INTRODUCTION

Phytochemistry is one of the rapidly expanding areas of Plant Taxonomy which utilizes chemical information to improve the classification of plants. Plants are endowed with various phytochemical molecules such as vitamins, flavonoids, phenolic acids, tannins, flavonoids, terpenoids, quinones, coumarins, alkaloids, amines and other metabolites which are rich in antioxidant activity. Natural compounds may be considered alternative means for medicine, because of low or little toxicity due to their dietary properties or their long history as herbal medicines.

These secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. The antioxidant compounds possess anti- inflammatory, anti-tumour, anti-mutagenic, anti-carcinogenic, antimicrobial and antiviral activities. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with aging.

Angiosperms are flowering plants that bear fruits and flowers. They include insect-pollinated herbs such as buttercups, pond plants such as water lilies, wind –pollinated grasses and tress such as apple and oak. Seeds are enclosed within a fruit. Angiosperms were formely called magnoliophyta. Angiosperms are the largest and the most diverse group within the kingdom plantae. They represent approximately 80% of all the known green plants now living. Plants belonging to angiosperms consists of ovary usually enclosed in a flower, that part of angiosperms plants that contain the males and females reproductive organs or both.

Angiosperms have evolved specialized cells and tissues that carry out these functions and have further evolved specialzed vascular tissues that translocate the water and nutrients to all areas of the plant body, which has evolved as an adaptation to a principally terrestrial habitat, includes extensive root systems that anchor the plant and absorb water and minerals from the soil. The basic angiosperms form is woody or herbaceous. Woody forms are rich in secondary tissues, while herbaceous forms rarely have any.

There are about 300,000 species of flowering plants and india is a home to roughly 20000 angiosperms including cultivated/naturalised ones with approximately 15% endemic species. Scientific explorations and studies are done worldwide to search new, unexplored species and increase the number of anigosperms.

The genus *syzgium* is one of the genera of the myrtle family of myrtaceae which is native to the tropics, particulary to tropical America and Australia. The herb is cultivated widely in Africa. In india *Syzygium cumini* is commonly known as black plum, jamun, kala jamun, nerale, kottai-nakam etc and with other words in different parts of india. It is a evergreen tropical tree native to india, Pakistan, Indonesia and sri lanka. It is also native to indian subcontinents and adjoining regions of southeast asia. It is distributed throughtout upper gangetic plains, bihar, Orissa. flowering starts usually during march and continues up april in north indian conditions. Fruits ripen during june-july or with the onset of rains. It takes about

3-5 months to ripen after full bloom. Fruits change their colour from green to deep red or bluish black.

Syzygium cumini

Syzygium cumini is a tree belonging to the myrtaceae family, originally from india and widely distributed in Asian countries such as Malaysia, Thailand and the Philippines. The *syzygium cumini* tree is a broad and densely foliated, reaching a height of 15m and a crown diameter of 4m. the immature bark is light brown, while the mature bark is dark brown and scaly. The leaves are 6-12cm long, leathery, dark green, smooth. Shiny and elongated or oblong shaped. The flowers are small (7-12mm), numerous, fragnant, white, creamy or greenish. The jamun fruits are berries, fleshy, elliptically shaped and composed of a single dark brown central seed. It's length varies from 1.5-3.5cm and are 2cm in diameter.

*Syzygium cumin*i is an important ayurvedic herb which has been used in Ayurveda for the treatment of diabetes, worm infection, diarrhea, asthma, cough and cold. It is also used by some indigenous people for local medicines as well. It can also used in preparations of kashya, madhura, amla. *Syzygium cumini* leaves has therapeutic properties such as antiemetic (prevents vomiting), anti-diabetes, anti-oxidant and anti-hemorrhagic. Leaves also contains phytochemicals such as taninns, anthocyanins, terpenes, flavonoids, saponins, alkaloids, proteins, glycosides and carbohydrates, etc and other chemicals. Present study was carried out in order to evaluate the presence of phytochemical constituents and anti-oxidant property of ethanol leaf extract of *syzygium cumini.*

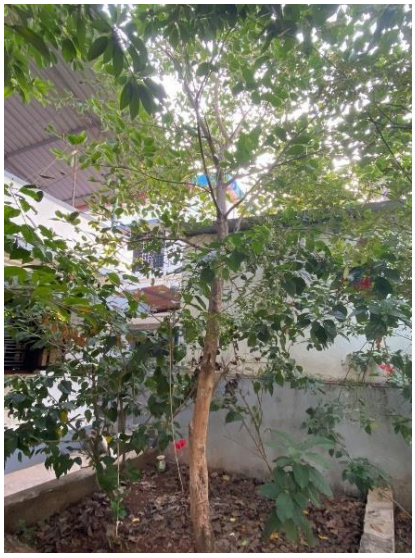

Fig no.1 *Syzygium cumini.*

Review of literature

A study was done on phytochemical analysis for alkaloids, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and quantitative phytochemical analysis for total alkaloids, total phenolics, total flavonoids and ascorbic acid. *In vitro* antioxidant properties were evaluated by assessing DPPH•, NO and ABTS, radical scavenging abilities and assaying the reducing power, β-carotene and antihemolytic activities by adapting standard method. This showed that the quantitative phytochemical analysis of this species exhibited the presence of alkaloids, total phenolics, total flavonoids, tannins, saponins and ascorbic acid in considerable quantity. The in vitro antioxidant activity of the species, *Hypochaeris radicata* clearly showed that both the leaf and root parts have prominent antioxidant property. From this study, it was concluded that the species is effective in scavenging free radicals and has the potential to be a powerful antioxidant. **(Senguttuvan et.al, 2014).**

Review was done on the evaluation of phytochemical constituents and antioxidant activites of aqueous extract of *Schotia latifolia* bark which was used for oxidative stress induced ailments in south Africa. Phytochemical tests for total phoenols, total flavonoids, total flavonols, tannins, saponins, alkaloids, steroids etc. and antioxidant activity estimation was done by DPPH method, scanenging activity was done by ABTS and nitric oxide tests. Results shown that glycosides and steroids were present in prominent content where as alkaloids and saponins were in least amount. And phenolics compounds were found to be very high in content thus responsible for strong antioxidant activity. **(Mbaebie et.al, 2012)**

Study was conducted to evaluate the chemical constituents of hydromethanolic macerating extract of *Chaptalia nutans* leaves and antioxidant, antimicrobial, cytotoxic and genotoixc activity of this plant. Antioxidant tests were done by DPPH and FRAP tests. Results showed that the plant leaves extracts contained alkaloids, phenolics, flavonoids, tannins and steroids where as Antibacterial results showed that it was positive results for hexane fraction and inhibited the microbes (*Candida krusei*, *Candida glabrata* etc). and the plant had high antoxidant capacity both in-vitro and in-vivo. **(De souza et.al, 2020).**

A literature review was done on phytochemical analysis and antimicrobial and antioxidant properties of methanol/chloroform and aqueous extracts of 61 medicinal plant species. Antioxidant activity were studied by reducing power (PR), total antioxidant capacity (TAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). phytochemical analysis was done for total phenols and total flavonoids Using of high performance liquid chromatography coupled with diode assay detector (HPLC-DAD). Results showed that maximum amount of gallic acid was detected in aqueous extract of *Syzygium cumini* and phenolics compounds were major contributors of antioxidant capacities in these plants. **(Akhtar et.al, 2018).**

A review of literature was done on phytochemical profiling and antioxidant activity of different parts of *Artocarpus heterophyllus* plant. It showed that the plant was rich in phytochemical compounds such as flavonoids, phenolics, terpenoids, steroids, glycosides, saponins, alkaloids, tannins, morin, artocarpin, artocarpesin, artocarpetin, artocarpenone etc. thus making the plant a potent antioxidant and anti-inflammatory fruit. Antioxidant tests were also conducted by DPPH, nitric oxide scavenging methods, ABTS and FRAP assays and the highest antioxidant activity shown was by scavenging hydroxyl radical activity followed by scavenging hydrogen peroxide and chelating of ferrous iron. **(Devi et.al, 2021).**

Study was done on phytochemical analysis and antioxidant properties of methanolic and other leaf extracts of *Carica papaya.* Study was aimed in determining the phytochemical constituents and antioxidant property in the plant leaves. Total phenolic content and total flavonoid content were determined by Folin-Ciocalteau and aluminium chloride methods respectively and Antioxidant by DPPH method. Results revealed the presence of bioactive compounds such as alkaloids, carbohydrates and aminoacids. and methanolic extract showed highest amount of phytochemical constituents. **(Nandini et.al, 2020).**

Review of literature was done on phytochemical screening and antioxidant, analgesic and antihelmintic effect of ethanolic extract of *Merremia umbellate* stems. Phytochemical investigation was done and antioxidant activity estimation was done by DPPH method. Total phenolic and tannin content was spectrophotometrically determined by folin-ciocalteu method and flavonoid was determined by aluminium chloride colorimetric assay. Analgesic and antihelmentic property was carried out in swiss albino mice and nematode of cattle. Phytochemical screening revealed that extracts of plants contained reducing sugar, alakloids, flavonoids, tannins, gums, steroids, glycosides and acidic compounds. Antianalgesi and antihelmentic properties were also excibited properly in the respective animals thus suggesting that the plant is a potential source of useful bioactive compounds**. (Acharzo et.al, 2020).**

A study on phytochemical profiling of methanolic extracts of *Gardenia latifolia* was done along with the evaluation of its antioxidant and antimicrobial property by DPPH activity, differential pulse voltammetry and resazurin microtiter assay. Phytochemical profiling revealed the presence of 22 bioactive compounds in methanol extracts such as alkaloids, saponins, glycosides, flavonoids, phenols and terpenoids. And no bioactive compounds in hexane and presence of phenols and flavonoids in chloroform extracts. Ethyl acetate extracts showed presence of phenols, flavonoids, glycosides and terpenoids. Antioxidant property showed that the plant has huge potential to be used in food industry**. (Reddy et.al, 2021).**

Evaluation was done of phytochemicals and bio-active properties in the mangrove associate *Suaeda monoica forssk* of indian sundarbans. Different leaves and roots extracts were prepared by water, ethanol, chloroform, acetone, hexane etc. bio-active properties such as anti-oxidant, anti-bacterial, anti-fungal assays was done. The study revealed the presence of different phytochemical compounds suchs as phenolics, flavonoids, phlobatannins, tannins, saponins, sterols, reducing sugar, steroids etc. total phenol content determination was done by callibaration curve of gallic acid. Antioxidant activity eastimation was done by DPPH method, ABTS radical cation decolorization assay and other methods**. (Dutta et al., 2021).**

Phytochemical profiling and antioxidant activity of methanolic leaf extract of *Cinnamomum tamala* was done. Qualitative and quantitative tests were done to determine the bio-active compounds in the leaf extracts and anti-oxidant activity was determined by DPPH, FRAF and ABST assays. The study revealed the presence of different phytochemicals present in the leaves such as polyphenols, flavones, alkaloids, steroids, terpenoids and glycosides. **(Sharma et.al, 2022).**

A review was done on the phytochemical screening and antioxidant activity from several parts of *Gardenia jasminoides.* A total of 104 compounds were isolated and identified from all parts of gardenia plants such as phenolics, flavonoids, glycosides, monoterpenoids, gallic acids, tannins, triterpenoids, carotenoids, organic acids and their derivatives and the phenolic compounds contained in gardenia plants have the potential as antioxidants. And can be used in traditional medicines. **(Andayani, 2022).**

Literature review was done on antioxidant potential, phytochemical composition and metal contents of *Dathura alba.* Investigation of phytochemical characterisitcs and anti oxidant activity in leaves, roots,stem, flowers and seed parts. It also assessed the heavy metals (Cr, Mn, Zn, and Cu) accumulation in each plant part. Cholorform, ethyl-acetate, n-hexane and methanol extracts were prepared in this work. The study showed the presence of bioactive compounds such as alkaloids, flavonoids, tannins and phenols. And highest amount were found in leaf parts. Methanol showed high antioxidant activity in the leaf extract due to the presence of high concentrations of polyphenolic consitituents including flavonoids and tannins. Thus the plant can be used in pharmaceuticals to improve the human health. **(Subhan et.al, 2019).**

A study was conducted on chemical composition and in-vitro evaluation of total phenolic, flavonoids and anti-oxidant properties of essential oil and solvent extract from the aerial parts of *Teucrium polium.* Anti-oxidant estimation was done by DPPH assay, radicle scavenging activity, β-carotene bleaching and reducing power. This study revealed the presence of bio-active compounds such as flavonoids, tannins, saponins, glycosides, terpenoids, steroids etc and the best anti-oxidant activity was found for acetone extracts. **(Bakari et.al, 2015).**

Study was conducted on in-vitro anti-oxidant activites of menthanolic extracts of *Caesalpinia volkensii*. Anti-oxidant estimation was done by DPPH, radicle scavenging activity and FRAP. This study concludes the presence of bio phytochemical compounds such as tannins, alkaloids, steroids, terpenoids, saponins. Methanolic leaf extracts of this plant had high anti-oxidant acitivity and anti-oxidant associated phytochemicals. **(Ngugi et.al, 2020).**

OBJECTIVES

- To collect the leaves of *syzygium cumini*
- To prepare the ethanol extract of *syzygium cumini* by maceration method.
- To evaluate the presence of phytochemical constituents in the ethanol leaf extract of *syzygium cumini.*
- To study the properties of *syzygium cumini* by estimating proteins, total sugars by standard methods.
- To analyze the anti-oxidant property by DPPH radical scavenging assay.

METHDOLOGY

A. PHYTOCHEMICAL ANALYSIS:

Qualitative phytochemical analysis: The extract was tested for the presence of phytochemical constituents by using following standard methods**.**

1.Test for proteins:

- **Biuret test:** 1ml of crude extract when mixed with 1ml of 50% of NaOH and 2 drops of copper sulphate solution a violet colour solution appeared which indicates the presence of proteins.
- **Ninhydrin test:** crude extract with 2ml of 0.2% solution of Ninhydrin, violet colour appeared indicates the presence of amino acids and proteins.

2.Test for carbohydrates:

- **Benedict's test:** Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish-brown precipitate formed which indicated the presence of carbohydrates.
- **Molish's test:** Crude extract was mixed with 2ml of Molish's reagent and the mixture was shaken properly. After that 2ml of concentrated sulphuric acid was poured carefully along the sides of the test tube. Violet colour at the interphase is formed indicating the presence of carbohydrates.

3.Test for flavonoids:

- **Lead acetate test:** Few drops of lead acetate is added to 2ml of extract. Yellow precipitate is formed indicating the presence of flavonoids.

4.Test for saponins:

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

6.Test for steroids:

Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

7.Test for terpenoids:

- **Copper acetate test:** Crude extract was mixed with 2ml of water and 3-4 drops of copper acetate solution was added. Emerald green colour indicates the presence of alkaloids.

8.Test for alkaloids:

- **Drgandroff's Test**: Crude extract was mixed with 2ml of 1%HCl and heated gently. To this mixture Dragandroff's reagent is added. A red precipitate indicates the presence of alkaloids.

9.Test for phenolics:

- **Ferric chloride test:** 1ml of crude extracts and ferric chloride solution added. Greenish yellow coloured solution appeared indicated the presence of phenolics.

10.Test for Tannin:

- **Tannin gelatin test:** To the extract 1% gelatin solution containing sodium chloride was added. White precipitate is formed which indicates the presence of tannin.

11. Test for anthocyanins:

To the extract, few drops of 10% of sodium hydroxide is added. Blue colour formed in the solution indicates the presence of anthocyanins.

12. Test for coumarins:

To the extract, 3ml of 10% NaOH is added. Presence of yellow colour in the solution indicates the presence of coumarins.

QUANTITATIVE ANALYSIS

A. ESTIMATION OF PROTEINS BY BIURET METHOD:

Proteins present in the leaf extract was determined by biuret method.

Reagents required: standard protein solution, sodium chloride solution and biuret reagent.

Preparation of standard solution: 1mg of crude extract of leaves was dissolved in 10ml of distilled water.

Preparation of reagents:

- **Standard protein solution**: 62.5mg of albumin dissolved in 25ml of distilled water.
- **Preparation of NaCl solution**: 0.45g of NaCl dissolved in 50ml of distilled water.

Procedure:

- Six dry test tubes were taken and labelled as 0.0, 0.4, 0.8, 1.2, 1.6, 2.0 and standard extract solution is added respectively.
- Two test tubes were taken for unknown 1ml and 2ml respectively.
- All the test tubes are made up to 3 ml by using NaCl solution.
- Then added 3ml of biuret reagent to all the test tubes.
- All test tubes are kept for incubation at room temperature for 10 minutes.
- Optical density is taken at 540 nm using colorimeter.

B. ESTIMATION OF TOTAL SUGARS BY PHENOL-SULPHURIC ACID METHOD:

Carbohydrates present in the leaf extract was determine by phenol-sulphuric acid method.

Reagents required: standard glucose solution, distilled water, 5% phenol reagent, concentrated sulphuric acid.

Preparation of standard solution: 1mg of crude extract of leaves was dissolved in 10ml of distilled water.

Preparation of reagents:

- Standard glucose solution: 2mg of glucose dissolved in 20ml of distilled water.
- 5% phenol reagent: 0.5g of phenol dissolved in 10ml of distilled water.

Procedure:

- Six dry test tubes were taken and labelled as 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and standard extract solution is added respectively.
- Two test tubes were taken for unknown 1ml and 2ml respectively.
- All the test tubes are made up to 1 ml by using distilled water.
- Then added 1ml of 5% phenol reagent to all the test tubes.
- Then added 5ml of concentrated sulphuric acid to all the test tubes.
- All test tubes are kept for incubation in boiling water bath at 25-30ºC for 20 minutes.
- Optical density is taken at 490 nm using colorimeter.

C. ESTIMATION OF ANTIOXIDANT PROPERTY BY DPPH RADICAL SCAVENGING METHOD:

Antioxidant property of ethanol extract was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method.

Preparation of standard solution: 0.04g of crude extract of leaves were dissolved in 100ml of ethanol.

Reagents required: DPPH(2,2-diphenyl-1-picrylhydrazyl).

Procedure:

- Seven dry test tubes were taken and labelled as 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and standard extract solution is added respectively.
- Two test tubes were taken for unknown 1ml and 2ml respectively.
- All the test tubes are made up to 1 ml by using methanol.
- Then add 2ml of DPPH to all test tubes.
- All test tubes are kept in dark for 30 minutes.
- Optical density is taken at 517 nm by using colorimeter.
- % Of inhibition is calculated by $\textbf{\% Of inhibition} = \frac{\textbf{O.D of C - O.D of E}}{\textbf{O. D of C}} \times 100$

where, C-Absorbance of Control.

E- Absorbance of test sample.

RESULT AND DISCUSSION

1. PLANT COLLECTION:

The leaves of *syzygium cumini* is collected from gowrikere, Moodubidire, Dakshina kannada and packed in a polythene bag to avoid decomposition of some bioactive compounds. Authentication of the plant was done by Mrs, ramya rai PD, dean, dept of botany. Then the leaves are washed and kept for shade drying for few days. . After drying leaves are finely grinded using mixer.

Place of collection: gowrikere, moodubidire.
Date of collection: 18-04-2023
Drying period: 22-04-2023 – 2-05-2023
Date of Powdering: 2-05-2023

Fig no2: drying of leaves

Fig no 3: powdered leaves

2. PREPARATION OF PLANT LEAF EXTRACT:

Ethanol extract: Crude plant extract was prepared by maceration method. About 10g of finely grinded leaf material is dissolved in 100ml of ethanol solvent. The mixture is kept for about 2 days for fermentation and constantly mixed at regular intervals. After that the extract was obtained by filteration by using whattmann no1 filter paper. The filterate is than stored in refrigerator for further use.

Incubation time: 4-05-2023 – 6-05-2023 (2 days) (12:40pm)
Filteration: 6-05-2023 (12:40pm)

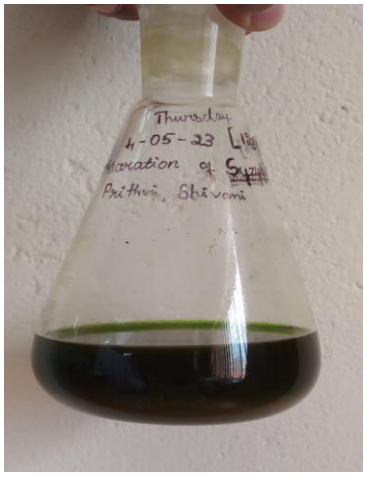

Fig no 2: maceration method

Fig no 3: Ethanol extract

3. PHYTOCHEMICAL ANALYSIS:

The phytochemical analysis of ethanol leaf extract of *Syzygium cumini* showed the presence of different phytochemicals such as proteins, carbohydrates, flavonoids, saponins, glycosides, steroids, terpenoids, alkaloids, coumarins, anthocyanins, tannins. The results are shown in table no 1.

Table no 1: Showing results for phytochemical analysis

Phytochemicals	Ethanol extract
Proteins	+
Carbohydrates	+
Flavonoids	-
Saponins	+
Glycosides	+
Steroids	+
Terpenoids	+
Alkaloids	+
Tannins	+
Phenols	+
Anthocyanins	+
Coumarins	-

Fig no 4: Test tube showing results for phytochemicals analysis

4. QUANTITATIVE ANALYSIS

A. Estimation of Protein by Biuret method:

Biuret method was done to determine the amount of soluble proteins in a solution. The presence of peptide bonds can be detected by observing a colour change from blue to violet in the solution. The protein concentration was found to be more in Test sample.2 compared to Test sample.1

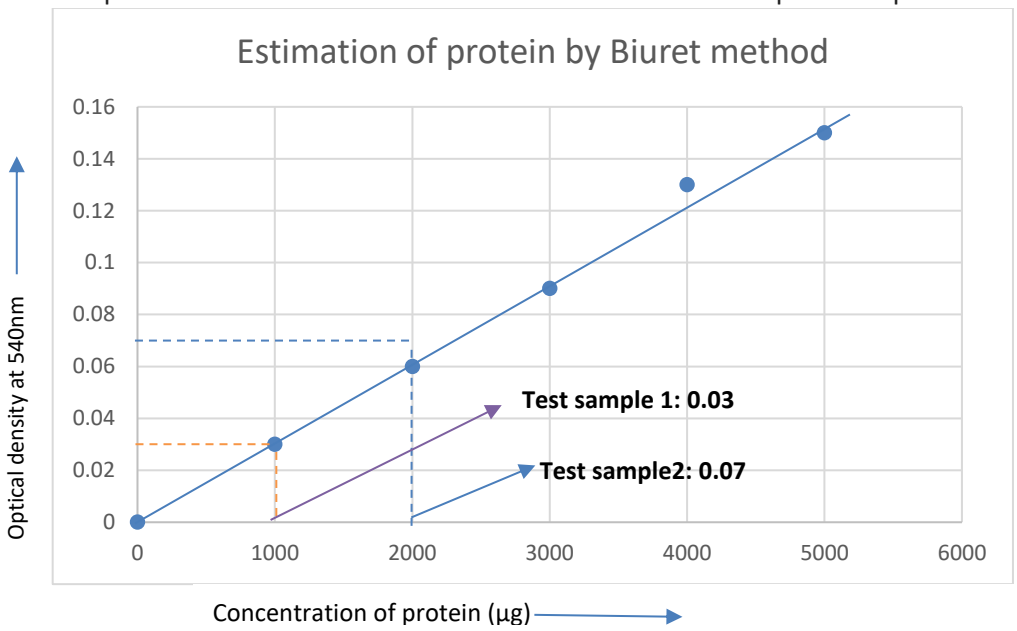

Fig no.6: Total protein concentration present in both the test samples in leaf extract of *Syzygium cumini.*

Result:

Plant extract	Concentration (µg/ml)				
Ethanol extract	1000	2000	3000	4000	5000
	0.03	0.06	0.09	0.13	0.15

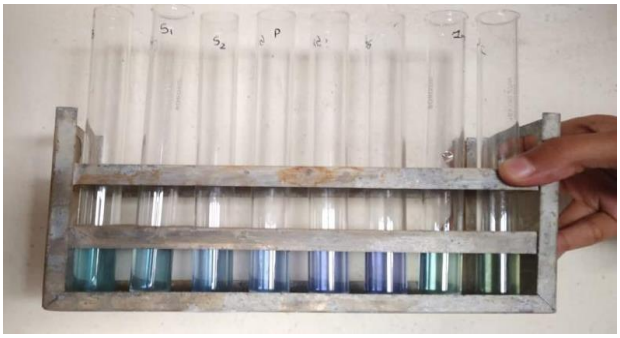

Fig no.7: Test tubes showing results for Quantitative analysis for Biuret test

B. Estimation of Total sugars by Phenol-Sulphuric acid method.

Phenol sulphuric acid method was done to determine the amount of carbohydrates present in the solution. Carbohydrates reacts in the presence of strong acid and heat to form furan derivatives that condenses with phenol to form stable yellow-gold compound that was measured at 590nm. This method showed the increasing sugar concentration. The total carbohydrate content was found to be more in Test sample.2 compared to Test sample.1.

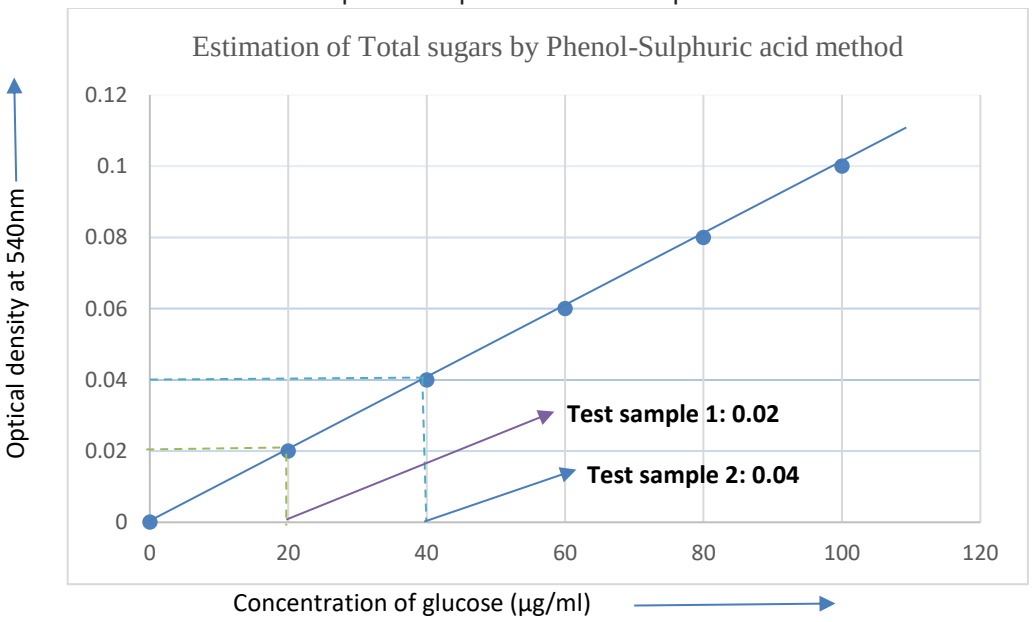

Fig no.8: Total carbohydrate concentration present in both the test samples in leaf extract of *Syzygium cumini.*

Result:

Plant extract	Concentration (µg/ml)				
Ethanol extract	20	40	60	80	100
	0.02	0.04	0.06	0.08	0.10

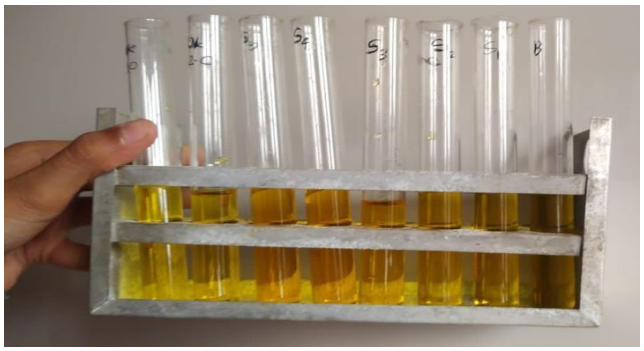

Fig no.9: Test tubes showing results for Quantitative analysis for Phenol Sulphuric acid test.

C. Estimation of Antioxidant property by DDPH radical scavenging method

In vitro Antioxdiant property of ethanolic extract were determined by DPPH (2,2-diphenyl-1picrylhydrazyl) radical scavenging method.

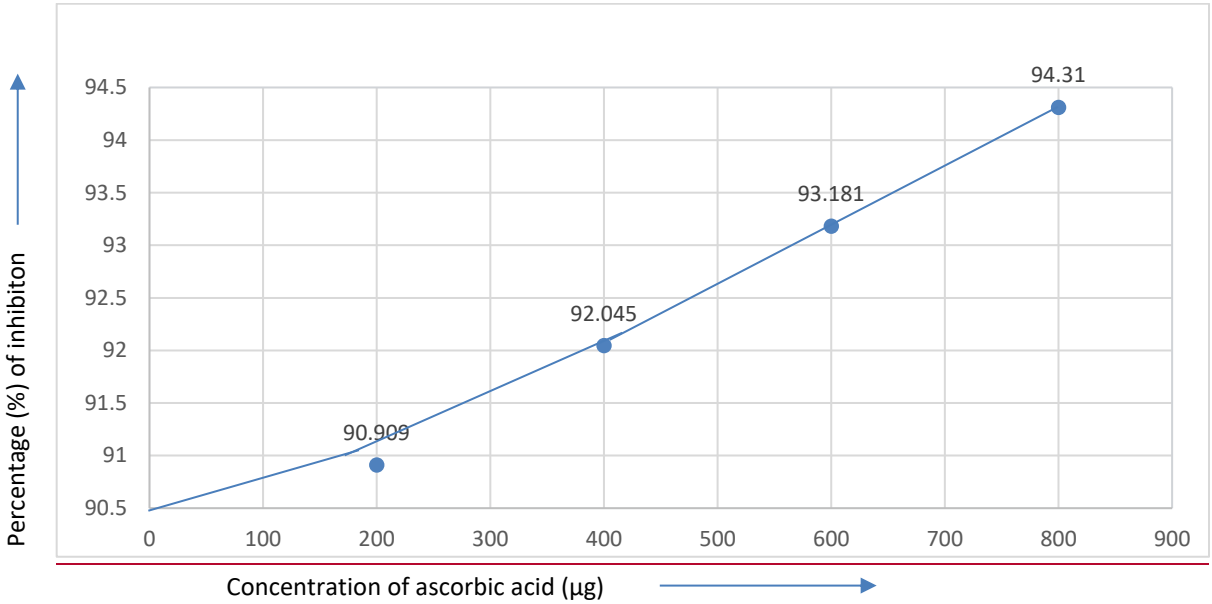

Fig no.9: Percentage of inhibition shown by standard ascorbic acid

Table no.2: Showing results for DPPH Radical scavenging activity for leaf extracts of *Syzygium cumini*

Plant extract	Extract	Volume of sample (ml)	Percentage (%) of inhibiton
Syzygium cumini	Ethanol	1	8.090
		2	79.545

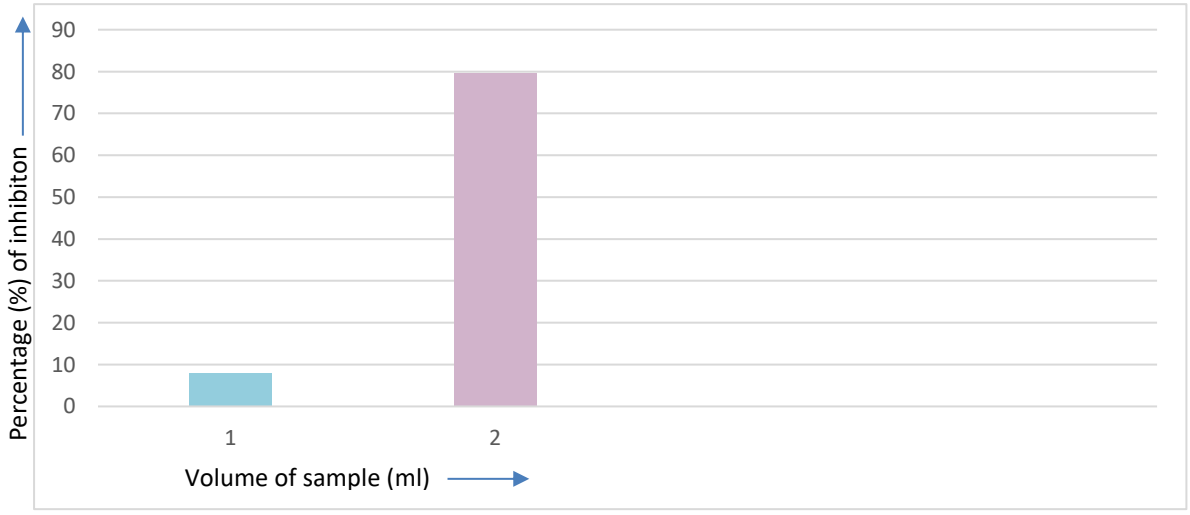

Fig no.10: Percentage of inhibiton shown by ethanolic extract

CONCLUSION

The present study revealed that ethanolic extract of *Syzygium cumini* have various phytochemical constituents such as saponins, alkaloids, proteins, glycosides, carbohydrates, tannins, phenolics and steroids. The extract was also present with higher protein content and carbohydrate contents which help the plant for its growth and development of seeds and fruits. The antioxidant property of ethanolic extract is may be due to the presence of higher Phyotchemical components. Therefore, it can be used as good source of antioxidant and can play important role in drug discovery.

The medicinal plant with antioxidant property terminates free radicals in the body and prevent or reduce the damage caused by oxidation such as aging, damaging cells, proteins and DNA and reduce the risk of many diseases including diabetes, cancer and nuerodegenrative diseases such as aAlzheimer's. Ascorbic acid is one of the anti-Oxidant which has highest amount of vitamin C, that protect the cells from damage, prevent oxidative dmage to DNA and help in maintaining proper health.

The present report reveals that the plant *Syzygium cumini* is having very good antioxidant property and large amount of secondary metabolites that can be used for curing many bacterial and fungal diseases. The leaves of *Syzgyium cumini* can also used in herbal medicines for its antiinflammatory, cardioprotective and antioxidant activites.

Based on these facts, this work highlights the role of jambolan in various treatments and recommend that furthur phytochemical and clinical research should be done on this traditional medicinal plant for the discovery of safer drugs.

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