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## DETERMINATION OF ANTIOXIDANT ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *FICUS VIRENS*

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### Keywords:

Antioxidant activity, *Ficusvirens*, DPPH assay, H<sub>2</sub>O<sub>2</sub> scavenging assay, nitric oxide scavenging assay

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**ABSTRACT:** In biological cells, antioxidants play a crucial role in preventing cellular damage caused by oxidation of other molecules. Their primary function is to neutralize free radicals, which can have detrimental effects on living organisms. Antioxidants achieve this by reacting with free radicals, thereby terminating the chain reactions that could harm cells. This study aimed to assess the antioxidant potential of ethanolic leaves extract of *Ficus virens* and compared it with a standard antioxidant, such as ascorbic acid. The plant material was subjected to sequential extraction using a solvent of 70% ethanol, utilizing a Soxhlet extraction method. The phytochemicals examination ethanolic leaves extract of *Ficus virens* exposed the presence of alkaloids, glycosides, flavonoids, proteins, carbohydrates, Tannins and phenolic compounds. The extract was tested for total phenolic content (TPC) and antioxidant activity. Antioxidant activities were determined spectrophotometrically using the DPPH assay, hydrogen peroxide assay, Ferric reducing antioxidant power assay, phosphomolybdate assay, nitric oxide scavenging assay. The result of ethanolic leaves extract shows the good antioxidant potency when compared with standard ascorbic acid.

**INTRODUCTION:** In present day's human civilization the most used, acceptable and recognized form of medicine is plant derived medicinal products throughout the world. Medicinal plants have been an essential component of human life since ancient times, aiding in the fight against various diseases. More than 80,000 plants are used as medicinal plant around the world and among these maximum numbers of plants are traditionally used from generation to generation. Since, ancient times, Indian medicinal plants have been recognized for their potential in treating various diseases.

Ethnobotanicals, natural products, and their derivatives have demonstrated promising therapeutic activities for these ailments. Medicinal plants serve as rich natural sources of diverse phytochemicals, including flavonoids, tannins, terpenoids, polyphenols, steroids, alkaloids, glycosides, chlorophyll, carotenoids, proteins, minerals, vitamins, and other essential nutrients. These compounds possess potent antioxidant properties and exhibit various biological activities<sup>1</sup>.

Oxidative stress (OS) arises from the disparity between the cellular generation of reactive oxygen species (ROS) and the capacity of cells to neutralize them<sup>2</sup>. ROS cause the damage of many cellular components including lipids, proteins and nucleic acids, such as DNA leading to subsequent cellular death by modes of necrosis or apoptosis<sup>3</sup>. Antioxidants are a class of chemical substances



naturally found in our food which can prevent or reduce the oxidative stress of the physiological system. Antioxidants are molecules that shield cells from damage caused by oxidation in other molecules. Oxidation, a chemical process, involves the transfer of electrons from one molecule to an oxidizing agent, often generating free radicals. As the body utilizes oxygen regularly, it continually produces free radicals highly reactive species with one or more unpaired electrons in their outermost shell initiating a chain reaction upon formation. Antioxidants intercept these radicals, halting the chain reaction by eliminating radical intermediates and sacrificing themselves to prevent further oxidation reactions. Numerous medicinal plants have undergone scrutiny for their antioxidant properties. Whether in the form of raw extracts or their chemical constituents, natural antioxidants prove highly effective in thwarting the detrimental effects of oxidative stress<sup>4</sup>. Therefore, the development and utilization of more effective antioxidants of natural origins are desired. Of various kinds of natural antioxidants, phenolic compounds have received much attention. Therefore, the phenolic constituents of plants are of interest as potential chemo preventive agents and plants may be an attractive alternative to currently available commercial antioxidants because they are biodegradable to non-toxic products<sup>4</sup>.

One of such plant is *Ficus virens* is commonly known as white fig, pilkhanand plaksa, one such plant that belongs to the family Moraceae and normally found in India, Southeast Asia, Malaysia, and northern Australia, this plant contains chemical constituents like phenols, flavonoids, tannins, saponins, vitamins, anthocyanins, glycosides, alkaloids, and amino acids. Not much scientific support was given to the folklore claims of the plant, and some of its traditional uses have been investigated, including anti-diabetic, anti-inflammatory, hepatoprotective effect, wound healing properties, anti-hyperlipidemic, anti-viral, anti-oxidant, anti-bacterial, anti-implantation, anti-ovulatory, anti-estrogenic, and anti-cancer activity<sup>6,7</sup>.

Hence, current study was designed to identify the phytoconstituent and evaluate the antioxidant activity of the ethanolic leaves extract by using the Total phenolic acid determination, DPPH free

radical scavenging assay, Phosphomolybdate assay, Ferric reducing antioxidant power assay, Hydrogen peroxide scavenging assay, Nitric oxide scavenging assay. The comparative analysis of antioxidant activities involved assessing them in relation to well-known antioxidants like ascorbic acid.

#### **MATERIALS AND METHODS:**

**Collection of Plant Material:** The plant material was collected from Chennipura, Malavallitaluk, Mandya district, Karnataka, India in the month of march 2024. The plant was identified and authenticated by Dr. Thejesh Kumar M.P. M.Sc., Ph.D, Co-ordinator Department of Botany (PG).Bharathi College, Bharathinagar.

**Extraction Procedure:** The leaves was dried under shade and grinded to fine powder. The powder was subjected to Soxhlet extraction with 70% ethanol and the extract was evaporated to dryness. The dark greenish mass obtained was stored in a well closed air tight resistant container.

**Preliminary Phytochemical Studies<sup>8, 9</sup>:** Preliminary phytochemical test for leaves of *Ficus virens* were performed and chemical constituents were determined by using standard procedures described by Kokate C.K., Purohit A.P., and Gokhale S.B.

**Antioxidant Assays<sup>10</sup>:** The assessment of antioxidant activity was done through various in-vitro assays. The free radical scavenging activity of various concentrations of ethanolic extract of plant and ascorbic acid was measured in terms of total Phenolic content, DPPH scavenging assay, phosphomolybdate assay, ferric reducing power assay, hydrogen peroxide scavenging assay, nitric oxide scavenging assay.

**Total Flavonoid Contents Determination<sup>11</sup>:** The total flavonoid content of *Ficus virens* was determined by using aluminium chloride calorimetric method based on the methodology reported by Afifty et al. (2012) with some modification. 0.1ml (1mg/ml) of plant extract was mixed with 1ml of 10% aluminium chloride, 1ml of potassium acetate (1M) and 2.5 ml of distilled water. Rutin was used to make the calibration curve. The absorbance of the mixtures was measured at 415nm by using the spectrophotometer.

**DPPH free Radical Scavenging Activity**<sup>13,14</sup>:

**Principle:** The molecule of 1, 1-diphenyl-2-picrylhydrazyl ( $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol/methanol solution centred at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present). Representing the DPPH radical by  $Z\cdot$  and the donor molecule by AH, the primary reaction is  $Z\cdot + AH = ZH + A\cdot$

**Procedure:** DPPH (2,2-diphenylpicrylhydrazyl) free radical scavenging activity was done following standard protocol with slight modifications. Different concentration of Standard and plant extract like 6, 12, 18, 24 and 30 $\mu$ g/ml were prepared by diluting with DMSO. 0.1ml different concentration of plant extract and Standard ascorbic acid were mixed with 2.9 ml of methanolic solution of DPPH in each test tube. Control was prepared by adding 0.1 ml of DMSO and 2.9 ml of DPPH. The test tubes were covered with aluminium foil to protect from light and kept in dark place for 15 min. DMSO was used as blank. Absorbance of standard, control and test extract was measured at 517nm using UV- Visible spectrophotometer. The % inhibition was calculated by using following formula and compared with the values of standard Ascorbic acid.

$$\% \text{ inhibition of DPPH activity} = [(A_0 - A_1) / A_0] \times 100$$

Where  $A_0$  is the absorbance of control and  $A_1$  is the absorbance of the extract/standard.

**Phosphomolybdate Assay**<sup>15,16</sup>:

**Principle:** This method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue/green complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite and this causes a reduction in intensity of the blue/green colour

which is measured spectrophotometrically. [prieto 1999].

**Procedure:** Different concentration of Standard and plant extract like 6, 12, 18, 24 and 35 $\mu$ g/ml were prepared by diluting with DMSO. 0.1ml different concentration of plant extract and Standard ascorbic acid was shaken with 1 mL of phosphomolybdate reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were covered and incubated in a water bath at 95 °C for 90 min. After the samples were cooled, the absorbance of the mixture was measured at 765 nm. Ascorbic acid was used as standard. The antioxidant capacity was estimated using the following formula:

$$\text{Total antioxidant capacity (\%)} = [(\text{Abs. of control} - \text{Abs. of sample}) / (\text{Abs. of control})] \times 100$$

**Ferric Reducing Antioxidant Power assay**<sup>12, 17, 18</sup>:

**Principle:** This method measures the ability of antioxidants to reduce ferric iron. It is based on the reduction of the complex of ferric iron and 2,3,5-triphenyl - 1, 3, 4 - triaza - 2 - azoniacyclopenta-1, 4- diene chloride (TPTZ) to the ferrous form Fe (TPTZ) 2+ complex (intensely blue in color) in the presence of anti-oxidant in an acidic pH. This reduction is monitored by measuring an increase in absorbance value at 593nm using spectrophotometer.

**Procedure:** The reducing power of plant extracts were determined by the method of Oyaizu's (1986) with some modification. The capacity of extract to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by re-cording the absorbance at 700 nm after incubation. For this purpose, Different concentration of Standard and plant extract like 6, 12, 18, 24 and 30 $\mu$ g/ml were prepared by diluting with DMSO and it is mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The resulting mixture was incubated at 50°C for 20 minutes and cooled it rapidly. Then, 2.5 ml of 10 % tri-chloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes. The supernatant was mixed with 2.5ml of deionised water and 1 ml of 0.1 % ferric chloride. Then the absorbance was measured at 700nm.

**Hydrogen Peroxide Scavenging Assay<sup>9,19</sup>:**

**Principle:** The UV light absorption of hydrogen peroxide can be easily measured at 230nm. On scavenging of hydrogen peroxide by the plant extracts, the absorption decreases at this wavelength.

**Procedure:** The plant extract radical scavenging activity against hydrogen peroxide was determined using the method of Ruch *et al* with some modification. Different concentration of Standard and plant extract like 6, 12, 18, 24 and 30µg/ml were prepared by diluting with DMSO. Samples of plant extract and standard ascorbic acid of different concentration were added to phosphate buffer solution (pH 7.4, 3.4 ml), respectively, and mixed with 43mM hydrogen peroxide solution (0.6 ml). After 10 min, the reaction mixture absorbance was determined at 230nm. The reaction mixture without sample was used as the blank. Ascorbic acid was used as a reference compound. The percentage inhibition activity was calculated.

**Nitric Oxide Scavenging Activity<sup>20, 21</sup>:**

**Principle:** Nitric oxide is a very unstable species under the aerobic condition. It reacts with O<sub>2</sub> to produce the stable product nitrates and nitrite through the intermediates NO<sub>2</sub>, N<sub>2</sub>O<sub>4</sub> and N<sub>3</sub>O<sub>4</sub>. It is estimated by using the Griess reagent. In the

presence of test compound, which is a scavenger, the amount of nitrous acid decreases. The extent of decrease reflects the extent of scavenging.

**Procedure:** Nitric oxide radical scavenging activity of plant extract and standard ascorbic acid was determined by Griess Ilosvay reaction using sodium nitroprusside. Different concentration of Standard and plant extract like 6, 12, 18, 24 and 30µg/ml were prepared by diluting with DMSO. 0.5ml different concentration of plant extract and Standard ascorbic acid were mixed with the 2 mL of sodium nitroprusside (10 mM) and 0.5 mL of phosphate buffer (pH-7.4) and incubated for 150 min at 25°C. After the incubation period was over, 0.5 mL of nitrite was pipetted out and 1mL of sulfanilic acid reagent (0.33% of sulfanilic acid in 2% glacial acetic acid) was added to it and kept for 5 min. Then, 1 mL of 1% naphthyl ethylene diaminedihydrochloride (NEDD) was added and allowed to stand for 30 min at 25 °C. The absorbance of pink colour of the solution was read at 540 nm. The percentage of nitric oxide inhibition was calculated using the following equation:

$$\text{Percentage (\%)} \text{ of nitric oxide radical scavenging assay} = \frac{[(A_0 - A_1) / A_0] \times 100}{1}$$

Where A<sub>0</sub> was the absorbance of control, and A<sub>1</sub> was the absorbance of the treated sample.

**RESULTS AND DISCUSSION:****TABLE 1: PRELIMINARY PHYTOCHEMICAL STUDIES**

Sl. no.	Phytoconstituents	Tests	Results
1	Flavonoids	Lead acetate test	+
		Alkaline reagent	+
2	Tannins and phenolic compounds	Ferric chloride tests	+
		Lead acetate test	+
3	Alkaloids	Dragendroffs tests	+
		Wagners tests	-
		Hagers tests	+
4	Proteins	Biuret tests	+
		Ninhydrin tests	-
5	Glycosides	Keller killani tests	+
6	Carbohydrates	Benedicts tests	+
		Fehlings test	-

Note: + = Present; - = Absent.

**In-vitro Anti-oxidant Study:****TABLE 2: TOTAL PHENOLIC CONTENT DETERMINATION**

Sample	Concentration(µg/ml)	Absorbance
Standard (Rutin)	10	0.265
	20	0.36
	30	0.452



	40	0.563
	50	0.643
Sample (ethanolic extract)	50	0.438

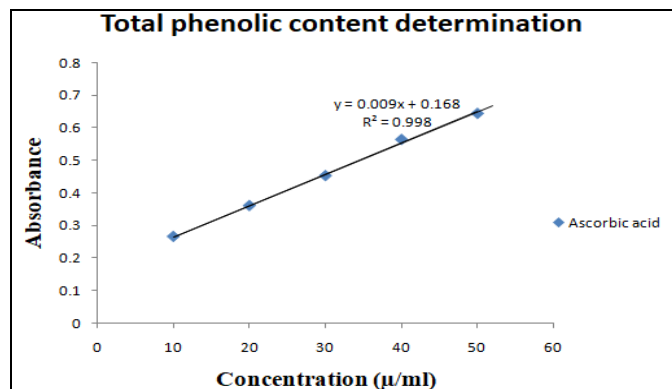


FIG. 1: STANDARD GRAPH OF ASCORBIC ACID FOR ESTIMATION OF TOTAL PHENOLIC CONTENT

TABLE 3: TOTAL FLAVONOID CONTENT OF PLANT EXTRACT OF LEAVES OF *FICUS VIRENS*

Extracts 100µg/ml	Phenolic content Ascorbic acid equivalent µg/ml
Plant extract	28.03

TABLE 4: TOTAL FLAVONOID CONTENT DETERMINATION

Sample	Concentration(µg/ml)	Absorbance
Standard (Rutin)	10	0.24
	20	0.338
	30	0.432
	40	0.502
	50	0.612
Sample (ethanolic extract)	50	0.462

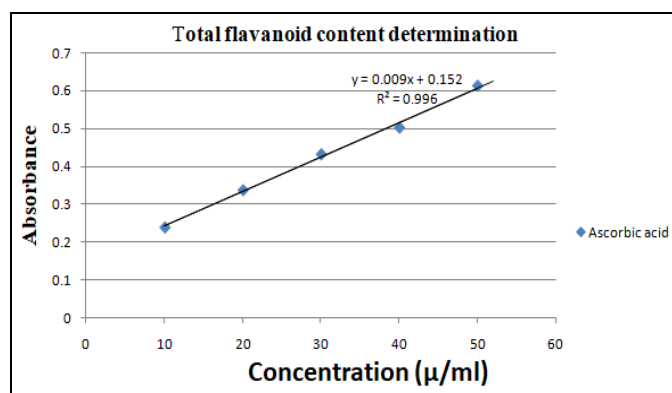


FIG. 2: STANDARD GRAPH OF ASCORBIC ACID FOR ESTIMATION OF TOTAL FLAVONOID CONTENT

TABLE 6: CONCENTRATION AND % RSA, IC50 VALUE OF STANDARD ASCORBIC ACID AND EXTRACT

Sample	Concentration (µg/ml)	%RSA	IC <sub>50</sub> Value (µg/ml)
Standard (Ascorbic acid)	6	37.10247	
	12	45.22968	
	18	62.89753	

TABLE 5: TOTAL FLAVONOID CONTENT OF PLANT EXTRACT OF LEAVES OF *FICUS VIRENS*

Extracts 100µg/ml	Flavonoid content Ascorbic acid equivalent µg/ml
Plant extract	34.02

**DPPH Free Radical Scavenging Activity:** The stable free radical DPPH undergoes reduction by accepting either hydrogen or electrons from a donor.

Plant extracts exhibit significant scavenging activity compared to the standard ascorbic acid. Both the plant extract and standard ascorbic acid showed an increase in DPPH scavenging activity in a dose-dependent manner.

The IC<sub>50</sub> value was determined to ascertain the concentration of the sample needed to inhibit 50% of the radical. Lower IC<sub>50</sub> values indicate higher antioxidant activity of the samples. The IC<sub>50</sub> value for standard ascorbic acid was determined to be 12.96 mcg/ml, demonstrating its remarkable antioxidant potency.

In contrast, the ethanolic extract of *Ficus virens* exhibited an IC<sub>50</sub> value of 19.29 mcg/ml, indicative of its substantial antioxidant effectiveness. The results indicate a notable reduction in DPPH radical concentration, attributed to the scavenging potential of the extract in comparison to the standard (ascorbic acid).

This study provides evidence supporting the strong antioxidant properties of the plant extract, which effectively scavenges DPPH radicals.

**Note:**

IC <sub>50</sub> value (mcg/ml)	Category
<50	Very strong
50-100	Strong
100-150	Medium
150-200	Weak

Sample (ethanolic extract)	24	71.14252	12.96441
	30	83.15665	
	6	10.01178	
	12	36.51355	
	18	48.05654	19.29435
	30	62.42638	
	30	75.97173	

%RSA= Percentage radical scavenging activity, IC<sub>50</sub>= Half-maximal inhibitory concentration.

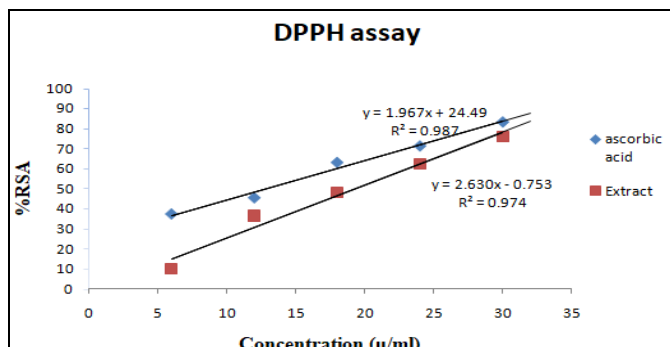


FIG. 3: DOSE-RESPONSE CURVE OF STANDARD ASCORBIC ACID AND EXTRACT

**Nitric Oxide Scavenging Assay:** The nitric oxide scavenging activity of the plant extract from *Ficus virens*, depicted in Fig. 2, demonstrates the percentage inhibition of nitric oxide generation by the extract. Ascorbic acid served as the reference. The plant extract exhibits significant scavenging activity in comparison to the standard ascorbic acid. Both the plant extract and standard ascorbic

acid display an increase in nitric oxide radical scavenging activity in a dose-dependent manner. The IC<sub>50</sub> value, calculated to ascertain the concentration of the sample necessary to inhibit 50% of radicals, serves as a measure of antioxidant potency. A lower IC<sub>50</sub> value indicates higher antioxidant activity in the samples. The IC<sub>50</sub> value of standard ascorbic acid was determined to be 13.09 mcg/ml, indicating very strong antioxidant activity, whereas the ethanolic extract of *Ficus virens* exhibited an IC<sub>50</sub> value of 24.07 mcg/ml, denoting strong antioxidant activity. The results indicate a notable reduction in nitric oxide concentration attributed to the scavenging capacity of the extract compared to the standard (ascorbic acid). This study provides evidence supporting the plant extract's commendable antioxidant properties and its ability to scavenge nitric oxide.

TABLE 7: CONCENTRATION AND % RSA, IC<sub>50</sub> VALUE OF STANDARD ASCORBIC ACID AND EXTRACT

Sample	Concentration (µg/ml)	%RSA	IC <sub>50</sub> Value (µg/ml)
Standard (Ascorbic acid)	6	41.15623	
	12	49.415	
	18	55.40262	
	24	63.17963	13.09552
	30	70.26841	
Sample (ethanolic extract)	6	22.50516	
	12	28.07983	
	18	38.26566	24.0796
	24	51.61734	
	30	59.8073	

%RSA= Percentage radical scavenging activity, IC<sub>50</sub> = Half-maximal inhibitory concentration.

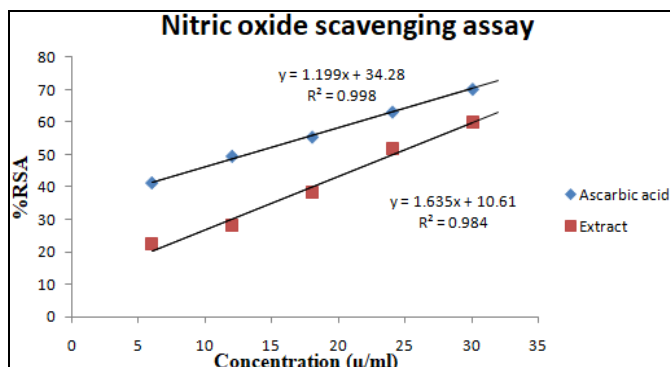


FIG. 4: DOSE-RESPONSE CURVE OF STANDARD ASCORBIC ACID AND EXTRACT

**Hydrogen Peroxide Scavenging Assay:** The hydroxyl radical scavenging activity of the plant extract from *Ficus virens*, depicted in Fig. 2, illustrates the percentage inhibition of hydroxyl radicals by the extract. Ascorbic acid served as the reference. Hydroxyl radicals are known to cause significant damage to nearby biomolecules like lipids, proteins, and DNA. The plant extract demonstrates remarkable scavenging activity against hydroxyl radicals. Furthermore, both the plant extract and standard ascorbic acid exhibit an

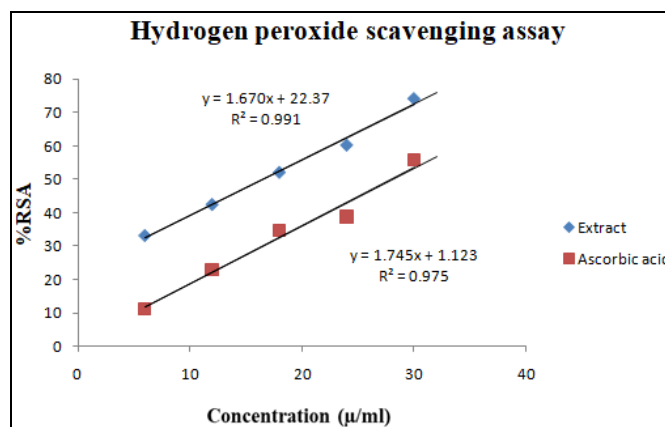
increase in hydroxyl radical scavenging activity in a dose-dependent manner. The  $IC_{50}$  value was calculated to ascertain the concentration of the sample necessary to inhibit 50% of radicals. A lower  $IC_{50}$  value indicates higher antioxidant activity in the samples. The  $IC_{50}$  value of standard ascorbic acid was determined to be 16.54 mcg/ml, indicative of its very strong antioxidant activity. Conversely, the  $IC_{50}$  value of the ethanolic extract

of *Ficus virens* was found to be 28 mcg/ml, suggesting strong antioxidant activity. The results shows a notable reduction in the concentration of hydroxyl radicals due to the scavenging ability of the extract compared to the standard (ascorbic acid). This study provides evidence supporting the plant extract's commendable antioxidant properties and its ability to scavenge hydroxyl radicals.

**TABLE 8: CONCENTRATION AND % RSA,  $IC_{50}$  VALUE OF STANDARD ASCORBIC ACID AND EXTRACT**

Sample	Concentration ( $\mu$ g/ml)	%RSA	$IC_{50}$ Value ( $\mu$ g/ml)
Standard (Ascorbic acid)	6	11.13445	16.54053
	12	22.79412	
	18	34.55882	
	24	38.65546	
	30	55.56723	
Sample (ethanolic extract)	6	33.08824	28.00281
	12	42.43697	
	18	52.10084	
	24	60.29412	
	30	74.26471	

%RSA= Percentage radical scavenging activity,  $IC_{50}$ = Half-maximal inhibitory concentration.



**FIG. 5: DOSE-RESPONSE CURVE OF STANDARD ASCORBIC ACID AND EXTRACT**

**Phosphomolybdate Assay:** This assay relies on the reduction of phosphomolybdate ions in the presence of an antioxidant, leading to the formation of a green phosphate/MoV complex, which is quantified spectrophotometrically.

Figure 0 shows the phosphomolybdate ion reducing potential of the ethanolic leaf extract of *Ficus virens* compared to the standard ascorbic acid at

765nm. The results indicate that the phosphomolybdate ion reducing activity of both the plant leaf extract and standard ascorbic acid increased with their concentration.

Notably, the plant extract exhibited antioxidant activity equivalent to that of ascorbic acid at a concentration of 13.57 mcg/ml.

**TABLE 9:**

Sample	Concentration( $\mu$ g/ml)	Absorbance
Standard (Ascorbic acid)	6	0.056
	12	0.064
	18	0.073
	24	0.084
	30	0.095
Sample (ethanolic extract)	30	0.067

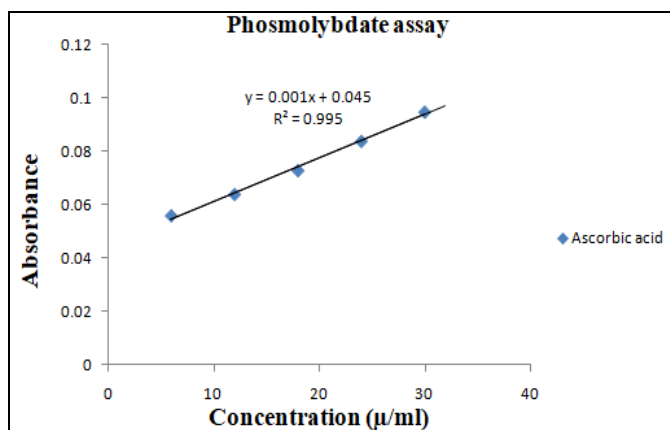


FIG. 6: DOSE-RESPONSE CURVE OF STANDARD ASCORBIC ACID

**Ferric Reducing Antioxidant Power Assay:** The ferric ion reducing power potentials of the ethanolic leaf extract of *Ficus virens* in comparison with the standard ascorbic acid at 700 nm, as result shows in the figure. The results demonstrate that the ferric reducing activity of both the plant leaves extract and standard ascorbic acid increased with their concentration. Notably, the antioxidant activity of the plant extract was found to be equivalent to that of ascorbic acid at a concentration of 12.99 mcg/ml.

TABLE 10:

Sample	Concentration (µg/ml)	Absorbance
Standard (Ascorbic acid)	6	0.476
	12	0.582
	18	0.656
	24	0.745
	30	0.867
Sample (ethanolic extract)	30	0.586

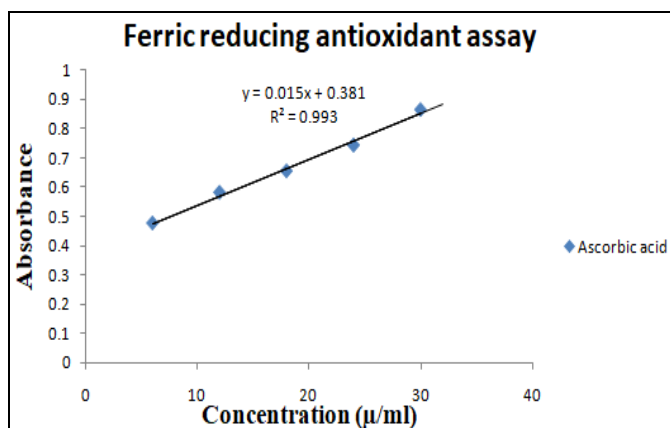


FIG. 7:

**CONCLUSION:** The phytochemical analysis unveiled that the ethanolic leaves extract of *Ficus virens* is rich in glycosides, flavonoids, phenols,

and saponins. This research specifically focused into the assessment of total phenolic and flavonoid levels in the ethanolic leaves extract, revealing notable concentrations of these compounds. *In-vitro* antioxidant evaluations of the plant extract were conducted through diverse methods including DPPH, hydrogen peroxide, nitric oxide radical, and reducing power scavenging assays. This results underscored the potent scavenging effect of the plant extract on free radicals, showcasing robust antioxidant activity comparable to ascorbic acid utilized as a standard reference. Both the plant extract and ascorbic acid demonstrated antioxidant activity in a manner dependent on dosage. Collectively, these findings *Ficus virens* as a promising natural antioxidant reservoir, potentially pivotal in mitigating or forestalling oxidative stress-related degenerative diseases.

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