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# Phytochemical screening and antioxidant activities of *Mangifera indica* leaves grown in temperate region of the Nepal

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**Abstract**
The present study was carried out to find out the preliminary phytochemical screening and antioxidant activity of 70% ethanolic extract of *Mangifera indica* grown in temperate zone of Nepal. The antioxidant properties were measured by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay. Total phenolic and Total Flavonoid content were carried out spectrophotometrically by using Folin-Ciocailteau reagent and aluminium chloride reagent. Gallic acid and quercetin were used as standard for the calibration of phenol and flavonoid respectively. The result showed that plant is potent antioxidant property so they could be the rich source of natural antioxidants.

**Keywords:** Antioxidant activity, *Mangifera indica*, phytochemical screening, total phenolic content, total flavonoid content

## 1. Introduction
Nepal is a storehouse of natural medicinal plants and ethnic groups are rich in indigenous knowledge. Knowledge on various plants acquired by their self-experience is rapidly eroding due to the gap of communication between the scientific and indigenous communities [1]. Plant medicine is used today since the time of Ayurveda [2]. Today thousands of drugs commonly prescribed are derived from plants sources [3, 4]. Phytochemical exhibit various important biological, pharmacological and antioxidant activities [5, 6, 7]. Plant as a source of therapeutic agents due to their antimicrobial and antioxidants properties [8]. The effectiveness of phytochemical in the treatment of various diseases may lie in their antioxidant effects because oxidative stress is associated with pathogenic mechanisms. Antioxidants can inhibit the oxidation by scavenging free radicals and diminishing oxidative damage [9]. *Mangifera indic*, a king of fruits, is a large evergreen tree (locally called Aanp, and in english mango). Fruits are highly nutritive and sweet in taste. Not only fruits but also the leaves, seeds and barks of this plant are also used for medicinal purposes [10].

## 2. Materials and Methods
### 2.1 Plant material
The leaves of *Mangifera indica* (Aanp) was collected from the premises of Patan Multiple College Lalitpur district. The plant collected was identified in department of Botany, PMC, Patan dhoka, Lalitpur, Tribhuvan University. The plant sample was air dried and the leaves were crushed into powder and stored in polythene bags for further use.

### 2.2 Chemicals and preparation of 70% ethanolic extract
Folin-Ciocalteu's phenol reagent, gallic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), was purchased from sigma chemical company, USA. All the solutions are prepared in distilled water. Chemicals and reagents used were of analytical grade. Extraction of the plant parts was done with ethanol and distilled water (70:30). Plant material 30 gm was weighed and kept in a conical flask then soaked in100 ml. of ethanol (70 %). The mouth of conical flask was covered with aluminum foil and kept on orbital shaker for 48 hrs. Then the extract was taken by filtering the content. The extract was pooled and concentrated on a water bath by keeping the temperature 60 °C. The concentrated extract was kept in the desiccator for further evaporation of the solvent. The solution then filtered with the help of filter paper and filtered extract of the selected plant sample was taken and used for further phytochemical analysis.

**2.3 Preliminary phytochemical Screening**
The extracts were used for the preliminary phytochemical analysis to identify the various chemical constituents. All the tests were performed in triplicate mode. The standard procedures taken for analysis [11, 12, 13].

**2.3.1 Spot test (volatile oil)**
2 ml solution was evaporated to get residue and it was mixed with 0.5 ml methanol. The solution was shaken vigorously and filtered. Few drops of filtrate were put on a filter paper by means of a capillary tube. A yellow spot on a filter paper if not persist after evaporation indicates the presence of volatile oil.

**2.3.2 Alkaloids: Mayer's Reagent**
In 1ml. of the plant extract, 2ml. of Mayer's reagent (potassium mercuric iodide solution) was added. Appearance of dull white precipitate indicated the presence of alkaloids.

**2.3.3 Flavonoid**
Crude extract was mixed with 2ml. of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoid.

**2.3.4 Keller-Kilani test**
A mixture of Acetic acid glacial (2 ml.) with 2 drops of 2% $FeCl_3$ solution was added to the plant extract and $H_2SO_4$ concentrated. A brown ring at the interface indicated the presence of cardiac glycosides.

**2.3.5 Lieberman-Burchard's Test (Steroids)**
The plant extract was dissolved in 2ml. of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red color through blue to green indicated the presence of steroids.

**2.3.6 Salkowski's Test (Triterpenoids)**
5 ml. of each extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml.) and it was carefully added to form a layer. A reddish brown precipitate of the interface indicated the presence of triterpenoids.

**2.3.7 Phenols and tannins**
Crude extract was mixed with 2ml. of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

**2.3.8 Saponins**
Crude extract was mixed with 5ml. of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

**2.3.9 Reducing Compounds (Fehling's test)**
0.5 ml alcoholic extract was added with 1 ml water and 0.5 ml Fehling's solution (A+B) then warm or gently boiled. A reddish brick precipitation denotes the presence of reducing compounds.

**2.3.10 Determination of Total Phenolic Content and Total Flavonoid content**
**a) Preparation of standard for phenolic content and flavonoid content**
The total phenolic content of extract was estimated by Folin-Ciocalteau reagent as method described by Singleton and Rossi [14]. Gallic acid stock solution was prepared by dissolving 1 mg gallic acid in 1 ml of methanol (1 mg/ml). Various concentrations of gallic acid such as 200, 100, 50 and 25 µg/ml were prepared by serial dilution of stock solution. An aliquot of 1 ml gallic acid of each concentration in methanol was added to 20 ml test tube. To that 5 ml of Folin-Ciocalteu reagent (10%) and 4 ml of 7% $Na_2CO_3$ were added to get a total of 10 ml. The blue colored mixture was shaken well and incubated for 30 minutes at 40ºC in a water bath. Then the absorbance was measured at 760 nm against blank. The absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.
Similarly, total flavonoid content was determined by aluminium chloride colorimetric assay [15]. Various concentration of standard quercetin viz, 2 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml were prepared by serial dilution of stock solution of concentration of 4 mg/ml. An aliquot of 1 ml quercetin of each concentration in methanol was added to 10 ml volumetric flask containing 4 ml of double distilled water. At the zero time, 0.3 ml, 5% sodium nitrite was added to the flask. After 5 min, 0.3 ml of 10% $AlCl_3$ was added to the flask. At 6 min, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the total volume of the mixture was made up to 10 ml by the addition of 2.4 ml double distilled water and mixed thoroughly. Absorbance of the pink colored mixture was determined at 510 nm versus a blank containing all reagents except quercetin. Absorbance values obtained at different concentrations of quercetin were used to plot the calibration curve.

**b) Preparation of samples for Phenolic content and flavonoids content**
The stock solutions of all the extracts were prepared by dissolving 1 mg in 1ml of methanol. Serial dilutions were carried out to get the concentration of 200, 100, 50 and 25 µg/ml. To these diluted solution FCR and $Na_2CO_3$ were added and incubated for 30 minutes as in the case of standard gallic acid preparation and absorbance was measured at 760 nm. Similarly, various concentrations of the extracts viz, 2 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml were prepared. Following the procedure described above in flavonoid, absorbance for each concentration of extract was recorded. Total Flavonoid Content of the extracts was expressed as mg quercetin equivalents (QE) per gram of extract in dry weight (mg/g).

**Calculation for total phenolic and flavonoid content and Statistical Analysis**
The total phenolic content and flavonoid content was calculated using the formula [15].

$$C = \frac{cV}{m} \quad \ldots\ldots\ldots\ldots (1)$$

C= total contents of compounds in mg/g, in mg GAE/ g or total flavonoid content mg QE/ g dry extract.
c= concentration of gallic acid established from the calibration curve in mg/ml or concentration of quercetin obtain from calibration curve, mg/ml, V= the volume of extract in ml, m= the weight of plant extract in g.
Calculation of linear correlation coefficient $R^2$ and correlation analysis were carried out using Microsoft Office Excel 2007. The linear regression equation is given as,

$$y = mx + c \quad \ldots\ldots\ldots (2)$$

where y = absorbance of extract, m= slope of the calibration curve, x= concentration of the extract, c=intercept

**2.3.11 Determination of antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH)**

DPPH radical scavenging activity of extracts was carried out according to Brands *et al* [16]. DPPH solution (0.1 mM) in methanol was prepared by dissolving 3.9 mg of DPPH in 100 ml methanol and stirred overnight at 4ºC. Thus prepared purple colored DPPH free radical solution was stored at -20˚C for further use. Three different concentrations (5, 10 and 15 µg/ml) of methanolic solutions of each extracts were prepared by the serial dilution of the stock solution of the respective extract. To each 0.5 ml extract solution, 2.5 ml, 0.1 mM methanolic DPPH solution was added. A control was prepared by mixing 0.5 ml distilled water and 2.5 ml 0.1 mM methanolic DPPH solution. These samples were well shaken and kept in dark for 30 min at a room temperature. The absorbance of the mixture was measured spectrophotometrically at 517 nm against the blank solution consisting 2.5 ml MeOH and 0.5 ml distilled water.

The radical scavenging activity was expressed as the radical scavenging percentage using the following equation: DPPH

$$\% \text{ scavenging activity} = \left[\frac{(A_c - A_s)}{A_c}\right] x\ 100$$

Where, $A_c$=absorbance of control and methanol,

$A_s$= absorbance of sample solution and DPPH radical.

$IC_{50}$ value is the concentration of sample required to scavenge 50% of DPPH free radical and was calculated from the plotted graph of radical scavenging activity against concentration of extracts. The antioxidant activity was determined by DPPH assay and the free radical scavenging activity ($IC_{50}$) value was calculated. The highest the phenolic content, the lowest the $IC_{50}$ Value.

## 3. Results and discussion

The research work was carried out on the medicinal plant which shows the potent phytochemical constituent's summarized in Table 1.

**Table 1:** Preliminary phytochemical screening of *Mangifera indica.*

| S.N | Experiment | Test | Colour | Result |
|---|---|---|---|---|
| 1 | Volatile oils | Spot Te/Residue test | No yellow colour persist | + |
| 2 | Alkaloids | Mayers Regent Test | White yellowish ppt. | + |
| 3 | Flavonoid | Shinoda test | Pink Scarlet | + |
| 4 | Steroids | Steroid test | Yellow with green fluorescence | - |
| 5 | Terpenoids | Terpenoids test | A grayish colour | - |
| 6 | Tannins | 0.1% $FeCl_3$ Test | Bluish black/greenish black | + |
| 7 | Reducing sugar | Fehlings Test | Reddish brick ppt. | + |
| 8 | Glycosides | Salkowski's test | Reddish brown | - |
| 9 | Phenols | Phenolic test | Blue green | + |
| 10 | Saponins | Froth/ Foam Test | Foam | - |

(+ = indicates presence of phytochemical, - indicates absence of phytochemical)

### 3.1 Calibration curve for Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The total phenolic content in plant extract was determined by using Folin-Ciocalteu colourimetric method. The absorbance values obtained at different concentrations of gallic acid was used for the construction of calibration curve. Absorbance values for gallic acid measured at 760 nm using Folin-Ciocalteu reagent. Similarly the total flavonoid contents were determined by a colorimetric assay using aluminum chloride. Absorbance values for quercetin measured at 430 nm using aluminum chloride coulometric assay were shown in Table 2 and calibration curve in Figure 1 and 2.

**Table 2**: Absorbance value for gallic acid and quercetin measured for calibration curve

| Concentration (µg/ml) | Absorbance for Gallic acid measured | Concentration (µg/ml) | Absorbance values for quercetin measured |
|---|---|---|---|
| 10 | 0.11 | 10 | 0.04 |
| 25 | 0.25 | 25 | 0.11 |
| 50 | 0.49 | 50 | 0.23 |
| 75 | 0.74 | 75 | 0.43 |
| 100 | 1.01 | 100 | 0.35 |

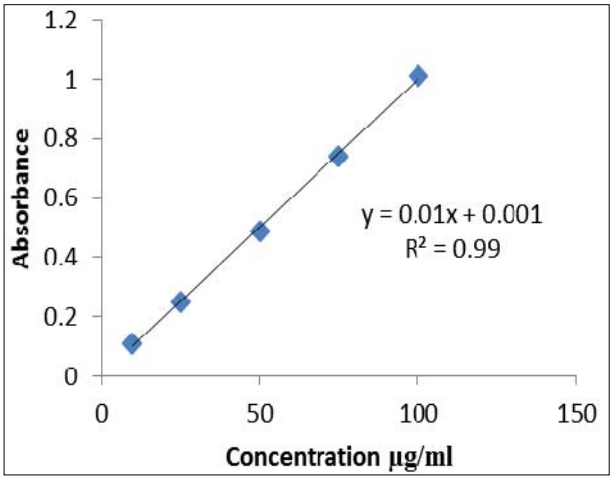


**Fig 1:** Calibration curve for authentic gallic acid

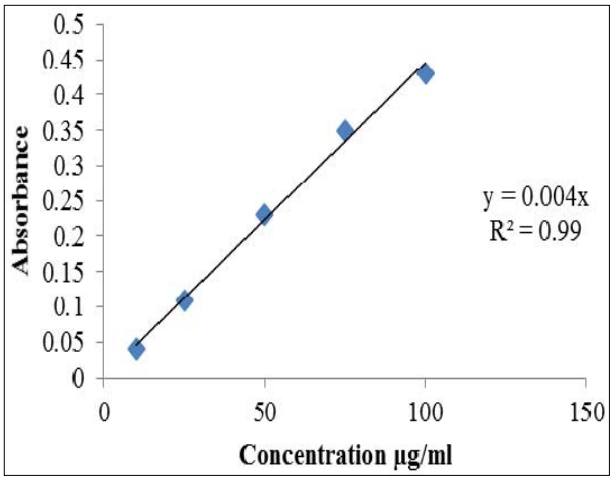


**Fig 2:** Calibration curve for authentic quercetin

### 3.2 Calculation of total phenolic and total flavonoid contents in extracts

The concentration of phenolic in extract was calculated from the calibration curve using the regression equation Y= 0.01x+0.001, $R^2$=0.999. The total phenolic content was calculated using the formula C=cV/m and expressed as mg gallic acid equivalents (GAE) per g of extract in (mg/g). The concentrations of flavonoid in each test samples were calculated from the calibration curve using regression equation Y=0.004x, $R^2$= 0.99. Total flavonoid contents of the extracts were calculated using the formula, C=cV/m and expressed as mg quercetin equivalents (QE) per gram extract in (mg/g). The total phenolic contents and flavonoid content was calculated given in Table 3.

**Table 3**: Total Phenolic and Total Flavonoid Content in different extract.

| Plant parts | 50% ethanol mg GAE/ g Phenolic content Mean TPC ± S.D | 50% ethanol QE mg/ g flavonoid content Mean TFC ± S.D. |
|---|---|---|
| Leaves | 360±0.00 | 55.63±3.69 |

The total phenolic content in 50% aqueous methanol was determined. The amount of phenolic is (360 mg/ GAE / g sample) and Flavonoid (55.63 mg/ GAE / g sample).

### 3.3 DPPH assay for antioxidant activities

The DPPH assay was carried out and absorbance values measured at wavelength 517 nm for different concentrations and the control are given in Table 4. The calculated percentage of inhibition showed that extract show antioxidant activity.

**Table 4:** Absorbance and a control measured at wavelength 517 nm in the DPPH assay, % inhibition and $IC_{50}$

| Plant extract | Conc. µg/ml | Absorbance | % inhibition | $IC_{50}$ | TPC(mg GAE/g) | TFC (mg QE/g) |
|---|---|---|---|---|---|---|
| 50 % ethanolic | 5 | 0.91 | 2.14 | 67.82 | 360±0.00 | 55.63±3.69 |
| | 10 | 0.86 | 7.50 | | | |
| | 15 | 0.84 | 9.60 | | | |

In the present study, a simple and reproducible Folin-Ciocalteu (FC) method was applied for the determination of total phenolic using gallic acid as a standard. FC method is based on the transfer of electrons in alkaline medium from the phenolic compounds to phosphomolybedic/phosphotungstic acid complexes to form blue coloured complexes, $(PMoW_{11}O_{40})^{4-}$ that are determined spectrophotometrically at 760 nm.

The DPPH assay is based on the capability of an antioxidant to donate hydrogen radical which is stable free radical with deep violet colour. When an odd electron become paired in the presence of free radical scavenger of antioxidant agent, DPPH radicals get reduced to corresponding hydrazine, DPPH-H form and the solution gets decolorized from its initial deep violet to light yellow colour.

## 4. Conclusion

The phytochemical screening, quantification of phenolic compounds and antioxidant activity in plant extracts is influenced by the chemical nature of the analyte, assay method, selection of standards and presence of interfering substances. Many plant of Nepal has not yet give attention as a source of antioxidant polyphenols due to lack of commercial application. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The result showed that plant is potent antioxidant property so they could be the rich source of natural antioxidants.

## 5. Acknowledgement

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