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UNVEILING THE PHYTOCHEMICAL PROFILE, SECONDARY METABOLITE QUANTIFICATION AND ANTIOXIDANT ACTIVITY OF *CLEMATIS WIGHTIANA* WALL. EX WIGHT & ARN.

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ABSTRACT

Traditionally, the leaves of *Clematis wightiana* have been used in the treatment of rheumatism, indigestion, headaches, varicose veins, bone problems, nasal congestion and sinus. The present study was conducted to evaluate the phytochemical profile, quantification of secondary metabolites and free radical scavenging capacity of *C. wightiana* leaf. The total phenolic, tannin and flavanoid content of *C. wightiana* leaves were quantified and were found to be higher in the ethyl acetate extract. Subsequently, the extracts were subjected to appraise their antioxidant capacity by availing various *in vitro* antioxidant assays namely DPPH radical scavenging assay, ABTS assay, Phosphomolybedenum assay, Ferric Reducing assay, Superoxide Radical Scavenging assay and Reducing power assay. The results of the antioxidant assays revealed that the ethyl acetate extract of *C. wightiana* leaves possess better free radical scavenging activity than other solvent extracts. Thus, the finding of the study elucidates the perception on phytochemical and bioactivity of *C. wightiana* which could be used in development of phytotherapeutics to enchance human health.

Keywords: *Clematis wightiana*, Antioxidant, Superoxide radical scavenging, *In vitro* antioxidant assay, DPPH, Ethnomedicine.

1. INTRODUCTION

Phytomedicine, the art of using plants to treat diseases in known from time immemorial. Right from the first health problem encountered by early human and until the recent corona viral infections that occurred as a pandemic, plants are one of the important resource's humans seek for medicine and treatment, the plants have provided many renowned drugs that act as life saviour at various situations to encounter the health problems. Apparently, there is a growing concern in the field of phytomedicine to develop the insights of the therapeutic potential of traditionally used and economically underused plants. Herbal interventions are much demanded in the globalized world since the existing conventional therapies and their synthetic drugs are of high cost and provide only symptomatic reliefs associated with detrimental side effects [1]. Consequently, the need and utility of the plant-based drugs and phytotherapeutics have increased considerably.

The current study focuses on evaluating the phytochemical constituents and exploring the antioxidant capability of *Clematis wightiana* extracts. The plant *Clematis wightiana* belongs to the family Ranunculaceae. The family comprises about 60 genera and 2,200 species which are mostly woody, climbing vines. Ranunculaceae members have a worldwide distribution, chiefly in the temperate zones of Northern hemisphere. Among all the genus belonging to the said family, the genus *Clematis* is the second largest genus and holds about 355 species. The *Clematis* genus can be differentiated from other genera by its phyllotaxy (opposite leave) and its habit (viney) [2]. *Clematis* species have been used enormously in the traditional medicine to cure a wide range of ailments. Some early reports have confirmed the anti-cancer, antibacterial, anti-inflammatory and analgesic effects of *Clematis* [3].

Traditionally, several *Clematis* species are used to treat rheumatism, arthritis, diuretic (*C. paruibola, C. manshurica, C. japonica, C. flammula, C. erecta, C. crassifolia, C. glycinoids*), asthma (*C. hirsuta, C. aprifolia, C. branchiata*) and skin diseases (*C. grata, C. roylei, C. montana*) [4].

The selected plant *Clematis wightiana* is used in folk medicine to treat rheumatism, indigestion, headaches, varicose veins, bone problems, nasal congestion and sinus [5-7]. Even though *C. wightiana* is used in folk medicine for its significant therapeutic values, it lacks scientific studies to substantiate the traditional claims. A systematic search of literature revealed that there are no previous scientific validations carried out or reported on the antioxidant activities of *C. wightiana* leaves. Therefore, this investigation was undertaken with the following objectives such as determination of the phytochemical profile, quantification of the secondary metabolites and evaluation of the free radical scavenging activity of various extracts of *C. wightiana*.

2. MATERIAL AND METHODS

2.1. Collection and extraction of plant material

The fresh plant material was collected during the month of December 2020 from Shola Forest Kotagiri (1,800 MSL), The Nilgiris district, Tamil Nadu, India. The plant sample was taxonomically identified and authenticated. The plant materials were washed under running tap water and they were air dried under shade. The dried leaf samples were powdered and used for further studies. The powdered leaf sample of *Clematis wightiana* was packed in thimbles and extracted successively with four organic solvents such as petroleum ether, chloroform, ethyl acetate and ethanol in the increasing order of polarity using Soxhlet apparatus. Finally, the sample was macerated with hot water and the aqueous extract was filtered.

2.2. Qualitative Phytochemical Analysis

All the extracted samples were assessed for preliminary phytochemical screening to determine the presence of carbohydrates, proteins, amino acids, alkaloids, saponins, phenols, flavanols, flavones glycosides and phytosterols using standard methods [8].

2.3. Quantitative Analysis of Secondary Metabolites

2.3.1. Determination of total phenolics and tannins

The total phenolic content was determined by using Folin-ciocalteu method [9] with gallic acid as equivalence. 100µl of the diluted leaf extract was mixed with 500µl of Folin-ciocalteu. 2.5ml sodium carbonate was added to the above mixture after few minutes and vortexed. The sample mixture was allowed to stand for 40 minutes in dark. The absorbance was recorded at 725 nm.

Using the same extracts, the total tannin content was also determined where, the reaction mixture containing 1ml of the sample, 1ml distilled water and 100mg of polyvinylpolypyrrolidine(PVPP) were freezed at 4°C for 15 min. Further, the mixture was centrifuged and the supernatant containing the non-tannin phenolics was used to assess the tannin content which was calculated by the following formula:

Tannin (%) = Total phenolics (%) - Non-tannin phenolics (%)

2.3.2. Determination of total flavonoids

The total flavonoid content was evaluated by aluminium trichloride method [10]. A calibration curve was constructed using quercetin as standard. The triplicates of 100µl of the extracts were added with 2ml of distilled water in each. 150µl of 5% sodium nitrate and 150 µl of 10% aluminium trichloride were added to the reaction mixtures and allowed to stand for 6 minutes. Subsequently, 2ml of 4% NaOH was added and the volume was made upto 5ml with distilled water. The appearance of pink colour after incubation period of 15 minutes was measured spectrophotometrically at wavelength of 510nm against the blank.

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging activity

The antioxidant activity of *C. wightiana* extracts were evaluated by determining the hydrogen or electron donating capacity of the sample to the stable radical DPPH ensuing in the decolourization of the dark purple DPPH solution [11]. The methanol solution of samples of various concentrations was added to 3ml of 0.1mM DPPH solution and incubated for 30 minutes. The methanol was considered as blank and the reaction mixture without sample served as control. The absorbance of the sample was recorded at 517nm.

2.4.2. ABTS radical cation scavenging activity

ABTS radical scavenging assay was carried out by following the method of Re *et al.* (1999) [12]. 30 µL sample was mixed with 1ml of diluted ABTS which was previously diluted with ethanol. The test tube containing reaction mixture other than the sample was

considered as control. All the test tubes were incubated at room temperature for 30 minutes. Absorbance of the sample was recorded at 734nm.

2.4.3. Phosphomolybdenum assay

The antioxidant activity of *C. wightiana* leaf was determined by the green phosphomolybdenum complex method [13]. 100 µl of the sample was added with 3ml of reagent solution (0.6 M sulfuric acid, 28mm sodium phosphate and 4mM ammonium molybdate) and the mixture was incubated in water bath at 95°C for 90 minutes. After cooling, absorbance was measured at 695nm.

2.4.4. Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of different extracts *C. wightiana* were evaluated by adopting the method of Pulido *et al.,* (2000) [14]. 30 µL of sample was mixed with 90 µL of distilled water along with freshly prepared FRAP reagent (900 µL). The test samples along with the blank were allowed to stand in a water bath maintained at 37°C for 30 minutes. The blue colour developed at the end of incubation was measured immediately at 593 nm against the reagent blank using spectrophotometer.

2.4.5. Superoxide radical scavenging activity

The assay was based on the ability of different extracts of *C. wightiana* to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system [15]. About 3 mL of reaction mixture containing 50 mM sodium phosphate buffer (pH-7.6), 20 µg riboflavin, 12 mM EDTA and 0.1 mg NBT was added to 100 µL sample solution, BHT and rutin. Reaction was started by illuminating each reaction mixture for 1.5 minutes. Immediately after illumination, the absorbance was measured at 590 nm against the blank (unilluminated reaction mixture without leaf sample).

2.4.6. Reducing power assay

The method reported by Pulido *et al.,* (2000) [14] was used to determine the reducing power of different *C. wightiana* extracts. The aliquots of different concentrations were made upto 1ml with methanol. All the samples were added with phosphate buffer (2.5ml, 0.2M, ph-6.6), potassium ferric cyanide (2.5ml 1%) and incubated for 20 minutes at 50°C. Followed by incubation, trichloroacetic acid (2.5ml 10%) was added and subjected to centrifugation. After centrifugation for 10 minutes at 3000rpm, 2.5ml distilled water and 0.5ml ferric chloride (0.1%) were mixed with 2.5ml of aliquot supernatant and allowed to stand for 10 minutes and the absorbance at 700nm was measured.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Analysis

3.1.1. Qualitative Phytochemical Screening

The phytochemical compounds play a major role in plants as these acts as first line of defense to protect themselves. The secondary metabolites have many positive and negative effects. Understanding the nature of these components will help in better utilization of these compounds in right way, this will further lead to explore them for their preventive effects. There are many reports on various medicinal plants that are used to cure various diseases and disorders such as cardiovascular, neuro degenerative diseases and cancer [16-19].

Table 1: Preliminary Phytochemical Screening of various extracts of *C. wightiana*

Extracts	Petroleum ether	Chloroform	Ethylacetate	Ethanol	Aqueous
Carbohydrate	+	+	+	+	+
Protein	+	+	+++	+	+
Amino acid	-	-	++	++	++
Alkaloid	+	+	+++	++	++
Flavanol glycosides	+	+	++	+	+
Phytosterol	+	+	++	++	++
Flavonoid	+	+	+++	++	++
Cardiac glycoside	-	++	++	+++	+
Phenolic compound	+	+	+++	+	++
Saponins	-	++	++	+	++

(+): Presence of chemical compound, (-): Absence of chemical compound, (+) < (++) < (+++): Based on the intensity of characteristic colour plants.

The qualitative phytochemical screening of *C. wightiana* for major primary and secondary phytochemicals were carried out and the results were shown in Table 1. The results revealed that the primary metabolites such as carbohydrates, proteins and amino acids showed positive result in all the extracts, except for amino acids in petroleum ether and chloroform extract. The results of the qualitative phytochemical screening showed the presence of all the phytochemical constitution of the present study such as alkaloid, saponins, phenol, flavonoid, flavonol glycosides, cardiac glycosides and phytosterol. The ++ sign indicates high concentration of particular secondary metabolites which was indicated by the high intensity of the colour developed and - sign indicates absence of chemical compound.

The results were in line with other species of *Clematis* as reported in *C. hedysarifolia* by previous researchers [20]. Similarly, Abdisa and Kenea (2021) [21] have also evaluated the phytochemical constituents of *C. hirsuta* and revealed the significant presence of alkaloids, saponins, tannins, flavanoid, phenols, glycosides and carbohydrates. This result also signifies the presence of similar components in *C. wightiana*.

3.2. Quantitative Analysis of Secondary Metabolites

3.2.1. Determination of Total Phenolics and Tannin Contents of C. wightiana

The amount of total phenolics of different extracts of *C. wightiana* were analysed and shown in Table 2. The total phenolics were found to be higher in ethyl acetate extract (436.8 mg GAE/g extract) followed by ethanol extract (396.33 mg GAE/g extract). The tannins were found to be higher in Ethyl acetate extract (426.73 mg GAE/g extract) followed by ethanol extract (377.33 mg GAE/g extract).

Table 2: Total Phenolic and Tannins Contents of *C. wightiana* Extracts

Extracts	Total Phenolics (mg GAE/g extract)	Total Tannins (mg GAE/g extract)
Petroleum ether	71.66±0.7	66.46±0.64
Chloroform	73.26±1.47	64.2±1.24
Ethyl acetate	436.8±0.34	426.73±0.23
Ethanol	396.33±0.7	377.33±0.64
Aqueous	234.06±2.73	228.53±2.84

*GAE - Gallic Acid Equivalents, *Values are mean of triplicate determination (n=3) ± standard deviation.*

Phenolics are aromatic compounds which protect the plants against stress and highly contribute to the antioxidant capacity [22, 23]. Phenolic compounds are relatively stable phenoxyl radicals, disrupting chain oxidation reactions in cellular components [24]. These play a specific role in scavenging of free radicals [25]. The phenolic content of the selected sample *C. wightiana*(436.8±0.34 mg GAE/g) was found to be higher than that of many former reports in *Clematis* species, namely *C. connata* (265.7±1.4 mg GAE/g) [26], *C. orientalis* (215.29 mg GAE/g) [27]. Menfei *et al.,* (2018) [28] reported that *C. chinensis* possess total phenolic content of 38.23±4.8 mg GAE/g and the results disclosed by Djawhara *et al.,*(2020) [29] revealed that *C. cirrhusa* has 84.05±0.80 mg GAE/g which are comparatively lower than that of *Clematis wightiana* (426.73 mg GAE/g extract) extracts. Thus, the high amounts of secondary metabolites are known to have high free radical scavenging activity.

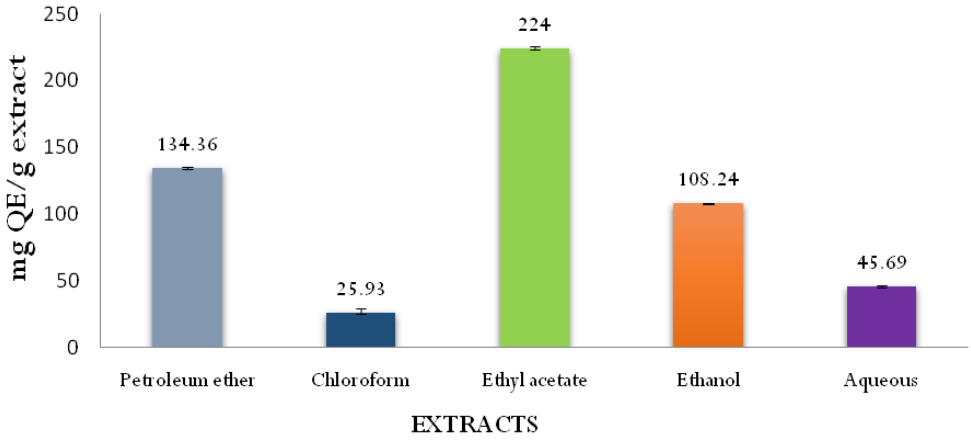
Tannins are bitter tasting organic compounds that have been reported to have strong astringent properties such as anti-microbial, anti-inflammatory and antioxidant activity causing protein precipitation [30]. Asma *et al* (2019) [31] analysed the total tannin content in *C. flammula* which is higher than the tannin content present in *C. wightiana.* The greater amount of tannins in the extracts of *C. wightiana* can be due to higher polymerization of existing polyphenolic compounds. It has been reported recently that the high molecular weight phenolics such as tannins acquires more ability to quench free radicals and protect the human body from the oxidative stress. Apart from that, the high amounts of tannins in *C. wightiana* may enhance the free radical scavenging activity of its extracts.

3.2.2. Quantification of Flavonoids

The flavonoid contents of *C. wightiana* was analysed and presented in Fig 1. Among all the extracts, the ethyl acetate extracts were found to have appreciable amount of flavonoid contents (224 mg QE/g extract) followed by petroleum ether extract (134.36 mg QE/g). From this data, it is clear that the ethyl acetate extract has higher flavonoid content when compared to other extracts.

Flavonoids are most common and widely distributed group of polyphenolic compounds. These play a major role as antioxidants derivative in scavenging the free radicals and chelate trace elements [32-35]. In the selected plant sample *C. wightiana*, the flavanoid content was found to be 224 mg QE/g extract and the results are compared with the earlier reports where Mostafa *et al.*, (2018)[36] have analyzed the total flavanoid content

of *Clematis brachiata* (135.11±1.2 mg QE/g extract). A comparative study carried out by Karimi *et al.,* (2017) [2] has reported that *C. ispahanica* (5.6 mg RE/g extract) holds more flavanoid content than *C. orientalis* (4.4 mg/g rutin). Mengfei *et al.*, (2018) [28] have reported the total flavanoid content in *Clematis chinensis* (2.91±0.02 mg CE/g DW) which is comparatively lower than that of selected study sample. This present study concluded that *C. wightiana* could be good source of natural antioxidant since it contains higher concentration of flavonoids with hydroxyl groups which are responsible for the radical scavenging activity.



*QE - Quercetin Equivalents, *Values are mean of triplicate determination (n=3)±standard deviation.*

Fig. 1: Flavonoid Contents of *C. Wightiana* Extracts

3.3. *In Vitro* Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the extracts of *C. wightiana* was determined and shown in Table 3. The lowest IC_{50} values indicated the highest free radial scavenging activity of the extract. The assay was compared with standard rutin and BHT. Among the extracts examined, ethyl acetate extract (46.92 µg/mL) exhibited superior IC_{50} values for DPPH radical scavenging activities than the other solvent extracts. The radical scavenging activities of the standard drug of rutin and BHT were found to be 7.43µg/mL and 8.8 µg/mL, respectively. This clearly indicated that the ethyl acetate extract possesses highest DPPH radical scavenging activity.

DPPH is an organic chemical compound composed of unwavering free radical molecule in an appropriate environment that has been extensively used to investigate antioxidant potential as it does not disintegrate in water, methanol or ethanol. DPPH free radicals can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule [37]. Wang *et al.* (2007)[38] have reported that the plant extract has the ability to reduce the stable DPPH radical which indicates a color change from purple color to the yellow color.

In the present investigation, the ethyl acetate extract of *C. wightiana* depicted highest radical scavenging with IC_{50} value of 46.92µg/mL. Similarly, many of the Clematis sp. such as *C. grata* (IC_{50} value 11.39 µg/mL) by Ashwani kumara *et al.*, (2019) [39] , *C. connata* (IC_{50} value of 1.5 µg/mL) by Qureshi *et* al., (2011) [26], *C. flammula* (IC_{50} value of 120 mg/mL) by Saidi *et al.,* (2019) [40] and *C. hirsuta* (IC_{50} 0.59 mg/mL) by Abdisa and Kenea (2021) [21] have been reported to possess antioxidant properties and radical scavenging activities. The findings of Mostafa *et al.,* (2018) [36] showed that the methanol extract of *C. brachiata* exhibited superior DPPH value with 180.45±2.4 µg/mL which is comparatively lower than that of the study sample *C. wightiana*. On comparing the earlier reports of DPPH radical scavenging activities of various *Clematis* species, the selected plant has promising radical scavenging activities, which might be due to the higher phenolic contents.

3.3.2. ABTS radical cation scavenging activity

The total antioxidant capacity of various *C. Wightiana* extracts using ABTS radical cation scavenging activity was determined and the results were shown in Table. 3. The results showed maximum scavenging activity in the ethyl acetate extract (37511.42±79.08 µM TE/g) followed

by the ethanol extract (36438.36±181.21 µM TE/g). In this assay, natural antioxidant rutin (86736.11±213 µM TE/g) and synthetic antioxidant BHT (84768.52±494 µM TE/g) were used as standards.

Table 3: DPPH radical scavenging activity ABTS radical cation scavenging activity of *C. wightiana*

Extracts	DPPH Radical Scavenging Activity (IC_{50} µg/mL)	ABTS Scavenging activity (µM TE/g extract)
Petroleum ether	98.78	11004.57±209.25
Chloroform	98.86	1269.06±377.23
Ethyl acetate	46.92	37511.42±79.08
Ethanol	74.4	36438.36±181.21
Aqueous	91.37	35776.26±79.08
Rutin	7.43	86736.11±213
BHT	8.8	84768.52±494

The ABTS (2, 2-azinobis-3-ethylbenzothiazolino-6-sulfonicacid) radicals were generated through an oxidation reaction with potassium persultate. ABTS cation radical scavenging activity was analysed for the selected sample *C. wightiana*, the ethyl acetate extract showed maximum activity (37511.42±79.08 µM TE/g). Qureshi *et al*. (2011) [26] have analysed the radical scavenging activity of *C. connata* (0.405 µM TE/g). Mostafa *et al*., (2018) [36] have done ABTS radical scavenging assay in *C. brachiata* which ranged from 60-170 µg/ml. On comparative analysis with other *Clematis* species *C. wigtiana* showed high radical scavenging capacity. The strong hydrogen donating ability of the extracts of C. *wightiana* shown from the ABTS assay could serve as free radical scavengers by portraying as main antioxidants when they are infused along with nutrients.

3.3.3. Phosphomolybdenum Reduction Assay

The total antioxidant volume of various solvent extracts of *C. wightiana* was determined and the maximum reduction was found in ethyl acetate extract (592.88 mg AAE/g extract) followed by ethanol extract (380 mg AAE/g extract), aqueous extract (186mg AAE/g extract), chloroform extract (56.44mg AAE/g extract) and petroleum ether extract (52.22mg AAE/g extract). The antioxidant capacity observed in the extracts of *C. wightiana* is equivalent to the natural antioxidant ascorbic acid.

It is a simple assay employed for antioxidant measurements. This method is dependent on the reduction of Mo(VI) to Mo(V) by the antioxidant agents and the subsequent formation of a green phosphate /Mo(V) complex with maximal absorption at 695nm [41]. In the present study, the sample possessed high reduction capacity in the ethyl acetate extract (592.88 mg AAE/g extract) which is higher than the previous work carried out by Mastewal *et al*., (2018)[42] in *C. simensis*. The reduction of Mo (VI) to Mo (V) by the extracts of *C. wightiana* may be due to the electron transfer or hydrogen ion transfer by the bioactive compounds, specifically phenolics and flavonoids present in the respective plant parts.

3.3.4. Ferric Reducing Antioxidant Power (FRAP) Assay

In FRAP assay, depletion of the ferric-tripridyltridyltriazine to the ferrous complex which appears with an intense blue colour and is measured at a wavelength of 593 nm in low pH. Higher absorbance power indicates a maximum ferric reducing power. The density of the colour is related to the amount of antioxidant reductants in the sample. The presence of reducers makes the conversion of the Fe^{3+} /ferricyanide complex used to reduce power of Fe^{3+} to Fe^{2+}. The result that shows ferric reducing capacity of ethyl acetate extract is maximum (797.80 mM Fe(II)/mg extract). This reducing capacity is found to be comparable with that of standard rutin (814.81 mM Fe (II)/mg) and BHT (826.17 mM Fe (II)/mg extract) (Table 4).

Table 4: FRAP and Superoxide radical scavenging activity of *C. wightiana*

Extracts	FRAP mM Fe (II)/mg extract	Superoxide radical scavenging activity Percentage of inhibition
Petroleum ether	206.2	20.26
Chloroform	212.22	17.14
Ethyl acetate	797.8	31.1
Ethanol	744.01	38.73
Aqueous	793.31	55.58
Rutin	814.81	94.7
BHT	826.17	94.2

Menfei *et al*., (2018) [28] has investigated the antioxidant capacities of several Chinese medicinal herbs including *C. chinensis* (177.02 µmol Fe(II)/mg extract) and the ferric reducing power of *C. connata* (6.3 µmol Fe(II)/mg extract) was studied by Qureshi *et al.,* 2011 [26]. Thus, the results from previous work concluded

that the study sample *C. wightiana* holds comparatively maximum ferric reducing ability than the other species. Compounds with reducing power shows that they are electron donors and can minimise the oxidized intermediates of lipid peroxidation processes, so that they can act as main and secondary antioxidants.

3.3.5. Superoxide Radical Scavenging Activity

Superoxide radical is known to be a dangerous species to cellular components as a precursor of more Reactive Oxygen Species. The extracts are seen to be an exceptional scavenger of superoxide radicals produced in the riboflavin-NBT-light system *in vitro*. The scavenging activity was maximal in aqueous extract of *C. wightiana* (55.58%). In this assay BHA and BHT exhibited better radical inhibition. The percentages of inhibition of various extracts are displayed in table 4. Zhang *et al.*, (2014) [43] have analysed the superoxide scavenging activity of *C. huchouensis* which was found to have similar results as of the selected plant sample *C. wightiana*. Thus, the results disclose that *C. wightiana* could be an efficient scavenger of superoxide radical and prevents the etiology of several diseases.

3.3.6. Reducing Power Assay

Reducing power assay is a good reflector of the antioxidant activity of the plant. The plant having higher reducing power generally reported to carry high antioxidant potential too. In this assay, the existence of reductions (antioxidants) in tested illustrative would result in the depletion of Fe^{3+} ferricyanide complex to the ferrous form. *C. wightiana* extract was analysed by the measurement of the reductive ability from Fe^{3+} to Fe^{2+} and the report is shown in Fig 2.

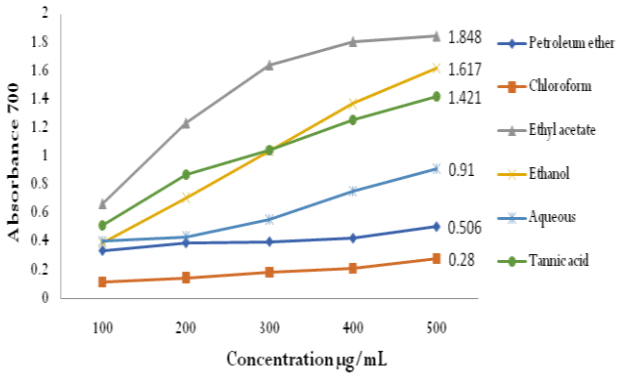


Fig 2: Reducing Power Assay of *C. Wightiana* extracts

The reductants observed in the extract displayed by blocking the peroxide formation or reacting with free radicals and concluding the free radical chain reaction. The diminishing power of all the extracts displayed a concentration reliant activity from 100 to 500 µg/mL of samples and absorbance at 700 nm.

Studies on the leaf extracts of *C. wightiana* on reducing power have revealed that the electron donors reduce the oxidized intermediates and which could act as primary antioxidant substances. Similarly, *C. graveolans* was also reported to acquire high antioxidant activity (Mushtaq *et al.,* 2013) [44] which could be definitely stated that the genus *Clematis* is a warehouse of phytocompounds which are great source of natural antioxidants.

4. CONCLUSION

The result of the present study clearly substantiated that the ethyl acetate extract of *C. wightiana* leaf exhibited higher antioxidant potential. Hence it could be concluded that *C. wightiana* is a natural source of antioxidant substances which could play a pivotal role in the advancement of new drugs. Further studies are assured to isolate the active compounds to display various pharmacological properties of *C. wightiana*.

5. ACKNOWLEDGEMENT

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Conflict of interest

The authors declare no conflict of interest.

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