grammes.

### 2. Determination of total Flavonoids [32]

The aluminium chloride colorimetric test was used to quantify the total flavonoid levels in the extracts. To the test tube containing 1 mL of plant solution of each Concentration,4 mL of distilled water was added. Simultaneously, 0.3 mL of 5% $NaNO_2$ and 0.3 mL of 10% AlCl3 were added to the test tube after 5 minutes. 2 mL of 1 M NaOH was added to the mixture after 6 minutes. The mixture was immediately increased to a volume of 10 millilitres by adding 4.4 millilitres of distilled water. All analyses were carried out in triplicate. Using the linear equation based on the calibration curve, the total flavonoid content was represented as quercetin equivalents.

## In-vitro antioxidant assay

### 1. Free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) [33]

In methanolic solution,0.1mM solution of DPPH was prepared. One millilitre of DPPH solution was added to three millilitres of Piper betle methanol extract at various concentrations, and the mixture was left to remain at room temperature for thirty minutes after shaking vigorously. The absorbance was then determined using the UV-VIS spectrophotometer at 517 nm. The reference standard compound utilised was ascorbic acid. An analysis of the sample's concentration needed to inhibit 50% of the DPPH free radical was done using a log dose-inhibition curve to determine its inhibitory concentration (IC50) value. Higher levels of free radical activity were indicated by the reaction mixture's reduced absorbance.

% Inhibition=

$$\frac{\text{Absorbance(control) -Absorbance (sample)} * 100}{\text{Absorbance(control)}}$$

## 2. Hydrogen peroxide scavenging assay [34]

PBME's capacity to scavenge $H_20_2$ was verified using spectrophotometry. Phosphate-buffered saline (PBS: pH 7.4) at 20°C was used to create a 4 mM solution of $H_2O_2$. Using spectrophotometry, the concentration of $H_2O_2$ was measured at 230 nm. To the final concentrations of 25, 50, 100, 200, and 400 ug/mL at 20°C, 2 mL plant extract was added to the $H_2O_2$ solution. After ten minutes, H202 A230 was measured in a spectrophotometer using blank solutions that contained plant extract. (25,50,100,200,400 ug/mL) in Phosphate buffer solution without $H_2O_2$.

The following formula is used to calculate hydrogen peroxide scavenging activity:

%Hydrogen scavenging = $[A_0\text{-}A_1/A_0]$ *100, where the control's absorbance is A0.

A1 is the sample's absorbance.

## Experimental

Microsoft Excel was used for data analysis, and the total flavonoid and phenolic content result would be expressed as Mean ± standard deviation (S.D.).

# Results

This investigation confirms the phenolic and flavonoid content and the potency of the methanolic extracts of Piper betle leaves as antioxidants. [35].

## Yield value:

Weight of dried leaves powder= 143 gram

Weight of extract= 10 gram

Percentage yield = Theoretical yield/ Practical yield * 100

Thus, it was discovered that the betel leaf extract yield was 6.993%.

## Phytochemical Analysis [27]

Qualitative phytochemical analysis of Piper betle leaves is shown in **Table 1.**

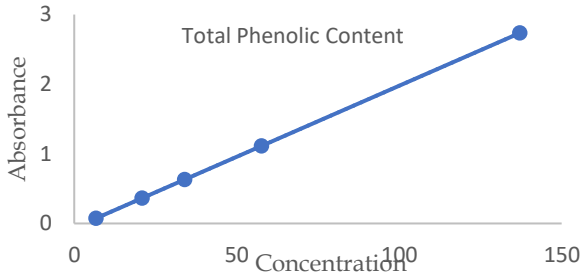
**Table 1.** Qualitative phytochemical analysis of Piper betle leaves

| Phyto-constituents | *P. betle* leaves extract | Observation |
|---|---|---|
| Alkaloids | Present | Reddish-brown coloration |
| Phenols | Present | Blue-green to black coloration |
| Saponins | Present | Stable, persistent froth |
| Flavonoid | Present | Yellow coloration |
| Glycosides | Present | Greenish coloration |
| Terpenoids | Present | Reddish-brown coloration |

## Total phenolic content

Gallic acid (0-200 ug/mL) was used as a reference standard and the Folin-Catechu reagent was used to quantify the spectrophotometric concentration of total phenols. The results were represented in gallic acid equivalents (GAE) per gramme dry extract weight (**Figure 2**).

The formula for the link between gallic acid concentration and absorbance is y = (0.0204x+0.0626), $R^2$=0.9915. Total phenolic content (TPC) was found to be




ranged between (6.696±0.009 to 137.137±0.002) mg GAE/gm. The calculated TPC was 51.2548 mg GAE/g.

**Figure 2**: Galic acid calibration curve. At 750 nm, the respective absorbance of gallic acid at various concentrations was determined.

## Total Content of Flavonoids

The total amount of flavonoids was investigated using quercetin (0-200 ug/mL) as a reference standard to find different samples of extract stated as quercetin equivalents (QE) per gramme of weight from the dry extract. The following equation describes the relationship between quercetin concentration and absorbance: y=0.0026x+0.0054. The whole content of flavonoids was

**Table 2:** Mean TPC of methanolic extract of Piper betle leaves ranged (6.696±0.009 to 137.137±0.002 mg GAE/gm). Each value was expressed in Mean± std. (n=3).

| Concentration | Mean Absorbance | Concentration Equivalent to GA (mg/mL) | SD | TPC | AverageTPC |
|---|---|---|---|---|---|
| 10 | 0.074 | 6.696 | 0.009 | 6.696±0.009 | |
| 20 | 0.362 | 20.814 | 0.003 | 20.814±0.003 | |
| 40 | 0.631 | 34 | 0.013 | 34±0.013 | 51.2548 |
| 80 | 1.113 | 57.627 | 0.009 | 57.627±0.009 | |
| 160 | 2.735 | 137.137 | 0.002 | 137.137±0.002 | |

**Table 3.** Mean TFC of methanolic extract of Piper betle leaves ranged (5.359±0.006 mg QE/gm to 94.5897±0.004 mg QE/gm. Each value was expressed in Mean± std. (n=3).

| Concentration | Mean Absorbance | Concentration Equivalent to QE(mg/mL) | SD | Total Flavonoid Content (TFC) | Avg TFC |
|---|---|---|---|---|---|
| 10 | 0.019333333 | 5.359 | 0.006 | 5.359±0.006 | |
| 20 | 0.029333333 | 9.2051 | 0.004 | 9.2051±0.004 | |
| 40 | 0.034666667 | 11.2564 | 0.003 | 11.2564±0.003 | 33.76922 |
| 80 | 0.131333333 | 48.4359 | 0.01 | 48.4359±0.01 | |
| 160 | 0.251333333 | 94.5897 | 0.004 | 94.5897±0.004 | |

found to be ranged between 5.359±0.006 to 94.5897±0.004) mg, equivalent to quercetin and average flavonoid content was 33.76922 mg QE/g.

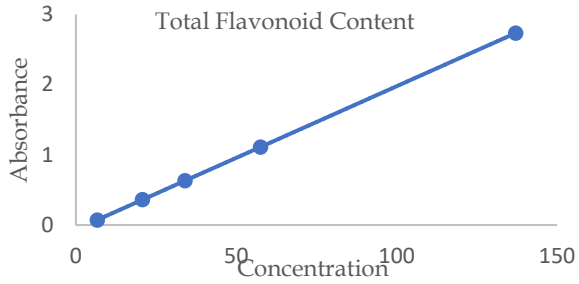



**Figure 3**. Calibration curve of Quercetin. Respective absorbance of Quercetin at different concentration was measured at 415nm.

## Radical Scavenging Activity of DPPH

A synthetic radical called DPPH is frequently employed to measure antiradical activity in vitro. [36]. The scavenging of free radicals by Ascorbic acid was used as a standard to quantify the extract of Piper betle with methanol. Dose-dependent action was observed in both ascorbic acid and Piper betle methanolic extract. The obtained % scavenging activity of DPPH of the methanolic section of *Piper betle* ranges were 39.74%, 43.3974%, 50.64102%,63.91026%, 94.67949% at concentration of 25,50,100,200,400 ug/mL respectively. Whereas for standard (ascorbic acid) scavenging activity was found as 44.487%, 50.3205%,53.4401%, 65.7435%, 96.1752% at above mentioned concentrations respectively. The highest % scavenging activity of sample was found 94.67949% at 400 ug/mL whereas for standard, was found 96.1752% at 400 ug/mL. The percentage of inhibition or scavenging activity was plotted versus log concentration, and linear regression analysis was used to determine the $IC_{50}$ (Inhibition concentration 50) value from the graph. Ascorbic acid's and Piper betle methanol extract's respective $IC_{50}$ values were determined to be 66.74124 µg/mL and 96.9273 µg/mL.

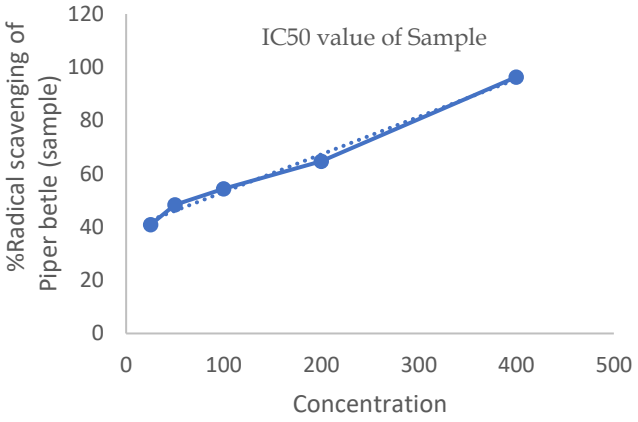



**Figure 4.** $IC_{50}$ value of Sample using DPPH

**Table 4.** The percentage of ascorbic acid (standard) and betel leaf extract (sample) that scavenges free radicals

| Test material | Concentration (ug/mL) | Mean Absorbance | % of scavenging action | $IC_{50}$ (ug/mL) |
|---|---|---|---|---|
| **Ascorbic Acid (Standard)** | 25 | 0.28867 | 44.487 | 66.7412 |
| | 50 | 0.2583 | 50.3205 | 66.7412 |
| | 100 | 0.24211 | 53.4401 | 66.7412 |
| | 200 | 0.18334 | 64.7435 | 66.7412 |
| | 400 | 0.01989 | 96.1752 | 66.7412 |
| ***Piper betle* (Sample)** | 25 | 0.31334 | 39.74359 | 96.9273 |
| | 50 | 0.294334 | 43.39744 | 96.9273 |
| | 100 | 0.25667 | 50.64102 | 96.9273 |
| | 200 | 0.187667 | 63.91026 | 96.9273 |
| | 400 | 0.0276667 | 94.67949 | 96.9273 |

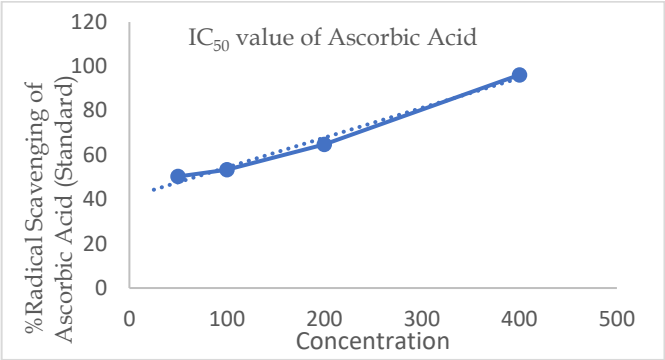



**Figure 5.** $IC_{50}$ value of Ascorbic Acid using DPPH

## Activity of Hydrogen Peroxide Scavenging

**Table 5.** Hydrogen scavenging activity of sample (betel leaf) and ascorbic acid (Standard).

| Test material | Concen-tration (ug/mL) | Mean Absorbance | % Activity of Scavenging | $IC_{50}$ (ug/mL) |
|---|---|---|---|---|
| Standard Ascorbic Acid | 25 | 0.058333 | 40.8333 | 77.8485 |
| | 50 | 0.056333 | 48.333 | 77.8485 |
| | 100 | 0.051222 | 54.333 | 77.8485 |
| | 200 | 0.03946667 | 64.6667 | 77.8485 |
| | 400 | 0.01711339 | 96.333 | 77.8485 |
| *Piper betle* | 25 | 0.05916 | 41.667 | 105.4627 |
| | 50 | 0.05166 | 43.667 | 105.4627 |
| | 100 | 0.04566 | 48.778 | 105.4627 |
| | 200 | 0.03533 | 60.5333 | 105.4627 |
| | 400 | 0.00366 | 82.8867 | 105.4627 |

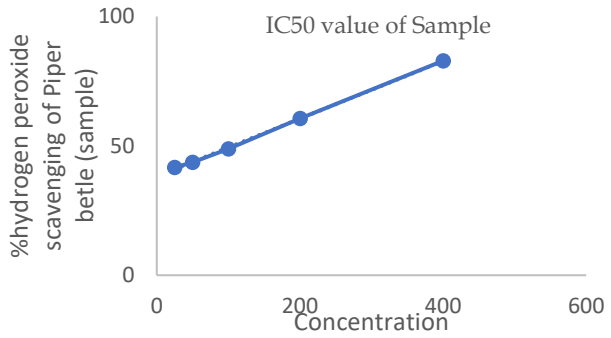



**Figure 6.** $IC_{50}$ value of Ascorbic Acid using $H_20_2$

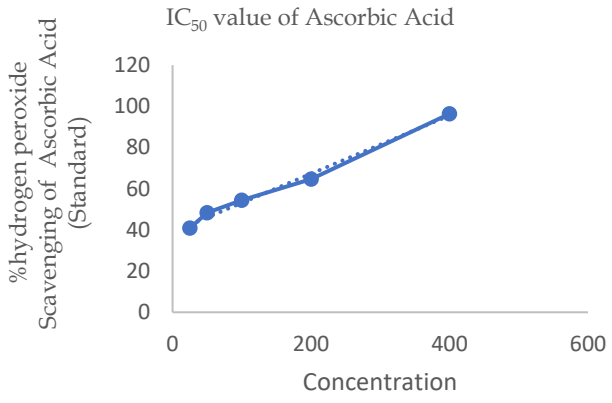



**Figure 7.** $IC_{50}$ value of sample using $H_20_2$

The ability of the extracts from Piper betle methanolic leaves to scavenge hydrogen was calculated serving as a positive control with ascorbic acid. The obtained % scavenging activity of hydrogen peroxide of a methanolic section of *Piper betle* Ranges were 41.667%, 43.667%, 48.7778%, 60.533%, 82.8867% at concentration of 25,50,100,200,400 ug/mL respectively. Whereas for standard (ascorbic acid) scavenging activity was found as 40.833%, 48.33%, 54.333%, 64.667%, 96.333% at above mentioned concentrations respectively. The highest % scavenging activity of sample was found 82.8867% at 400 ug/mL whereas for standard, was found 96.333% at 400 ug/mL.

The percentage of inhibition or scavenging activity was plotted versus log concentration, and linear regression analysis was used to determine the IC50 (Inhibition concentration 50) value from the graph. Ascorbic acid's and Piper betle methanol extract's respective IC50 values were determined to be 77.8485 µg/mL and 105.4627 µg/mL.

## Correlation coefficient of the Antioxidant Activity of TPC and TFC

The linear regression coefficient ($R^2$) of TPC and TFC exhibiting antioxidant properties analyzed using Microsoft Excel.

**Table 6.** Correlation between TPC and TFC with Antioxidant Activity.

| TPC | TFC | %DPPH | $\%H_20_2$ |
|---|---|---|---|
| 6.696 | 5.359 | 39.743 | 40.833 |
| 20.814 | 9.205 | 43.397 | 48.333 |
| 34.00 | 11.256 | 50.641 | 54.333 |
| 57.627 | 48.435 | 63.910 | 64.667 |
| 237.137 | 94.679 | 94.679 | 96.333 |

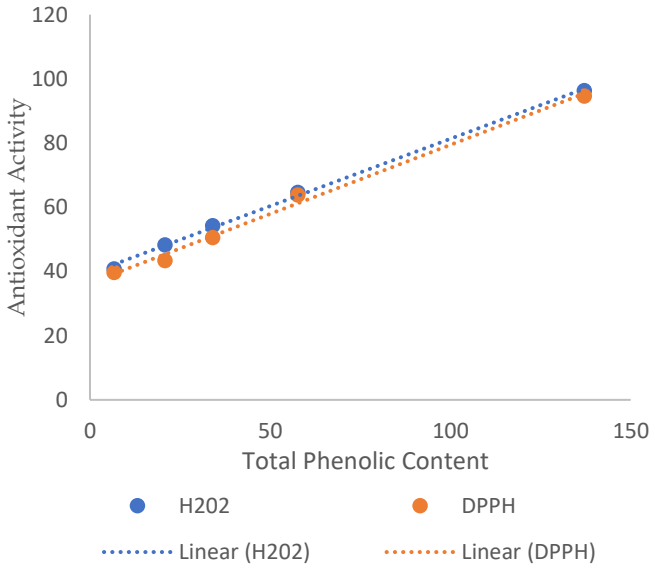



**Figure 8.** Correlations of TPC with Antioxidant activity where linear regression coefficient of DPPH ($R^2$=0.9939), H202 ($R^2$=0.9979), respectively

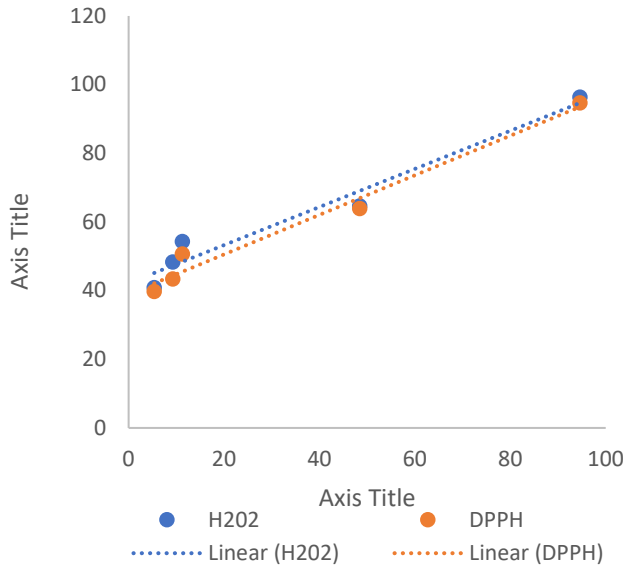



**Figure 9**. Correlations of TFC with Antioxidant Activity where linear regression coefficient of DPPH ($R^2$=0.97819, H202 ($R^2$=0.9591) respectively

## Discussion

In this study, the antioxidant (free radical scavenging value) using Piper betle leaves was found to be 94.67% leaves which is quite higher than % inhibition activity using ethanol as solvent (87.34%)[37) . The total flavonoid content, total phenolic content, and antioxidant capacity of the methanolic extract of the Piper betle plant were investigated by the use of an in-vitro hydrogen peroxide scavenging action and the 1,1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging approach.These are the most popular assays used by scientists to evaluate the antioxidant capacity of plant materials.The current investigation found that PBME exhibited concentration-dependent scavenging activity against DPPH and H202 radicals [38).

According to [39), phenolic compounds are vital to plant constituents with redox characteristics that are in charge of antioxidant action. Plant extracts' hydroxyl groups are what make free radical scavenging possible. The overall content of phenols is presented in table 2 . This outcome agrees with Noor Nazirahanie Abrahim et al.'s findings. [30]. The highest phenolic concentration in the Betle leaf methanolic extract was determined to be 136.33 mg Gallic acid equivalent/gm in a study by Badrul Alam [41] where as in a study by Hardik Savsani et al. using ethyl acetate as a solvent for extraction discovered that the overall phenolic content was 54.0 mg of equivalent gallic acid [34]. In another study done using an aqueous extract of betel leaf shows an entire phenolic content of 77.2 (µg TAE/mg) [42]. The phenolic content in our study differed slightly from that of the above two kinds of literature. This may be due to different geographical variation or solvents used, which could change the phenolic content [43].

Due to the presence and arrangement of free OH groups, flavonoids are secondary metabolic products that exhibit strong antioxidant properties [44]. The protective action of flavonoids is seen against DNA damage caused by hydroxyl radicals. [32]. The presence of chelating metal ions such as iron or copper is the mechanism underlying the protective action of flavonoids. Complex flavonoid of copper or irons are responsible for preventing the generation of ROS. [45].Our study found that total flavonoid content ranged between (5.39- 106.1282) mg, equivalent to quercetin, and the average flavonoid content was 39.666 mg, equal to quercetin. In a study done by Suryasnata Das et al., total flavonoid content was found to be 49.79(sonication), 32.10(maceration), and 40.89(soxhlet) mg QE/gm extract, which is in line with the results of our study [3], but 52.16 mg of quercetin equivalents (QE) were found in a study by Badrul Alam et al.'s total flavonoid content in the methanolic extract of betel leaf [41]. In another study on ethyl acetate solvent extraction, the flavonoid contents of PBEA was 45.22 mg quercetin equivalent/gram[34]. The flavonoid content in our study differed slightly from that of the above two kinds of literature. Vegetable flavonoid concentration was found to be highly influenced by biological, environmental, seasonal, and year-to-year fluctuations in addition to genetic diversity, as documented in the literature [46].

DPPH radical produces a new bond by reacting with a suitable reducing agent and changing the solution's color. Being a steady free radical, DPPH becomes a stable diamagnetic molecule. Because it contains phenolic chemicals such as eugenol, hydroxychavicol, and iso-eugenol, it undergoes a colour change from purple to yellow when it accepts an electron or hydrogen radical from PBME [47]. Therefore, DPPH is primarily helpful for figuring out a compound's antioxidant properties [46]. The methanolic solution of DPPH exhibits a prominent absorption band at 517 nm due to its odd electron. The solution becomes less coloured as the concentration of antioxidants rises because the antioxidant's DPPH radical absorbs electrons from the solution [32]. The standard ascorbic acid value in our investigation was determined to be 66.7414 µg/mL, while the IC50 value of the plant extract was 96.9273 µg/mL., which shows that plant extract has lower antioxidant properties compared to that of standard. Similarly, another study followed our findings [42]. Similar results were observed in a survey by Hardik Savsani et al., with the IC50 value of plant

extract being 100. 1 ug/mL, whereas ascorbic acid had a value of 53.66 ug/mL.

Strong oxidising agents like hydrogen peroxide [$H_2O_2$) can increase cellular proliferation or differentiation by activating the signalling pathway [48] or differentiation [49]. Superoxide dismutase, one of the oxidizing enzymes, produces $H_20_2$ in a biological system [50]. However, inflammation reactions and oxidative stress result due to the accumulation of a large amount of $H_2O_2$, which results in ailments such as diabetes, cancer, and cardiovascular disorders [[51],[52]]. This happens due to the $H_2O_2$ quickly breaking down and producing the hydroxyl radical [•OH), which commences damage to cellular components and lipid peroxidation [53] In biological study, the regulation of $H_2O_2$ age by plant antioxidants is fascinating. In biological study, the regulation of $H_2O_2$ age by plant antioxidants is fascinating. The obtained % scavenging activity of hydrogen peroxide in our study ranged between [41.667-82.8867%). At the same time, that of ascorbic acid ran between [40.8333%-96.333%) The percentage [%) scavenging of the extract was found to be concentration-dependent with the IC50 value of 77.014388 µg/mL, but normal ascorbic acid's IC50 weight was discovered to be 111.9151 µg/mL.This shows ascorbic acid has a comparatively higher antioxidant properties than a plant extract. While in a study conducted using ethylacetate as a solvent for extraction, the highest enhanced scavenging activity for PBEA and ascorbic acid was determined to be 98% and 97% at 500 µg/mL concentration, respectively. [34]. The above-used solvent antioxidant property is more significant than our findings at similar Concentrations of sample and standard. This may be because the best solvent for removing antioxidants from P. betle is ethyl acetate, which suggests that the majority of P. betle is medium polarity. [40].

Due to their ability to give free radicals hydrogen atoms, phenolic and flavonoid molecules are the main antioxidant components that deactivate free radicals. Additionally, they have structural traits that enable them to scavenge free radicals [54]. There is a strong linear relationship between the total phenolic content and its antioxidant activity, according to many literature findings. [40][49]. Figure 9 illustrates the relationship between the antioxidant capacity of betel leaf and its total phenolic and flavonoid content. There is a strong correlation between Total Phenolic Content with DPPH [$R^2$=0.9939) [p value= 0.0002), $H_20_2$ [$R^2$=0.9979) [p value<0.0001) respectively at a 95% confidence level which is consistent with the above literature reports. There is a significant relationship between TFC and DPPH [$R^2$=0.9890) [p value= 0.0014), $H_20_2$ [$R^2$=0.9591) [p value= 0.0036) respectively similarly with a 95% degree of certainty. It is feasible to infer that phenolic and flavonoid groups have a major role in the antioxidant activity of the chosen plant extracts by analysing the correlation coefficients (R2 values).

## Conclusion

Our study's findings indicate that betel leaves contain higher levels of flavonoids and phenolic compounds, which may make them a valuable source of natural antioxidants. Measuring antioxidant qualities can help determine how best to employ these plants for disorders connected to reactive oxygen species (ROS), even though the study's criteria were not disease-specific. The investigation of P. betle leaf extracts' total phenolic and flavonoid contents and antioxidant activity raises awareness of the plant's potential applications in medicine. commercially. To gain a better understanding of antioxidants' potential to prevent diseases that have a major impact on life quality, more research must be done on the separation, identification, and mechanism of action of these components.

## List of abbreviations

TPC: Total phenolic content; TFC: Total flavonoid content DPPH: 2,2 -Diphenyl-1-picryl- hydrazyl- hydrate; $H_20_2$: Hydrogen peroxide; P-value: Probability value; R: Coefficient of determination; GAE: Gallic acid equivalent; $H_2SO_4$: Sulphuric acid; HCl: Hydrochloric acid; $AlCl_3$: Aluminium trichloride; $NaN0_2$: Sodium nitrite; NaOH: Sodium hydroxide ; UV-VIS: Ultra violet-visible; IC: Inhibitory concentration; QE: Quercitin equivalent; Mg: Milligram

## Ethics Approval and Participant Consent

Ethical Approval for the research work was given by Institutional Review Committee of Universal College of Medical Sciences (UCMS).

## Conflicts of Interest

No conflicts of interest are disclosed by the authors.

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Conceptualization, Melina Poudel; Data Curation, Roshan Kumar Mehta, Melina Poudel and Ashish Lamsal; Methodology, Melina Poudel, Niranjan Koirala; Software, Melina Poudel; Supervision: Niranjan Koirala, Roshan Kumar Mehta, Ashish Lamsal; Writing-Review

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