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PHYTOCHEMICAL AND *IN VITRO* ANTIOXIDANT EVALUATION OF *PERICALLIS CRUENTA* (L HER.) BOLLE

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Abstract

The present study deals with the phytochemical screening and *In Vitro* antioxidant activities of leaves extract of *Pericallis cruenta*. The methanolic extract of the leaves was prepared by maceration. Different tests were performed to find out the phytochemicals present in the leaves. Five assays i.e. DPPH Radical Scavenging Activity, Ferric Reducing Antioxidant Power (FRAP), Total Flavonoid Content (TFC), Total Antioxidant Activity (TAA) and Metal Chelating Activity were used to assess the antioxidant activity. The phytochemical screening exposed the existence of saponins, proteins & amino acids, carbohydrates, phenols & tannins, flavonoids and sterols. DPPH Radical Scavenging Activity was calculated for different concentrations i.e. 1ml, 0.75ml, 0.50ml and 0.25ml. It was maximum for 0.25ml concentration i.e. 92.06% and lowest as 48.83% for 1ml concentration. Trolox Equivalent (TE) for FRAP was calculated as 4.52 µM/ml. The value for Total Antioxidant Activity (TAA) was found to be 108.5mg/ml. Moreover, value for Total Flavonoid Content (TFC) was 40.8 mg Ru./g. The percentage iron bound value for Metal Chelating Activity was very high and found to be 1211.20%. This high value indicates that the leaves of *P. cruenta* are a source of potential antioxidants. Therefore, this plant is recommended for further study to find out its use for the treatment of diseases.

Key words: *Pericallis cruenta* (L Her.) Bolle, antioxidant, phytochemical, maceration.

Introduction

The pharmaceutical industry continues to find novel pharmaceuticals and lead molecules through the scientific study of traditional medicines, or ethnopharmacology. The study of how both contemporary and traditional communities interpret and use plants is known as ethnobotany (Balick & Cox, 1996). Minerals, animal products, and plant products served as the primary sources of medications for a very long time. The usage of natural goods with curative characteristics predates the development of human civilization (Rates, 2001).

Plants are the primary source of medicine for the majority of rural inhabitants and play a important role in the local healthcare system. In addition to their nutritional values, plants sometimes have magical or ceremonial uses (Abbink, 1995). By acting as a springboard for the creation of new medication developments, they play a crucial part in the growth and advancement of contemporary research (Pramono, 2002). A growing belief in herbal therapy is one of the many factors contributing to the continued acknowledgement of medicinal herbs. Various diseases may be treated by allopathic medicine. However, because of its high cost and unpleasant side effects, people are switching back to use the herbal remedies (Kala, 2005).

According to a broad definition, any substance which prevents or lessens oxidative damage to a target molecule is called an antioxidant (Yamagishi & Mutsai, 2011). Antioxidants reduce cellular oxidative stress, proving effective in addressing a range of human illnesses, including cardiovascular issues, cancer, and inflammatory disorders. As natural chemicals with antioxidant and anti-microbial characteristics become more and more important for curing many chronic disorders like cancer and cardiovascular disease, more research is being done on them. It is anticipated that plant derivatives will make up about two thirds of all medications licensed globally (Patridge *et al.*, 2016).

Utilization of natural antioxidants has been linked to low chances of diabetes, cancer, cardiovascular disorders, and other aging-related conditions (Ashokkumar *et al.*, 2008). From ancient times, plant derivatives have formed an integral part of phytomedicines. Barks, flowers, leaves, fruits, roots, and seeds all serve as reservoirs of these substances (Criagg & David, 2001). Natural antioxidants enhance the antioxidant capacity of plasma and reduce the likelihood of disease development (Prior & Cao, 2000). Plant secondary metabolites like phenolics and flavonoids are effective free radical scavengers. Every component of plants, encompassing leaves, seeds, fruits, roots, and

bark, holds these metabolites (Mathew & Abraham, 2006). The use of synthetic antioxidants is widespread. They are said to have a number of negative effects, though, including the potential for liver damage and the development of cancer in lab animals (Williams *et al.,* 1999; Gao *et al.,* 2007). Therefore, there is a need for antioxidants that are more potent, less harmful, and more affordable (Chanda & Dave, 2009).

Medicinal plants containing phytochemical components are helpful for treating human ailments (Nostro *et al.,* 2000). Phytochemicals exist naturally and have defense mechanisms and protective properties and are found in medicinal plants, vegetables, leaves, and roots. Phytochemicals encompass both primary and secondary compounds. Primary ingredients include common sugars, proteins and chlorophyll, while Terpenoids, alkaloids, and phenolic compounds are included within secondary chemicals (Krishnaiah *et al.,* 2007). Terpenoids possess a diverse array of important pharmacological characteristics, encompassing anti-inflammatory, anti-malarial, inhibition of cholesterol synthesis, anti-viral, anti-cancer, and anti-bacterial properties (Mahato & Sen, 1997). Terpenoids play a vital role in attracting beneficial mites that prey on herbivorous insects (Kappers *et al.,* 2005). Alkaloids are compounds found in medicinal plants and are employed for their anesthetic properties (Herouart *et al.,* 1988).

Various applications of medicinal plants in conventional medicine continue to provide inspiration for the creation of fresh pharmacological substances such as alkaloids, flavonoids and terpenoids. Many of these medicinal plants also exhibit antioxidant or radical-scavenging qualities in addition to hepatoprotective, neuroprotective, and anti-inflammatory effects (Lin & Huang, 2000). Since many years, alternative treatments based on Medicinal plants have been widely employed in the treatment of diverse infectious ailments. The emergence of drug resistance in human and animal pathogenic bacteria together with the unfavorable negative effects of some antibiotics has sparked intense interest in the search for new plant-based antimicrobial alternatives. The effectiveness, affordability and accessibility of medicinal plants are thought to be their three most important advantages for curing a variety of diseases (Khan *et al.,* 2009; Mallikarjun *et al.,* 2016).

Pakistan's wonderful climate makes it a rich source of medicinal plants. Nearly 400–600 of the 6000 species are medicinal species, and these are all located in Pakistan. Natural defenses against various ailments are provided by medicinal plants, which are seen as a gift from nature (Baqi *et al.,* 2018). There are various naturally occurring phytochemicals in these therapeutic plants. For many years, it has been known that bioactive substances such alkaloids, flavonoids, glycosides, phenols, steroids, terpenoids, and tannins have beneficial therapeutic effects (Khan *et al.,* 2019; Kamal *et al.,* 2021). Their widespread therapeutic use is primarily motivated by the fact that natural substances are safer and less expensive than synthetic ones. The pharmacological and biological effects of these phytochemicals include antibacterial, antioxidant, and anti-inflammatory properties (Asif *et al.,* 2023).

The small genus *Pericallis* belongs to family Asteraceae and contains roughly 14 species of flowering plants. Herbaceous plants and subshrubs are included in this genus. *Pericallis cruenta* belonging to this genus produces typically purple daisy like flowers. It is used mostly for ornamental purposes in houses and offices. It is native to Pakistan and bloom from winter to spring. It needs bright light and humid conditions to grow. *Pericallis* is usually 30-45cm long and 30-60cm wide. The leaves of *Pericallis cruenta* are simple, stalked, broad, deciduous and green in color. They possess tiny hairs that give them a rough texture. The margins are lobed to serrate while apices are acute. *Pericallis cruenta* likes well-drained, moist and organic soil to grow.

As the literature reviewed, no significant work has been done for the screening of phytochemicals and the antioxidant properties of the leaves of *Pericallis cruenta.* So the present research was formulated to find out medicinal importance of this plant.

Methodology

2.1 Sampling

Pericallis cruenta was collected from main nursery of Government College University, Lahore for the phytochemical analysis and *In Vitro* antioxidant evaluation. The leaves of the plant were separated, washed and dried. They were then grinded to a fine powder.

2.2 Maceration

About 40g of the fine powder of the leaves was added in 500ml of methanol. It was mixed properly and kept for 7 days. It was then filtered using Whatman filter paper. The extract was kept for 4-5 days to get concentrated. It was stored in a glass jar

for future use for phytochemical screening and antioxidant evaluation.

2.3 Phytochemical Screening

Qualitative tests for phytochemical screening of the leaf extract was performed using the procedure by Shaikh & Patil (2020) for Saponins, Reducing sugars, flavonoids, Alkaloids, proteins and amino acids, carbohydrates, phenols and tannins, glycosides and sterols.

2.3.1 Tests for reducing sugars

- **Fehling test**

Fehling reagent was prepared by mixing 1ml each of Fehling solution A and B. 1ml of this solution was added in 1ml of the extract in a test tube and boiled on water bath. Red precipitates were observed as end point.

2.3.2 Test for carbohydrates

- **Starch test**

Starch test was performed by adding 5ml of 5% KOH solution in the extract. Canary color was observed indicating the presence of carbohydrates.

2.3.3 Test for alkaloids

- **Mayer's test**

Mayer's solution A and B were prepared and mixed. The final volume was raised to 100ml. 2-3 drops of the reagent are dissolved in 1ml of the extract and a creamy white layer was observed.

- **Wagner's test**

1.27g of I_2 and 2g of KI dissolved in a small amount of distilled water and then diluted to a final volume of 100ml. 2-3 drops of the reagent are dissolved in 1ml of extract and brown/reddish precipitates were observed as indication of presence of alkaloids.

- **Iodine test**

Few drops of iodine solution were added in 3ml of the extract. Blue color of the solution appeared which disappeared on boiling and reappeared on cooling.

2.3.4 Test for proteins and amino acids

- **Ninhydrin test**

Ninhydrin solution was prepared by the addition of 10mg of ninhydrin in 200ml acetone. 2-3 drops of ninhydrin solution were added in 1ml of the extract in a test tube. Appearance of purple color showed the existence of proteins and amino acids.

2.3.5 Test for phenols and tannins

Precisely measured 2ml of 2% $FeCl_3$ was dissolved in 1ml of the extract. Appearance of blue green or black color of the solution confirms the existence of phenols and tannins.

2.3.6 Tests for flavonoids

- **Alkaline reagent test**

Amount of 2ml of 2% NaOH was added to 1ml of the leaf extract. The solution turned yellow which again became colorless when few drops of dilute H_2SO_4 were dissolved.

- **Concentrated H_2SO_4 test**

Few drops of the concentrated H_2SO_4 were dissolved in 1ml of plant extract. Orange color of the solution suggested the existence of flavonoids compounds.

2.3.7 Test for glycosides

- **Keller-killani test**

Glacial acetic acid about 2ml was added in 1ml of the extract. 1 drops of ferric chloride solution and 2-3 drops of concentrated H_2SO_4 were added. A brown ring pointed out the existence of glycosides.

- **10% NaOH test**

Sulphuric acid about 1ml was added in 0.2ml of the extract. The solution was boiled for about 15 minutes. It was then allowed to cool. Few drops of 10% NaOH and 0.2ml of Fehling solution was added. The appearance of brick red precipitates indicated the existence of glycosides.

2.3.8 Test for saponins

- **Foam test**

Distilled water of about 5ml was added in 2ml of the extract. The solution was shaken well. Formation of the stable foam indicated presence of saponins.

2.3.9 Test for sterols

- **Salkowski's test**

Amount of 2ml of chloroform along with 2ml of Sulphuric acid was added in 2ml of the extract. After thorough shaking, a greenish yellow fluorescent layer was formed at the bottom which showed the presence of sterols.

2.4 ANTIOXIDANT ACTIVITIES

The antioxidant activity of the leaf extract was evaluated using the following assays.

2.4.1 DPPH Radical scavenging activity

DPPH radical scavenging activity of the extract of the leaves of *Pericallis cruenta* was evaluated by comparing it with known antioxidant, i.e. Butylated hydroxytoluene (BHT) using the procedure by Lee *et al.* (1998).

Preparation of reagent

Amount of 0.1mM methanolic solution of DPPH was prepared by mixing 2.4mg of DPPH in 100ml of methanol.

Procedure

Plant extract of different concentrations i.e. 1ml, 0.75ml, 0.50ml and 0.25ml were mixed with 3ml of DPPH solution in separate test tubes. They were kept at normal temperature for 1 hour in dark. The absorbance was noted at 517nm in spectrophotometer with methanol as blank solution. %age scavenging activity was assessed using the formula;

$$\textbf{Percentage scavenging activity} = \frac{A\,(control) - A\,(sample)}{A\,(control)} \times 100$$

Whereas;

$A_{(control)}$ = Absorbance of control

$A_{(sample)}$ = Absorbance of sample

1.4.1 Ferric Reducing Antioxidant Power (FRAP)

FRAP analysis was evaluated following the standard method by Benzie and Strain (1996).

Preparation of reagent

- HCl of 40mM was prepared by mixing 146µl of HCl with 40ml of the distilled water. The Volume was increased to 100ml by adding further distilled water.
- TPTZ of 10mM was prepared by dissolving 0.312g of 2,4,6- Tripyridyl-s-triazine in 50ml of 40mM HCl. The volume was made to 100ml by dissolving 40mM HCl solution.
- To prepare 20mM of ferric chloride solution, 0.54g of $FeCl_3.6H_2O$ was added in 50ml of distilled water and the volume was made to 100ml.
- For the preparation of 300mM Acetate buffer, 0.31g of sodium acetate was mixed in 10ml of the distilled water to make a homogeneous mixture. To this solution, 1.6ml of glacial acetic acid was added. The total volume was raised to 100ml by distilled water. Finally, pH of the solution was made to 3.6 by using 0.1N NaOH or 0.1N HCl according to requirement.

The working solution for FRAP Assay was made by mixing 2.5ml of 10mM TPTZ solution, 2.5ml of $FeCl_3.6H_2O$ solution and 25ml of 300mM acetic buffer.

Procedure:

The plant extract of 10µl was mixed with 2990µl of the freshly prepared FRAP solution in a test tube. After thorough shaking, it was kept in dark for about 30 minutes and the absorbance was measured at 593nm using spectrophotometer. The outcomes were documented in micromoles of Trolox Equivalents (TE) per milliliter of the sample. FRAP values were computed utilizing the subsequent equation;

$$\mathbf{X} = \frac{Y - 0.058}{0.4809}$$

Whereas;
Y= Absorbance of sample
X= TE (µM/ml)

2.4.2 Total Antioxidant Activity (TAA)

For evaluation of Total Antioxidant Activity, phosphomolybdenum complex formation method Prieto *et al.* (1999) was used.

Preparation of reagent

About 5.32g of sodium phosphate and 2.47g of ammonium molybedate were added in 100ml of the distilled water. In the solution, 16.7ml of Sulphuric acid was mixed and volume was made to 500ml by using distilled water.

Procedure

Amount of 4ml of prepared solution was dissolved in a test tube in 1ml of the extract. The test tube was cotton plugged. It was then incubated for 90 minutes in a water bath at 95°C. The sample was then cooled at room temperature. 4ml of the reagent was used as control and absorbance was noted at 695nm.

The result was described as milligram per milliliter of the ascorbic acid and determined by using the following equation;

$$\mathbf{X = \frac{Y + 0.0328}{0.0112}}$$

Whereas;
Y= Absorbance of sample
X =?

1.4.2 Metal Chelating Activity

For the determination of Metal Chelating Activity of leaf extract of *Pericallis cruenta*, the standard procedure by Dinis *et al.* (1994) was used.

Preparation of solutions

- For preparing 2mM of Ferrous Chloride ($FeCl_2$), 0.025g of $FeCl_2$ was added in small amount of the distilled water. The solution was diluted to 100ml.
- To prepare 5mM Ferrozine solution, 0.257g of ferrozine was dissolved in small amount of water. The solution was diluted to 100ml.

Procedure

Plant extract of 1ml was introduced into a test tube. About 50 µl of 2mM of ferrous Chloride as well as 0.2ml of 5mM Ferrozine solution were added in test tube. The solution was mixed thoroughly and kept for 10 minutes at normal temperature. Absorbance was noted at wavelength of 562nm by spectrophotometer.

%age inhibition of Ferrozine-iron complex configuration was considered by using formula;

$$\textbf{Percentage inhibition} = \frac{(Ab\_As)}{Ab} \times 100$$

Whereas;
A_b = Absorbance of blank
A_s = Absorbance of extract

1.4.3 Total Flavonoid Content (TFC)

Total Flavonoid activity evaluation of plant extract was performed by the procedure used by Zhishen *et al.* (1999).

Preparation of Solutions

- About 5% $NaNO_2$ was prepared by mixing 5g of $NaNO_2$ with small quantity of distilled water and volume was made to 100ml.
- To prepare 10% $AlCl_3$ solution, 10g of $AlCl_3$ was mixed in small quantity of water. It was then diluted to 100ml.
- To prepare 4% sodium hydroxide, 4g of NaOH was dissolved in small quantity of water. The solution was diluted to 100ml.

Procedure

About 1ml of extract and 0.2ml of distilled water were taken in the test tube. 0.15ml of 5% $NaNO_2$ was added into the mixture and allowed to sit for 5 minutes at the room temperature. 0.15ml of 10% Aluminum chloride was added in it and kept for 6 minutes at normal temperature. After that, 2ml of already prepared 4% sodium hydroxide was dissolved in the solution. The final volume of the solution was raised to 5ml with distilled water. It was then shaken thoroughly and kept for 15 minutes at normal temperature. The absorbance was noted at 510nm wavelength. Result was determined in terms of milligram equivalent of Rutin (mg Ru./g) using the formula;

$$\mathbf{X = \frac{Y + 0.027}{0.0129}}$$

Whereas;
Y= Absorbance of sample
X= mg Ru. /g

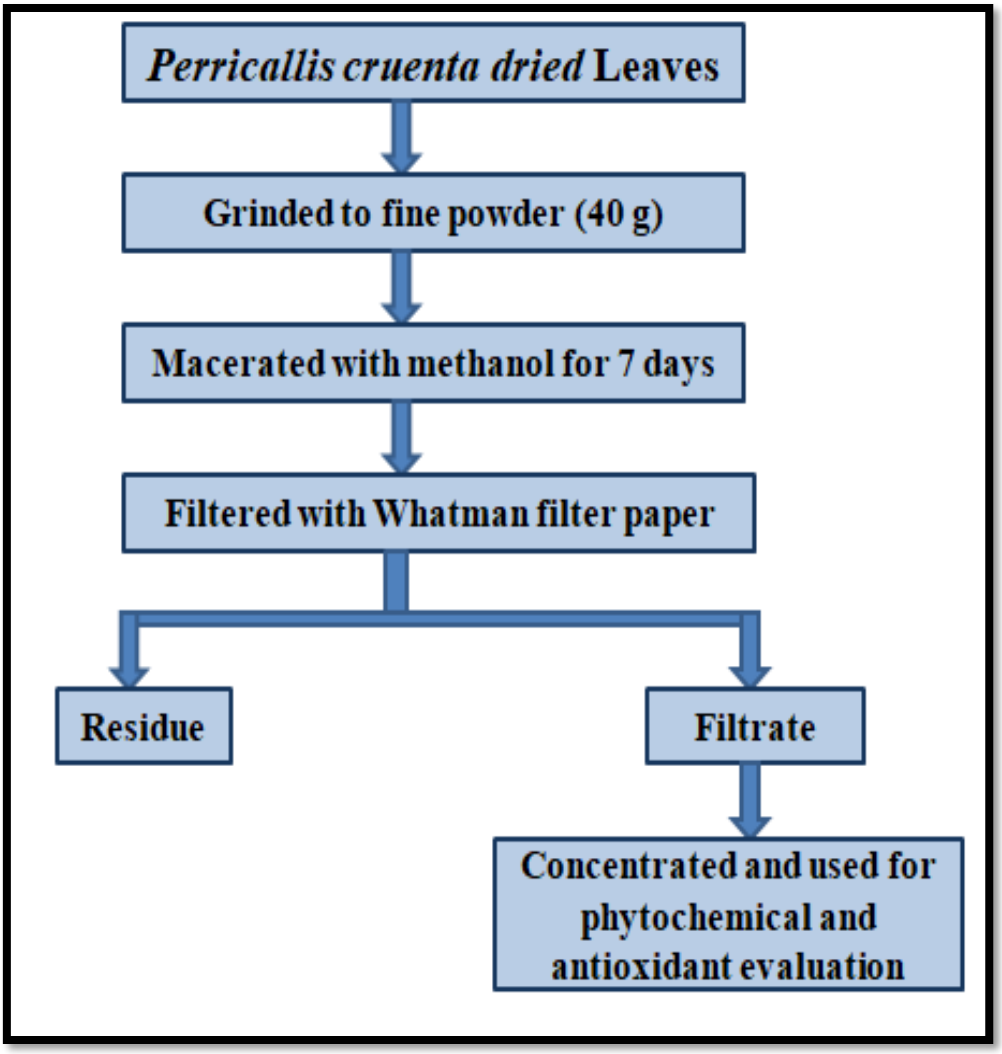

Fig.1: Pericallis cruenta dried leaves extraction schematic procedure

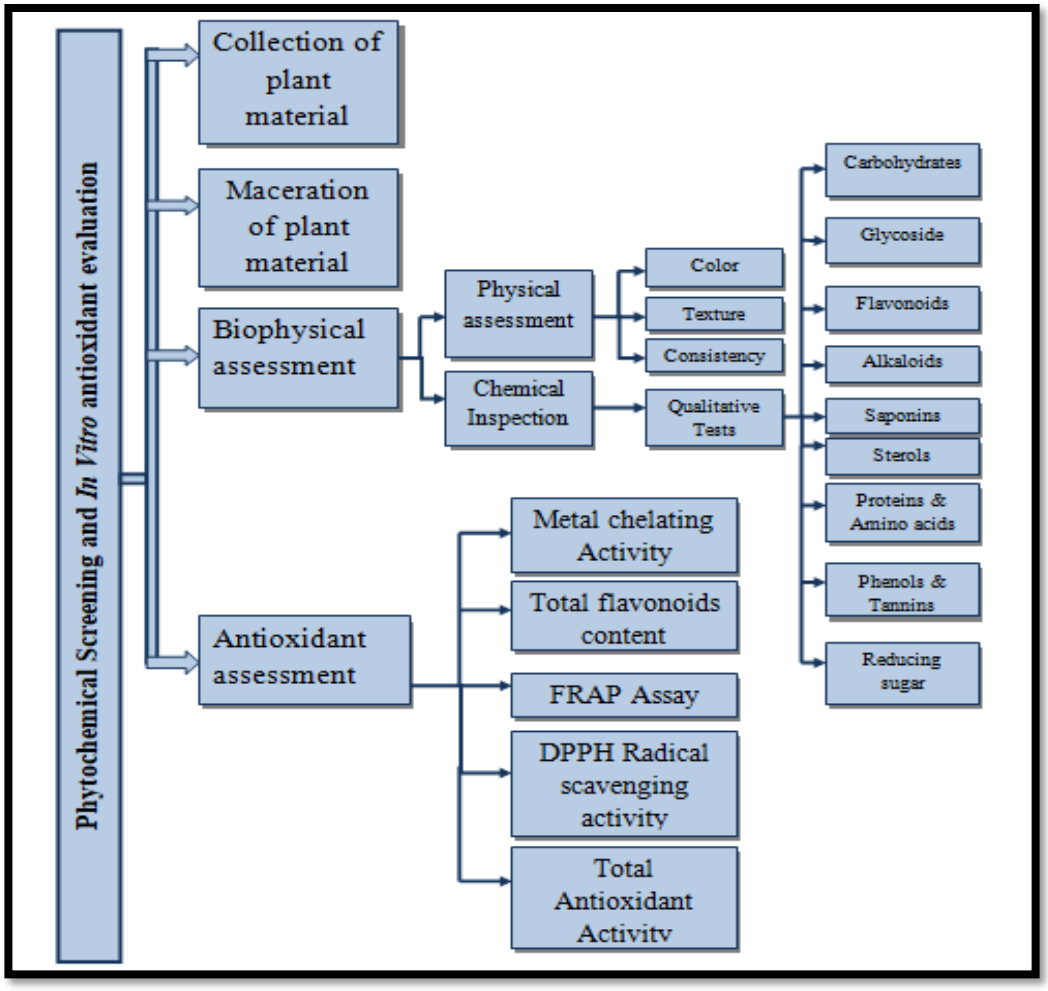

Fig. 2.2: Flow chart for phytochemical screening and In Vitro antioxidant evaluation of plant sampl

Result

The phytochemical screening along with antioxidant potential of leaves of *Pericallis cruenta* was carried out according to the standard methodology in the current study. Here are the results.

3.1 Physical analysis of Extract

The color, texture and appearance of methanol extract of the leaves were noted to analyze the physical properties of the extract. These properties are mentioned in the **Table 3.1**.

Table 3.1: Physical properties of methanolic extract of leaves of *Pericallis cruenta*

Properties	**Extract**
Solvent	Methanol
Color	Dark green
Texture	Non-sticky
Appearance	Liquid

3.2 Phytochemical screening

Phytochemical screening involves the qualitative assessment of the constituents possessed by the plant. In the methanolic extract of leaves of *Pericallis cruenta*, flavonoids, proteins, amino acids, sterols, carbohydrates, phenols & tannins and saponins are present. **Table 3.2** shows the results of phytochemical screening.

Table 3.2: Phytochemical screening

Tests	Methanolic extract
Carbohydrates	+
Glycosides	_
flavonoids	+
Alkaloids	_
Saponins	+
Reducing sugars	_
Sterols	+
Proteins and amino acids	+
Phenols and tannins	+

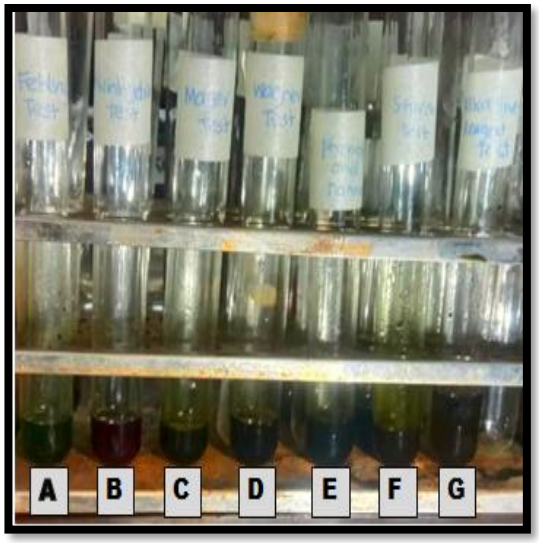

Fig. 2.3: (A) Fehling test (B) Ninhydrin test (C)Mayer Test (D) Wagner test (E) Test for phenols and Tannins (F) Starch Test (G) Alkaline reagent test

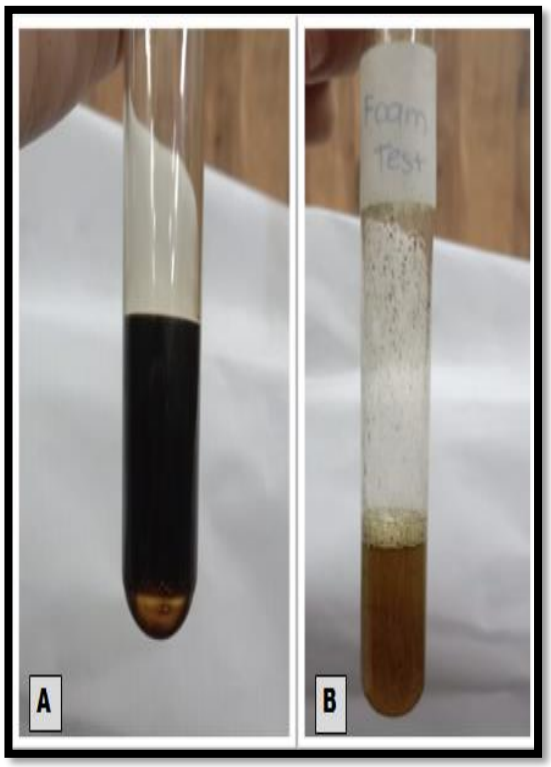

Fig. 2.4: (A) Test for Sterols (B) Test for Saponins

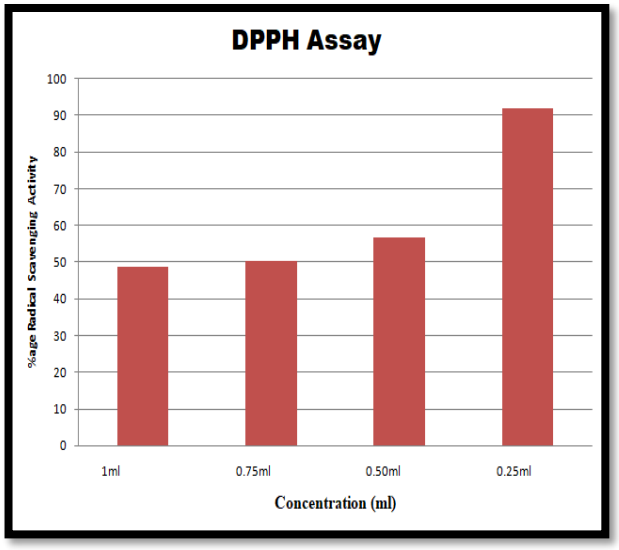

Fig. 2.5: Graphical representation of analysis of DPPH free radical scavenging Activity of Leaves of Pericallis cruenta

Antioxidant Activities

Standard methods were used to find the antioxidant potential of plant.

3.3.1 DPPH Radical scavenging activity

DPPH radical scavenging activity was assessed at different concentration of plant extract using the standard procedure. Absorbance of samples of different concentrations was noted at 517nm and %age scavenging activity was determined. **Table 3.3** shows the absorbance and percentage scavenging activity of the leaves extract.

Table 3.3: DPPH Radical Scavenging Activity

Sr. No.	Concentration of samples	Absorbance at 517nm	%age scavenging activity
1	1ml	0.154	48.83
2	0.75ml	0.150	50.16
3	0.50ml	0.130	56.81
4	0.25ml	0.024	92.06

3.3.2 Ferric Reducing Antioxidant Power (FRAP)

FRAP analysis was performed using the standard procedure for assessment of the antioxidant potential of plant extract. Absorbance was observed at wavelength 593nm and outcomes were calculated using the formula. These results are shown in **Table 3.4**.

Table 3.4: Ferric Reducing Antioxidant Power (FRAP)

Sr. No.	Solvent	Absorbance at 593nm	TE (µM/ml)
1	Methanol	2.240	4.537326±0.08
2	Methanol	2.164	4.379289±0.08
3	Methanol	2.311	4.684966±0.0

3.3.3 Total Antioxidant Activity (TAA)

Total Antioxidant Activity of the leaves methanolic extract of *Pericallis cruenta* was calculated as milligram per milliliter of the ascorbic acid after noting the absorbance at 695nm. The result is shown in **Table 3.5**

Table 3.5: Total Antioxidant Activity (TAA)

Sr. No.	Solvent	Absorbance at 695nm	TAA values (mg/ml of Ascorbic Acid)
1	Methanol	1.476	132.07±12.30

2	Methanol	1.119	102.83±12.30
3	Methanol	0.982	90.60±12.30246

3.3.4 Metal Chelating Activity

Metal chelating activity was performed to observe the antioxidant activity of leaves extract of *Pericallis cruenta*. Absorbance was observed at 562nm and %age inhibition of Ferrozine-ferrous complex formation was calculated. The outcomes are shown in **Table 3.6**

Table 3.6: Metal Chelating Activity

Sr. No.	Solvent	Absorbance at 562nm	%age inhibition
1	Methanol	2.287	1266.83±28.985
2	Methanol	2.098	1170.40±28.985
3	Methanol	2.145	1194.38±28.985

3.3.5 Total flavonoids content

Total Flavonoids Content of leaves of *Pericallis cruenta* was evaluated using the standard procedure phytochemical composition of methanol extract of the seeds of *Helianthus annuus.* Their results appeared to have tannins, carbohydrates, alkaloids, flavonoids, saponins, phytosterols, and fixed fats and oils.

The antioxidant potential of leaves extract of *Pericallis cruenta* was estimated using the five Assays, i.e. Metal Chelating Activity, DPPH Radical Scavenging Activity, Total Antioxidant Activity (TAA), Ferric Reducing Antioxidant Power and Total Flavonoid Content (TFC).

DPPH is a stable and widely used organic radical based on nitrogen and has a lambda maximum of 517 nm. When reduced, the solutions initial purple color disappears, demonstrating the samples antioxidant and absorbance was evaluated at 510nm. The outcome was noted in terms of milligram equivalent of Rutin as shown in **Table 3.7**.

Table 3.7: Total Flavonoid Content

Sr. No.	Solvent	Absorbance at 510nm	TFC values (mg Ru./g)
1	Methanol	0.291	24.65116±8.879
2	Methanol	0.686	55.27132±8.879
3	Methanol	0.521	42.48062±8.879

Discussion

Present study uses the methanolic extract of leaves of *Pericallis cruenta* for the purpose of phytochemical screening and antioxidant evaluation. Maceration procedure was used for the extraction. The extract obtained was dark green in color, non-sticky to touch and liquid in appearance.

After the phytochemical screening of the extract, it was observed that leaves extract of *Pericallis cruenta* contain carbohydrates, flavonoids, saponins, sterols, proteins, amino acids, phenols and tannins. These results were quite similar to those recorded by Subashini & Rakshitha (2012) while studying the

capabilities. The outcomes of the DPPH radical scavenging activity were displayed as a percentage of the residual DPPH. This assay was performed at different concentrations i.e. 1ml, 0.75ml, 0.50ml and 0.25ml. The highest absorption was shown by the solution of 1ml concentration and lowest by solution of 0.25ml concentration. However, the percentage Scavenging activity was highest for 0.25ml solution i.e. 92.06% and lowest for 1ml solution i.e. 48.83%. The results were slightly different from those documented by Aliyu *et al.* (2009) after evaluating the antioxidant activity of methanolic extract of *Ardisia crispa* L. They ranged between 59.588% - 94.732% at different concentrations.

Ferric Reducing Antioxidant Power assessment was performed to analyze the antioxidant capacity of leaves of *Pericallis cruenta*. The value of Trolox equivalent per ml of the extract was found to be 4.52 µM/ml average. The results were slightly different from those found by Yu *et al.* 2021 while carrying out the phytochemical and antioxidant study of food and medicinal plants. They found the value of 2.33±0.04 while evaluating the antioxidant properties methanolic extract of *Punica granatum* L.

Total antioxidant activity analyses the antioxidant potential of methanol extract of plant part. The average value for TAA was found out to be 108.5mg/ml. while doing the similar work Aliyu *et al.* (2013) calculated the total antioxidant capacity of n-Butanol extract of roots of *Anchomanes difformis* as 90mg/ml.

In case of Metal Chelating Activity, absorbance was noted at 562nm and percentage inhibition was calculated as 1211.20% average. This shows that the methanolic extract of leaves of *Pericallis cruenta* contain high value of %age iron bound. Same results were displayed by Serteset *et al.* (2009) when they were evaluating the antioxidant activity of some plants in Turkey.

Total Flavonoid Content was also conducted in order to analyze the antioxidant activity of plant extract. The average value for TFC was calculated as 40.8 mg Ru./ g. Same results were displayed by Atanassova *et al.* (2011) while finding out Total Flavonoid Content of methanolic extract of *Melissa officinalis.* Their value was found out to be 45.06 mg/g.

Vascular plants contain more than 4000 phenolic chemicals, including flavonoids, monophenols, and polyphenols. Phenolic substances including chlorogenic acid, quercetin, narigin, caffeic acid, catechin, gallic acid, rutin and quercetin are very significant for the plants (Qusti *et al.*, 2010).

From the above values obtained from different phytochemical tests and antioxidant assays, it is found that leaves of *Pericallis cruenta* have important medicinal properties and can be used to cure diseases.

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