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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF METHANOLIC ROOT EXTRACT OF *ANNONA GLABRA* LINN.

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Keywords:

Antioxidant activity, *Annona glabra Linn*, Phytochemicals, Folin-Ciocalteus reagent, Aluminium chloride

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ABSTRACT: Antioxidants are crucial in combating oxidative stress and related diseases. This study aimed to assess the antioxidant potential of methanolic root extract of *Annona glabra Linn.* and ascorbic acid is used as a reference. The plant material was subjected to sequential extraction using a solvent of varying polarities, utilizing a Soxhlet extraction method. The extract was tested for phytochemicals (secondary metabolites), total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity. The phytochemicals examination of root extract of *Annona glabra Linn*. exposed the presence of alkaloids, glycosides, flavonoids, saponins, phenols, steroids, terpenoids. TPC, TFC of crude methanolic root extract of plant was determined using Folin-Ciocalteus reagent and Aluminum chloride colorimetric method respectively. Antioxidant activities were determined spectrophotometrically using the DPPH assay, nitric oxide scavenging assay, hydrogen peroxide scavenging assay, phosphomolybdate assay, ferric reducing antioxidant power assay. The result of methanolic root extract shows the good antioxidant potency.

INTRODUCTION: Humans have used plants for medicinal purposes since ancient times [1]. Many medicinal plants, which are currently used to cure diseases, were identified by the well-known ancient civilisations which is Ayurveda. Ayurveda, an old Indian medicine system, has found almost 8000 plants useful for healing, which are being used successfully in the classical formulation of the ayurvedic system of medicine [2]. Based on the survey done by the World Health Organization (WHO), 80% of world population depends on herbal medicines for their health care.

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Scientifically, medicinal plants naturally produce and accumulate some secondary metabolites, such as alkaloids, glycosides, flavonoids, terpenes, steroids, tannins and saponins. The effectiveness of the medicinal plant is determined by the composition of chemicals present in it [3]. Oxidative stress (OS) is the imbalance between cellular production of reactive oxygen species (ROS) and the ability of cells to scavenge them. OS is one of the contributors to the pathogenesis of several diseases, such as cancer, diabetes and heart disease [4].

ROS cause the damage of many cellular components including, proteins, lipids and nucleic acids, such as DNA leading to subsequent cellular death by modes of necrosis or apoptosis [5]. The damage can become more widespread due to weakened cellular antioxidant defense systems.

Hence, dietary intake of antioxidant compounds is important. Intake of vegetables and fruits is known to lower the risk of several diseases [6]. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule [7]. The main characteristic of an antioxidant is its ability to capture free radicals. Antioxidant compounds like, polyphenols, phenolic acids and flavonoids scavenge free radicals such as hydroperoxide, peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Since, ancient times, people have recognized herbal plants as powerful antioxidants [8]. Currently, synthetic antioxidants, such asbutylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), and tert-butylhydroquinone (TBHQ) are widely used in the food industry. However, restriction on the synthetic antioxidants is being imposed because of their toxicity to liver and carcinogenicity. 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 powerful chemo preventive agents and plants maybe an attractive alternative to currently available commercial antioxidants because they are biodegradable to non-toxic products [9].

One such plant is *Annona glabra Linn,* belonging to the genus Annona and the family Annonaceae. It is a very large family of plants, comprising about 120 genera and more than 2000 species. These seem to be one of the least chemically as well as pharmacologically known families compared with other families. Many active compounds have been found in *A. glabra*, mainly alkaloids, flavonoids, glycosides, saponins, phenols, terpenoids, tannins, steroids, and anthraquinones. Annona plants have several scientifically proven pharmacological effects, such as anticancer, antidiabetic, antidiarrhea, antiulcer, antimalarial, anti-inflammatory, antioxidant, antileishmanial, antifungal, antibacterial, anticonvulsant, anti-depressant, antinociceptive, anti-acetylcholinesterase, and dengue vector control activity [10]. Hence, current study was designed to identify the phytoconstituent and evaluate the antioxidant activity of the methanolic root extract of plant by using the total phenolic content, total flavonoid content, DPPH free radical scavenging assay, nitric oxide scavenging assay, hydrogen peroxide scavenging assay, phosphomolybdate assay, ferric reducing antioxidant power assay. Standard ascorbic acid is used as a reference.

MATERIALS AND METHODS:

Collection of Plant Material: The plant material was collected from H.G doddi, Mandya district, Karnataka, India in the month of March 2024. The plant was identified and authenticated by Dr. V. Rama Rao, Research officer (Botany), Central Ayurveda Research Institute, Bengaluru. An herbarium voucher specimen was preserved in the department of Pharmacognosy, Bharathi College of Pharmacy, Bharathinagar for further reference.

Extraction Procedure [11]: 150gm of powdered drug was charged into the thimble of Soxhlet extractor individually and extracted with petroleum ether, chloroform, ethyl acetate and methanol successively in increasing order of polarity. Before extracting with the next solvent, marc was pressed to remove the residual solvent, combined and concentrated to get the concentrated extract. The percentage was calculated in terms of air-dried weight of plant material. Soxhlet apparatus used for the extraction of selected powdered herbal material using different solvents of increasing polarity and percentage yield was calculated.

Preliminary Phytochemical Studies [12]: Preliminary phytochemical test for root of *Annona glabra Linn.* were performed and chemical constutients were determined by using standard procedures described by Kokate C.K., Purohit A.P., and Gokhale S.B.

Antioxidant Assays [13]: The assessment of antioxidant activity was done through various in-vitro assays. The free radical scavenging activity of various concentrations of methanolic root extract of plant and ascorbic acid was measured in terms of total Phenolic content, total flavonoid content, DPPH scavenging assay, phosphomolybdate assay, ferric reducing power assay, hydrogen peroxide scavenging assay, nitric oxide scavenging assay.

Determination of Total Phenolic Contents [14]: The total phenolic content of the extract was determined by the Folin–Ciocalteu method with some modification. Briefly, 0.1ml of crude extract

(1 mg/mL) were made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. Rutin was used to make the calibration curve. The absorbance of the mixtures was measured at 650nm by using the spectrophotometer.

Determination of Total Flavonoid Contents [15]: The total flavonoid content of *A. glabra L.* was determined by using aluminium chloride calorimetric method based on the methodology reported by a fifty *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 [16, 17]:

Principle: The molecule of 1, 1-diphenyl-2-picrylhydrazyl (α, α-diphenyl-β-picryl hydrazyl; 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 colour, 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 colour (although there would be expected to be a residual pale-yellow colour from the picryl group still present). Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is Z• + AH = ZH + A•

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 15, 30, 45, 60 and 75µg/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 30min. Absorbance 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.

$$\% \text{ radical scavenging activity} = [(A_0 - A_1)/ A_0)] \times 100$$

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

Nitric Oxide Scavenging Activity [18, 19]:

Principle: Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through the intermediates NO_2, N_2O_4 and N_3O_4. 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 15, 30, 45, 60 and 75µg/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 ^{o}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 diamine dihydrochloride (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:

$$\text{Percentage (\%) of nitric oxide radical scavenging assay} = [(A0-A1)/A0] \times 100.$$

Where A0 was the absorbance of control, and A1 was the absorbance of the treated sample.

Hydrogen Peroxide Scavenging Assay [20, 13]:

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 15, 30, 45, 60 and 75µg/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.

Phosphomolybdate Assay [21]:

Principle: This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically

Procedure: Different concentration of Standard and plant extract like 15, 30, 45, 60 and 75µg/ml were prepared by diluting with DMSO. 0.1ml different concentration of plant extract and Standard ascorbic acid was shaken with 1 mL of phosphomolybdat**e** 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:

Total antioxidant capacity (%) = [(Abs. of control − Abs. of sample) / (Abs. of control] × 100

Ferric Reducing Antioxidant Power Assay [13, 16, 22]:

Principle: The basic principle involved in the assay is the reduction of the ferric complex of Fe (TPTZ) 3+ i.e.: tripyridyl triazine (a ferroin analogue) to Fe (TPTZ) 2+ complex (intensely blue in colour) in the presence of anti-oxidant in an acidic ph. There will be an increase in absorbance value at 593nm.

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-ferricya-nide 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 15, 30, 45, 60 and 75µg/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.

RESULTS AND DISCUSSION:

Percentage Yield of Extracts Obtained by Successive Solvent Extraction:

TABLE 1: PERCENTAGE YIELD VALUES

Plant Name	Part used	Method of Extraction	Solvents	Average extractive value (% w/w)
Annona glabra linn	Root	Continuous hot Percolation by Soxhlet Apparatus	Petroleum ether	1.16
			Chloroform	2.73
			Ethyl acetate	3.83
			Methanol	5.30

Preliminary Phytochemical Studies:

TABLE 2: PHYTOCHEMICAL SCREENING RESULT

Phytoconstituents	PE	CL	EA	ME
Alkaloids	-	+++	+	-

Glycosides	-	-	-	+++
Saponin	-	-	+	-
Flavanoid	-	-	-	+++
Carbohydrate	-	-	++	-
Fat and oil	+	-	-	-
Phenols	-	-	-	+++
Proteins& amino acid	-	-	-	-
Steroid	++	+	-	-
Triterpenoid	++	+	-	-

Note: + = Present; - = Absent.

In-vitro Anti-oxidant Study:

Total Phenolic Content Determination:

TABLE 3: CONCENTRATION AND ABSORBANCE VALUE OF ASCORBIC ACID AND EXTRACT

Sample	Concentration(µg/ml)	Absorbance
Standard (Rutin)	20	0.249
	40	0.421
	60	0.632
	80	0.815
	100	0.998
Sample (methanolic extract)	100	0.321

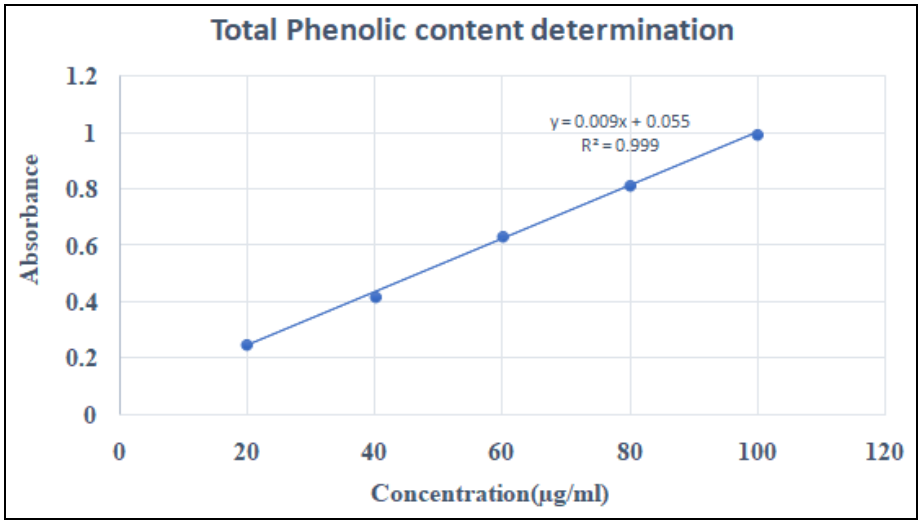

FIG. 1: STANDARD GRAPH OF ASCORBIC ACID FOR ESTIMATION OF TOTAL PHENOLIC CONTENT

TABLE 4: TOTAL PHENOLIC CONTENT OF METHANOLIC ROOT EXTRACT OF *A. GLABRA L.*

Extracts 100µg/ml	Phenolic content Ascorbic acid equivalent µg/ml
Plant extract	27.95

Total Flavonoid Content Determination:

TABLE 5: CONCENTRATION AND ABSORBANCE VALUES OF ASCORBIC ACID AND EXTRACT

Sample	Concentration(µg/ml)	Absorbance
Standard (Rutin)	20	0.340
	40	0.438
	60	0.537
	80	0.602
	100	0.712
Sample (methanolic extract)	100	0.441

TABLE 6: TOTAL FLAVONOID CONTENT OF METHANOLIC ROOT EXTRACT OF *A. GLABRA L.*

Extracts 100µg/ml	Flavonoid content Ascorbic acid equivalent µg/ml
Plant extract	41.91

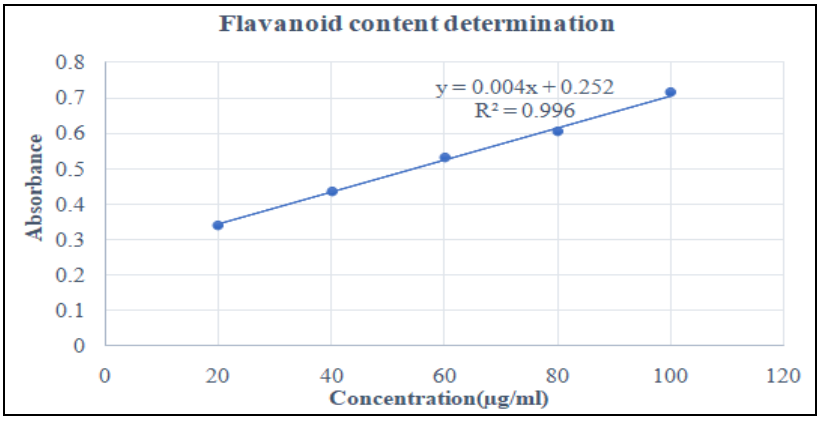

FIG. 2: STANDARD GRAPH OF ASCORBIC ACID FOR ESTIMATION OF TOTAL FLAVONOID CONTENT

DPPH Free Radical Scavenging Activity: DPPH is stable free radical which get reduced by accepting hydrogen or electron from donor. Plant extract shows remarkable scavenging activity when compared with standard ascorbic acid. DPPH scavenging activity of both plant extract and standard ascorbic acid was found to be increase in dose dependent manner. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC_{50} value, the higher the antioxidant activity of samples. IC_{50} value of standard ascorbic acid was found to be 11.43 mcg/ml and it exhibit very strong antioxidant activity where as IC_{50} value of methanolic extract of *Annona glabra* was found to be 51.49 mcg/ml and it exhibit strong antioxidant activity. The results showed a significant decrease in the concentration of DPPH radical due to the scavenging ability of extract as compared to standard (ascorbic acid). In the present study support, plant extract has good antioxidant and scavenges DPPH radicals.

Note:

IC_{50} value (mcg/ml)	Category
<50	Very strong
50-100	Strong
100-150	Medium
150-200	Weak

TABLE 7: CONCENTRATION AND % RSA, IC_{50} VALUE OF STANDARD ASCORBIC ACID AND EXTRACT

Sample	Concentration (µg/ml)	%RSA	IC_{50} Value (µg/ml)	Category
Standard (Ascorbic acid)	15	52.01	11.43	Very strong
	30	63.43		
	45	74.93		
	60	83.05		
	75	95.21		
Sample (methanolic extract)	15	03.85	51.59	Strong
	30	22.58		
	45	40.00		
	60	61.02		
	75	80.44		

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

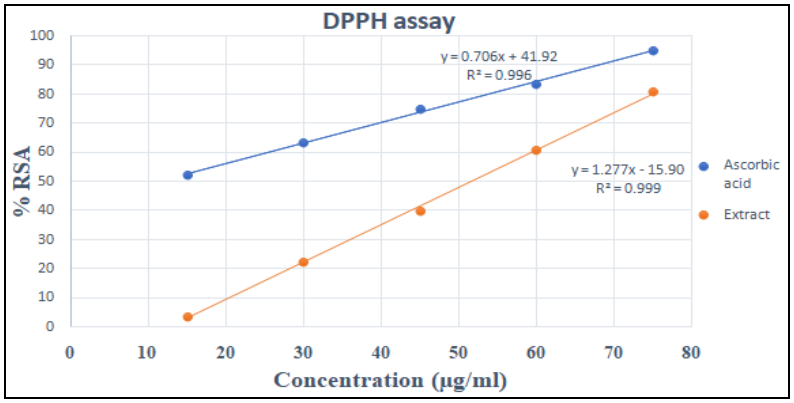

FIG. 3: DOSE-RESPONSE CURVE OF STANDARD ASCORBIC ACID AND PLANT EXTRACTS

Nitric Oxide Scavenging Assay: The nitric oxide scavenging activity of plant extract of *Annona glabra* and ascorbic acid shown in Figure 2 illustrates the % inhibition of nitric oxide generation by plant extract. Ascorbic acid was used as a reference. Plant extract shows remarkable scavenging activity when compared with standard ascorbic acid. Nitric oxide radical scavenging activity of both plant extract and standard ascorbic acid was found to be increase in dose dependent manner. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC_{50} value, the higher the antioxidant activity of samples. IC_{50} value of standard ascorbic acid was found to be 30.97 mcg/ml and it exhibit very strong antioxidant activity where as IC_{50} value of methanolic extract of *Annona glabra* was found to be 65.50 mcg/ml and it exhibit strong antioxidant activity. The results showed a significant decrease in the concentration of nitric oxide due to the scavenging ability of extract as compared to standard (ascorbic acid). In the present study support, plant extract has good antioxidant and scavenges nitric oxide.

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

Sample	Concentration(µg/ml)	% Inhibition	IC50 Value (µg/ml)	Category
Standard (Ascorbic acid)	15	40.88	30.97	Very strong
	30	50.17		
	45	57.7		
	60	65.16		
	75	74.8		
Sample (methanolic extract)	15	12.03	65.50	Strong
	30	22.24		
	45	36.23		
	60	45.39		
	75	56.92		

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

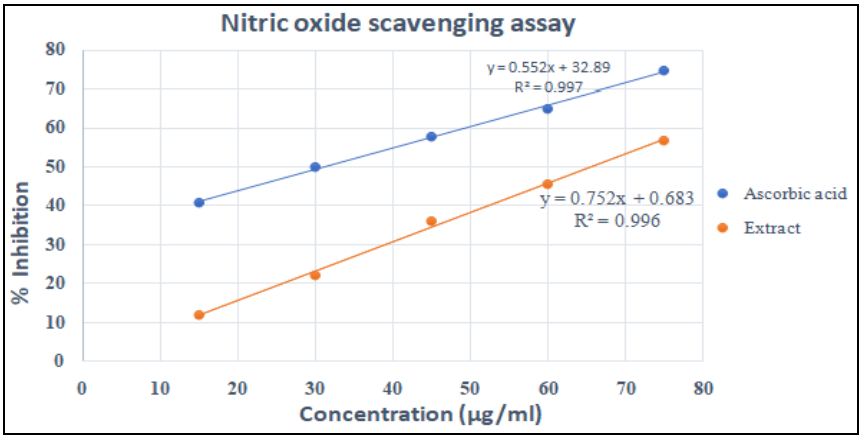

FIG. 4: DOSE-RESPONSE CURVE OF STANDARD ASCORBIC ACID AND PLANT EXTRACTS

Hydrogen Peroxide Scavenging Assay: The hydroxyl radical scavenging activity of plant extract of *Annona glabra* and ascorbic acid shown in **Fig. 2** illustrates the % inhibition of hydroxyl radical by plant extract. Ascorbic acid was used as a reference. The hydroxyl radical induces severe damage to adjacent biomolecules such as lipids, proteins and DNA. Plant extract shows remarkable hydroxyl radical scavenging activity. Hydroxyl radical scavenging activity of both plant extract and standard ascorbic acid was found to be increase in dose dependent manner. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC_{50} value, the higher the antioxidant activity of samples. IC_{50} value of standard ascorbic acid was found to be 41.24 mcg/ml and it exhibit very strong antioxidant activity where as IC_{50} value of methanolic extract of *Annona glabra* was found to be 66.15 mcg/ml and it exhibit strong antioxidant activity. The results showed a significant decrease in the concentration of hydroxyl radical due to the scavenging ability of extract as compared to standard (ascorbic acid). In the present study support, plant extract has good antioxidant and scavenges hydroxyl radicle.

TABLE 9: CONCENTRATION AND % RSA, IC_{50} VALUE OF STANDARD ASCORBIC ACID AND EXTRACT

Sample	Concentration(µg/ml)	% Inhibition	IC_{50} Value(µg/ml)	Category
Standard (Ascorbic acid)	15	31.48	41.24	Very strong
	30	42.23		
	45	52.87		
	60	61.78		
	75	74.91		
Sample (methanolic extract)	15	12.92	66.15	Strong
	30	22.69		
	45	32.46		
	60	44.40		
	75	55.15		

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

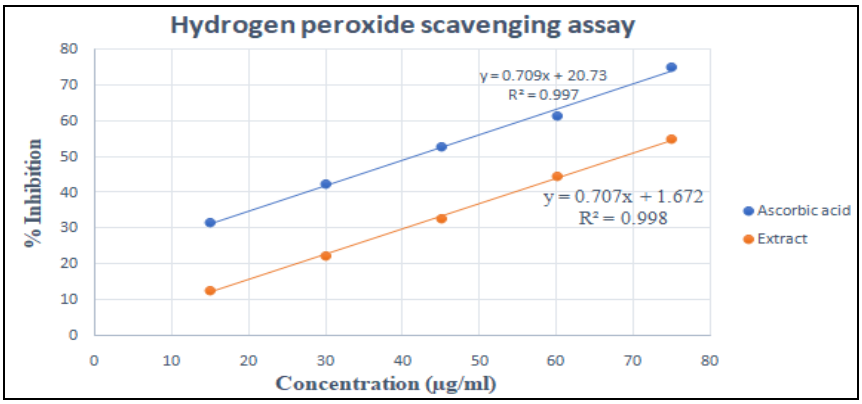

FIG. 5: DOSE-RESPONSE CURVE OF STANDARD ASCORBIC ACID AND PLANT EXTRACTS

Phosphomolybdate Assay: This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically. The phosphomolybdate ion reducing power potentials of the methanolic root extract of *Annona glabra* in comparison with the standard ascorbic acid at 765nm is explained in figure. The result indicates that phosphomolybdate ion reducing activity of both plant root extract and standard ascorbic acid increased with the increase in their concentration. Plant extract shown antioxidant activity which is equivalent to ascorbic acid at the concentration of 31.50mcg/ml.

TABLE 10: CONCENTRATION AND ABSORBANCE VALUES OF STANDARD ASCORBIC ACID AND EXTRACT

Sample	Concentration(µg/ml)	Absorbance
Standard (Ascorbic acid)	15	0.069
	30	0.077
	45	0.085
	60	0.094
	75	0.103
Sample (methanolic extract)	75	0.079

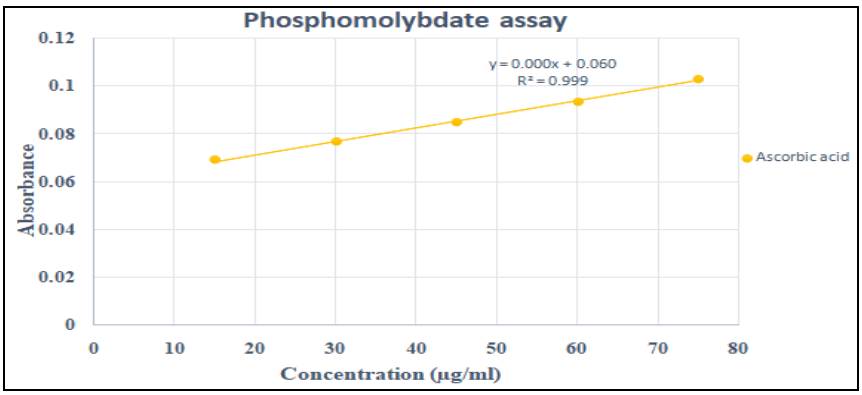

FIG. 6: DOSE-RESPONSE CURVE OF STANDARD ASCORBIC ACID

Ferric Reducing Antioxidant Power Assay: The ferricion reducing power potentials of the methanolic root extract of *Annona glabra* in comparison with the standard ascorbic acid at 700 nm is explained in figure. The result indicates that ferric reducing activity of both plant root extract and standard ascorbic acid increased with the increase in their concentration. Plant extract antioxidant activity equivalent to ascorbic acid at the concentration of 15.82mcg/ml.

TABLE 11: CONCENTRATION AND ABSORBANCE VALUES OF STANDARD ASCORBIC ACID AND EXTRACT

Sample	Concentration(µg/ml)	Absorbance
Standard (Ascorbic acid)	15	0.531
	30	0.623
	45	0.713
	60	0.822
	75	0.915
Sample (methanolic extract)	75	0.532

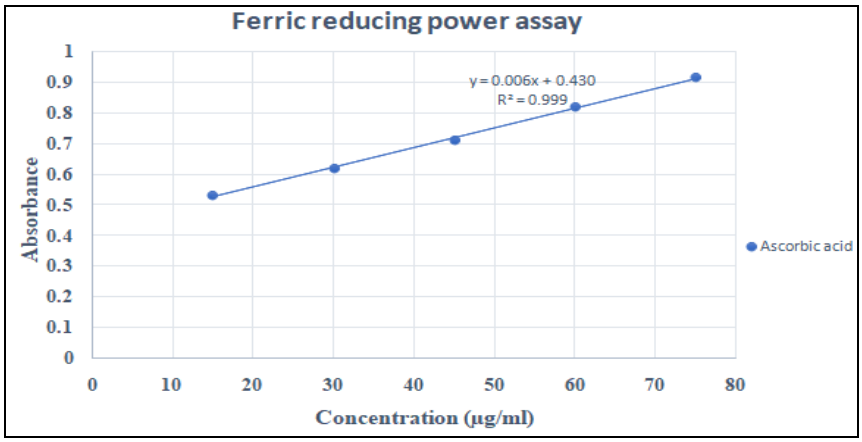

FIG. 7: DOSE-RESPONSE CURVE OF STANDARD ASCORBIC ACID

CONCLUSION: The phytochemical screening revealed that the methanolic root extract of *A. glabra L.* comprise of high amount of glycosides, flavonoids, phenols, saponins content. In this study, total phenolic and flavonoids content of the methanolic root extract of the plant were investigated. Methanolic extract showed high contents of phenol and flavonoid. *In-vitro* antioxidant activity of plant extract was carried by different methods such as DPPH, hydrogen peroxide, nitric oxide radical, reducing power scavenging assays. Our result shows that the free radicals scavenging effect of plant extract have displayed strong antioxidant activity.

Ascorbic acid is used as a reference. Both plant extract and standard ascorbic acid shows antioxidant activity in a dose-depended manner. The overall findings of this study concluded that *A. glabra L.* could be a potential source of natural antioxidant that would have a great importance as therapeutic agents in preventing or slowing the progress of reactive oxygen species and associated oxidative stress related degenerative diseases.

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REFERENCES:

1. Petrovska BB and Cekovska S: Extracts from the history and medical properties of garlic. Pharmacognosy Reviews 2010; 4(7): 106–110.
2. Shukla N: Role of Ayurveda in emergency treatment. Emergency Medicine 2012; 2(8): 1-5.
3. Kiang AK, Douglas B and Morsingh F: A phytochemical survey of malaya: Part II. Alkaloids. Journal of Pharmacy and Pharmacology 1961; 13(1): 98-104.
4. Gilgun-Sherki Y, Rosenbaum Z, Melamed E and Offen D: Antioxidant therapy in acute central nervous system injury: current state. Pharmacol Rev 2002; 54(2): 271–284.
5. Evans PH: Free radicals in brain metabolism and pathology. Br Med Bull 1993; 49(3): 577–587.
6. Willett WC: Balancing life-style and genomics research for disease prevention. Science 2002; 296(5568): 695-8.
7. Yamagishi S and Matsui T: Nitric oxide, a Janus-faced therapeutic target for diabetic microangiopathy-Friend or foe? Pharmacol Res 2011; 64(3): 187–194.

8. Wu YY, Li W, Xu Y, Jin EH and Tu YY: Evaluation of the antioxidant effects of four main the flavin derivatives through chemiluminescence and DNA damage analyses. J Zhejiang Univ Sci B 2011; 12(9): 744–751.
9. Borra SK, Prema Gurumurthy PG, Jaideep Mahendra JM, Jayamathi KM, Cherian CN and Ram Chand RC: Antioxidant and free radical scavenging activity of curcumin determined by using different *in-vitro* and *ex-vivo* models. J Med Plant Res 2013; 7(36): 2680-2690.
10. Sinchana R, Mani TT, Pavithra T and Shiju L: A review on a miracle plant *Annona glabra* linn. International jJournal of Pharmacognosy 2024; 11(3): 65-77.
11. Vadivel K, Thangabalan B, Veera NK, Chetanajessygrace B, Praveen KD and Manohar Babu S: Preliminary Phytochemical evaluation of leaf extracts of *Lannea coromandelica*. Int J of Pharm Res 2012; 2(2): 64-68.
12. Rahar S, Nagpal N, Swami G, Nagpal MA and Kapoor R: Pharmacognostical studies of *Saccharum munja* roxb, Root. Int J Pharm Tech Res 2011; 3(2): 792-800.
13. Pawa RS, Jain A, Sharma P, Chaurasiya PK and Singour PK: *In-vitro* studies on *Sida cordifolia* Linn for anthelmintic and antioxidant properties. Chinese Medicine 2011; 2(2): 47.
14. Baba SA and Malik SA: Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J of Taibah University for Science 2015; 9(4): 449-54.
15. Khan MS, Yusufzai SK, Rafatullah M, Sarjadi MS and Razlan M: Determination of total phenolic content, total flavonoid content and antioxidant activity of various organic crude extracts of *Licuala spinosa* leaves from Sabah, Malaysia ASM Sci J 2018;
16. Kanupriya J and Arihara Sivakumar G: Antioxidant potential and Phytochemical analysis of fruit extract of *Cucurbita pepo*. Int J Curr Res Chem Pharm Sci 2019; 6(3): 22-32.
17. Choudhury J, Majumdar S, Roy S and Chakraborty U: Antioxidant activity and phytochemical screening of two edible wetland pteridophytes *Diplazium esculentum* (Retz) Sw and *Marsilea minuta* L.-a comparative study. World Journal of Pharmaceutical and Medical Research 2017; 3(9): 195-203.
18. Patel A, Patel A and Patel NM: Detrmination of polyphenols and free radical scavenging activity of *Tephorsia purpureal* inn leaves (Leguminosae). Pharmacognosy Res 2010; 2(3): 152-158.
19. Ali BM, Boothapandi M and Nasar AS: Nitric oxide, DPPH and hydrogen peroxide radical scavenging activity of TEMPO terminated polyurethane dendrimers: Data supporting antioxidant activity of radical dendrimers. Data in Brief 2020; 28: 104972.
20. Palaksha MN, Ravishankar K and Girijasastry V: Preliminary phytochemical screening and *in-vitro* free radical scavenging activity of *Melochia corchorifolia* plant extracts. Int J of Res Pharm and Chem 2013; 3(2): 378-83.
21. Jan S, Khan MR, Rashid U and Bokhari J: Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong Public Health and Research Perspectives 2013; 4(5): 246-54.
22. Patil S, Rajiv P and Sivaraj R: An investigation of antioxidant and cytotoxic properties of green synthesized silver nanoparticles. Indo American Journal of Pharmaceutical Sciences 2015; 2(10): 1453-9.

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