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COMPREHENSIVE PHYTOCHEMICAL PROFILING, ANTIOXIDANT POTENTIAL, AND ANTICANCER ACTIVITY EVALUATION OF *SYZYGIUM CUMINI* SEED EXTRACTS

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Abstract

Syzygium cumini, a prevalent plant recognised for its therapeutic attributes, was examined for its phytochemical composition, antioxidant capacity, and anticancer effects in seed extracts. Ethanol extracts of Syzygium cumini seeds were made for phytochemical analysis, demonstrating the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, phenols, and carbohydrates. Quantitative examination verified the existence of total flavonoids (2.1 mg of quercetin equivalents per gram) and phenols (6.3 mg of gallic acid equivalents per gram). The extract exhibited notable antioxidant activity, achieving a 62.41% DPPH scavenging effect at a concentration of 1000 µg in comparison to ascorbic acid. The catalase activity was quantified at 3.6 mg of hydrogen peroxide consumed per minute, whereas the superoxide dismutase activity was recorded at 11.2 µg of pyrogallol autooxidation inhibition per minute. Non-enzymatic antioxidants comprised tocopherol (0.86 mg/g) and ascorbic acid (7.2 mg/g). The ethanolic seed extract demonstrated significant antioxidant properties in multiple experiments, including DPPH and reducing power assays. Additionally, the anticancer activity was evaluated using the colon cancer cell line HP 29, revealing that the ethanolic extract of Syzygium cumini has substantial anticancer characteristics. The results indicate that Syzygium cumini seed extracts possess significant antioxidant properties and exhibit potential anticancer effects.

Keywords: *Syzygium cumini seeds, DPPH Assay, FRAP Assay, In Vitro antioxidant Assay, MTT Assay,*

1. INTRODUCTION

Syzygium cumini, belonging to the Myrtaceae family, is also referred to as Syzygium jambolanum, Eugenia cumini, Jambul, Black Plum, Java Plum, Indian Blackberry, and Jamun. Different constituents of this plant demonstrate significant antioxidant and anti-inflammatory properties, underscoring its potential for medicinal use. Moreover, Syzygium cumini has nitric oxide scavenging and free radical scavenging properties, which aid in mitigating oxidative stress. [1]. Furthermore, the plant exhibits anti-diarrheal, antifertility, anorexiagenic, gastroprotective, antiulcerogenic, and radioprotective properties, highlighting its multifaceted nature as a therapeutic plant. Recent studies have examined the specific cytotoxic effects of jamun fruit extract on different breast cancer cell lines. The pro-apoptotic and antiproliferative effects were specifically examined in estrogen-dependent/aromatase-positive (MCF-7aro) cells, estrogen-independent (MDA-MB-231) breast cancer cells, and normal/nontumorigenic (MCF-10A) breast cell lines. Their findings indicate that Syzygium cumini may play a substantial role in cancer therapy, especially in breast cancer. Subsequent studies on human cervical cancer cells revealed growth suppression rates of 11.8% in SiHa (HPV-16 positive) cells and 14.4% in HeLa (HPV-18 positive) cells when a 40% Syzygium cumini extract was utilised to examine its anti-cancer effects. [2]. These data highlight the efficacy of Syzygium cumini extracts in the management of cervical cancer [3]. Multiple in vitro assays have

demonstrated that extracts from the leaves and seeds of Syzygium cumini possess significant antioxidant properties. The tests include total reducing antioxidant potential, total antioxidant activity, reducing power, hydroxyl radical scavenging activity, nitric oxide radical scavenging, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, ABTS assay, and Ferric Reducing Antioxidant Power (FRAP) assay. Collectively, these assays illustrate the substantial antioxidant potential of Syzygium cumini, endorsing its application as a natural source of antioxidants for the management of conditions associated with oxidative stress. [2]. In Ayurvedic medicine, several components of the Syzygium cumini tree have been employed for generations to address numerous illnesses. The fruits are conventionally ingested for their alleged advantages in diabetes management, attributed to their hypoglycemic qualities. The seeds, bark, and leaves have been utilised to mitigate digestive disorders, regulate diarrhoea, and offer relief from inflammatory ailments. Moreover, traditional healers have utilised Syzygium cumini formulations to facilitate wound healing and improve general vigour [3]. The phytochemical investigation of Syzygium cumini has identified a diverse array of bioactive substances that enhance its therapeutic attributes. Phytoconstituents present in various plant parts comprise alkaloids, flavonoids, tannins, terpenoids, saponins, phenols, and sugars. These compounds possess many properties, including antibacterial, antioxidant, anti-inflammatory, and anticancer effects. Syzygium cumini seeds possess flavonoids and phenols, potent antioxidants that mitigate oxidative stress and neutralise free radicals. These attributes not only validate Syzygium cumini's traditional applications but also illustrate the plant's potential as a significant source of natural antioxidants for therapeutic use [4]. Besides its antioxidant qualities, the phytochemicals in Syzygium cumini exhibit promise anti-diabetic actions. Studies have shown that extracts from various plant sections can block alpha-amylase, an enzyme that catalyses the conversion of starch into sugars. This inhibition aids in the regulation of blood glucose levels, indicating a potential role for Syzygium cumini in the management of diabetes mellitus. Furthermore, preliminary cytotoxicity studies indicate that certain extracts possess the capability to eradicate cancer cell lines through cytotoxic activity, implying their potential application in cancer treatment. These findings underscore the importance of a thorough examination of the bioactive compounds in Syzygium cumini and their mechanisms of action, paving the way for the potential development of these substances into therapeutic interventions for various medical conditions.

2 MATERIALS AND METHODS

2.1 Plant Materials and Chemical Reagents

All of the chemical reagents used in this experiment were of analytical grade and were acquired from Loba Chemicals in India. In Kancheepuram, Tamil Nadu, Syzygium cumini seeds were collected from a local pharmacy.

2.2 Preparation of Ethanol Extract

A Soxhlet apparatus extracted chemicals from 10 grams of dried Syzygium cumini seed powder in 200 mL of ethanol over 18 cycles. The extract was then concentrated for three hours in a rotary vacuum evaporator at 30–40°C. The extract was dried in a vacuum desiccator and kept at -20°C after concentration.

2.3 Preliminary Screening

Comprehensive phytochemical investigation of Syzygium cumini identified many beneficial chemicals. Mayer, Wagner, and Dragendorff's experiments proved alkaloids by precipitate forms. Shinoda's test showed flavonoids as pink or red, and the alkaline reagent test showed yellow that turned colourless with acid. The ferric chloride test showed a blue-black or greenish-black colour, while the potassium dichromate test showed a brownish precipitate [4]. Tannins were discovered. Salkowski found terpenoids, resulting in a reddish-brown interface. Folin-Ciocalteu colorimetric analysis identified polyphenols. Proteins were detected using the Ninhydrin test, which became blue or purple when heated. The Froth test produced a steady froth, while the Lead acetate test produced a white precipitate, identifying saponins. Standard procedures also found anthraquinones, glycosides, polysaccharides, and phytosterols. This thorough screening emphasises Syzygium cumini's extensive phytochemical profile, laying the groundwork for its pharmacological and medicinal uses [5].

2.4 Quantitative Analysis (Estimation of Flavonoids)

Standard quercetin solution was used to measure flavonoids. First, we combined 0.2 mL homogenate with 3 mL 95% ethanol. Next, we added 0.1 mL of 1M potassium acetate and aluminium chloride hexahydrate. To combine the flavonoids with aluminium chloride, we incubated the mixture at room temperature for 40 minutes. Using a spectrophotometer, we examined the solution's 430 nm absorbance after incubation. Using a calibration curve with known quercetin concentrations, the sample's flavonoid content was calculated in microgrammes of quercetin equivalents per gram or millilitre [5, 6].

2.5 Estimation of Total Phenolic Compounds

The Folin-Ciocalteu method measured total phenol. After mixing the Folin-Ciocalteu reagent with a sample extract, a reaction occurred. To make the mixture alkaline, sodium carbonate (Na2CO3) was added. A blue complex was analysed at 760 nm using spectrophotometry after phenolic chemicals reduced the Folin-Ciocalteu

reagent. Gallic acid concentrations were used to create a standard curve to assess the sample's total phenolic components. Results were given in milligrams of gallic acid equivalents per gram or millilitre [6].

2.6 Antioxidant analysis

Antioxidant assays measure substances' ability to fight oxidative stress and neutralise free radicals. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay measures antioxidant capability by measuring the stable DPPH radical's decrease at 517 nm using spectrophotometry. The ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay examines antioxidants' ability to block the 734 nm ABTS radical cation. Antioxidants' ability to spectrophotometrically convert ferric ions (Fe3+) to ferrous ions (Fe2+) at 593 nm is measured by the FRAP assay. As a final test, the ORAC assay measures antioxidant activity against peroxyl radicals produced by AAPH breakdown. These assays reveal a substance's antioxidant activity, which is vital for understanding its potential uses in treating and preventing oxidative stress disorders [7–9].

2.7 FT-IR Analysis

Pulverised Syzygium cumini ethanol extract was oven-dried at 60°C and mixed with KBr to make a salt disc for FT-IR spectral analysis. The spectra were taken from 500–4000 cm–1.

2.8 GC-MS Analysis

A mass spectrometer-connected GC-AGILENT system analysed crude ethanol extracts. Helium carried the sample into the GC-MS system. An inert capillary column of 30.0 meters and 250 micrometres was used to regulate column temperature between 60°C and 300°C [8, 13].

2.9 Estimation of Ascorbic Acid (Vitamin C)

A modified method determines ascorbic acid concentrations. To reduce a dye like 2,6-dichlorophenolindophenol (DCPIP) with ascorbic acid, titrate to the endpoint or observe the absorbance drop at 520 nm using spectrophotometry. Ascorbic acid was measured in milligrams per gram or millilitres using a standard curve built from recognised values [10].

2.10 Estimation of Tocopherol (Vitamin E)

Tocopherol was measured using Baker and Frank's technique. The material is extracted in a non-polar solvent such as hexane, then quantified by chromatography or spectrophotometry at tocopherol wavelengths. The tocopherol content of the samples was evaluated using a standard curve generated from known levels of alpha-tocopherol expressed in micrograms per gram or millilitre [11].

2.11 Assay of Catalase Activity

Catalase activity was tested using Sinha's technique. In this method, the sample catalase tracks hydrogen peroxide decomposition. Decreased absorbance at 240 nm over time was used to calculate the breakdown rate. Based on protein concentration, catalase activity was normalised and given as units per milligram [11].

2.12 Assay of Superoxide Dismutase Activity

The evaluation of superoxide dismutase (SOD) activity followed the protocol. After inhibiting a superoxide radical reaction, nitroblue tetrazolium reduction was measured at 560 nm. The amount of enzyme needed to 50% block the reaction was used to calculate SOD activity in units per milligram of protein or ml of sample [10].

2.13 Free Radical Scavenging Activity

The plant extract was tested for its antioxidant activity against the stable 1,1'-Diphenyl-2-picrylhydrazyl (DPPH) free radical. During DPPH-antioxidant interaction, a spectrophotometer measured the colour shift at 517 nm, indicating the antioxidant compounds' ability to neutralise reactive oxygen species [8, 9].

DPPH+ H-A → DPPH-H + (A)

Yellow purple

% inhibition was computed with the following formula:

$$\textbf{DPPH Scavenging activity (\%)} = \frac{A_{control} - A_{extract} \times 100}{A_{control}}$$

where the optical density of DPPH with methanol serves as the control.

2.14 Determination of Ferric Reducing Antioxidant Power (FRAP)

The plant extract (10-100 µg) was combined with 2.5 mL of 1% potassium ferricyanide in 1 mL of distilled water. After incubating at 37°C for 20 minutes, 2.5 mL of 10% trichloroacetic acid was added. Next, the mixture was centrifuged for 10 minutes at 1800 rpm [8]. After adding 0.1% $FeCl_3$ and distilled water, the supernatant's absorbance was 700 nm. Percent inhibition was utilized to calculate the reducing activity using the following formula:

% Inhibition = $((A_0-A_e)/A_o)$ x100

A_o = Absorbance of without extract

A_e = Absorbance without standard

As a common antioxidant, ascorbic acid was employed.

2.15 Cancer Cell Line Therapy Using Seed Extract

The study used crude Syzygium cumini extracts to treat cancer cell lines. The extracts were filtered through a 0.2 µm Whatman syringe filter, diluted, and mixed with 1 mL of MEM containing sodium pyruvate and 10% FCS to generate a stock solution. Add 100 µL of diluted extracts to 96-well plates, incubate at 36°C with 5% CO2, and observe after 72 hours [12].

2.16 Cytotoxicity Test of *Syzygium cumini*

A colorimetric experiment utilising MTT reduction assessed the cytotoxic effects of Syzygium cumini leaf, seed, and flower extracts on HCT15 colon cancer cell lines [9]. On an ELISA reader, cell viability and % inhibition were assessed by colour intensity [8].

3. RESULT AND DISCUSSION

3.1 Phytochemical constituents of ethanolic seed extracts *Syzygium cumini*

The examination revealed that glycosides and anthraquinones were not present; however, alkaloids, flavonoids, tannins, saponins, polyphenols, terpenoids, proteins, and carbohydrates were found to be present. Different portions of the plant contain these compounds, which are used to treat major ailments such as cancer, heart failure, and hypertension [12]. These substances can be discovered from the plant. Not only are they utilised in the production of euphoric and addictive drugs, but they are also utilised in the manufacturing of pesticides and insect repellents.

Table. 1: Preliminary phytochemical composition of ethanolic seed extracts of *Syzygium cumini* ethanolic seed extract

S. NO	Phytochemicals	Ethanolic Extract
1	Alkaloids	+
2	Anthraquinone	-
3	Carbohydrate	+
4	Flavonoids	+
5	Glycosides	-
6	Polyphenol	+
7	Proteins	+
8	Saponin	+
9	Tannin	+
10	Terpenoids	+

3.2 Total phenolic and flavonoid content of ethanolic seed extract of *Syzygium cumini*

Syzygium cumini's ethanolic seed extract contains 2.1 mg quercetin equivalents per gram (mg QE/g) and 6.3 mg gallic acid equivalents per gram (mg GAE/g) of phenols. Phenolic-rich foods delay atherosclerosis and reduce heart disease risk due to their antioxidant properties. This study shows that flavonoids, a subtype of phenolic compounds, have antioxidant activity, but the plant's main antioxidant supply, phenolic chemicals, outnumber them. Instead of flavonoids, phenolic compounds in Syzygium cumini seed ethanolic extract are responsible for its antioxidant properties [8].

Table. 2: Total amount of phenols and flavonoids in *Syzygium cumini* ethanolic seed extract

S. No	Major Phytochemical	Quantity (mg/g)
1	Phenol	6.3
2	Flavonoid	2.1

3.3 FTIR Absorption Spectra of Ag Ion Reduction

The FTIR absorption spectra of the soluble extract during the reduction of Ag ions exhibited absorbance bands at 3287, 2902, 1776, 1732, 1701, 1624, 1525, 1361, 1338, 1209, 1189, and 497 cm^{-1} within the 500–4000 cm^{-1}

range. The stretching vibrations corresponding to these bands are as follows: The O-H stretching of carboxylic acids occurs at 3287 cm^{-1}, C-H stretching of alkanes at 2902 cm^{-1}, anhydride $(RC(O))_2O$ stretching at 1776 cm^{-1}, C=C stretching of alkenes at 1732 cm^{-1}, C=O stretching of anhydrides and esters at 1701 cm^{-1}, ketone (CH_3—C(=O)—CH_3) stretching at 1624 cm^{-1}, NO_2 stretching of nitro compounds at 1525 cm^{-1}, O-H stretching of alcohols and phenols at 1361 cm^{-1}, ether (R–O–R) stretching at 1338 cm^{-1}, and monosubstituted benzene stretching at 1209, 1189, and 497 cm^{-1}. The total absence of these bands indicates the bio-reduction of Ag ions, with the reduction process predominantly influenced by a significant broad peak observed at 3287 cm^{-1}[12].

Table. 3: FT-IR analysis of the ethanolic extract of *Syzygium cumini* seed

BOND		FUNCTIONAL GROUP	FREQUENCY RANGE
C-H stretching		Alkanes	2974.29
C-H stretching		Alkanes	2891.34
O-H stretching		Carboxylic acid	2365.73
C=C=C Stretching		Allene	1969.35
C=C Stretching		Alkenes	1649.17
C-H Bending		Alkane	1450.49
S=O Stretching		sulphate	1417.70
S=O Stretching		Sulphonamide	1381.06
S=O Stretching		Sulfone	1328.98
C-O Stretching		Aromatic Ester	1274.00
C-O Stretching		Aliphatic Ether	1085.94
C-C Bending		Alkene	878.59

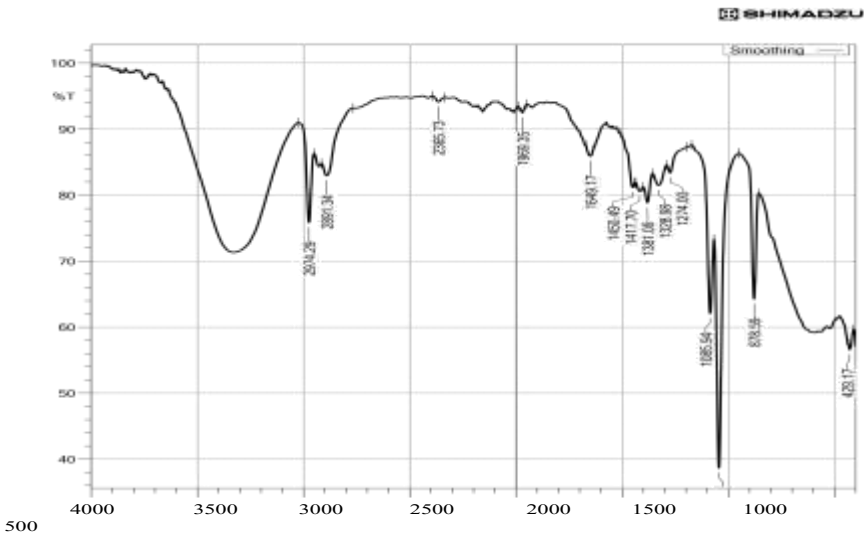

Fig. 1: FT-IR analysis of the ethanolic extract of *Syzygium cumini* seed

3.4 GC-MS analysis of ethanolic seed extract of *Syzygium cumini*

The ethanolic extract of *Syzygium cumini* seeds was analyzed using GC-MS, revealing ten compounds with antimicrobial, antioxidant, anti-inflammatory, antiarthritic, and diuretic properties. These compounds have potential therapeutic applications.

Table. 4: GC-MS analysis of ethanolic seed extract of *Syzygium cumini*

S. No	Compound name	RT	Molecular weight	Molecular formula	Probability	Biological activity
1	1-Pentanol	4.342	88	$C_5H_{12}O$	70.2%	Antioxidant, corrosives inhibitor
2	Butanoic acid, 3methyl-	4.437	102	$C_5H_{10}O_2$	44.3 %	Antimicrobial, antioxidant

3	Furfural	4.972	96	$C_5H_4O_2$	53.9 %	Anti tyrosinase, antimicrobial
4	4H-Pyran- 4-one, 2,3dihydro-3,5dihydroxy-6-methyl	9.943	144	$C_6H_8O_4$	92.1%	Antimicrobial, anti-inflammatory, Antioxidant hypoglycaemic
5	5Hydroxym ethyl furfural	11.664	126	C6H6O3	89.6 %	Anti proliferative, Antioxidant
6	Levomenol	16.416	222	$C_{15}H_{26}O$	4.89%	Anti apoptotic, anti-inflammatory, antidiabetic, antioxidant
7	Trans α Bergamotene	18.679	204	$C_{15}H_{24}$	14.7 %	Anti proliferative, antioxidant
8	Hexadecenoic acid, ethyl ester	19.491	284	$C_{18}H_{36}O_2$	46.7 %	Hypoglycaemic, hypo cholesterol emic , Antioxidant
9	n-Hexadecenoic acid	19.565	256	$C_{16}H_{32}O_2$	23.9 %	pesticides, nematicide
1 0	Hexadecenoic acid, 2hydroxy-1(hydroxy methyl) ethyl ester	24.192	330	$C_{19}H_{38}O_4$	41.6%	Haemolytic, flavour, pesticides, Antioxidant

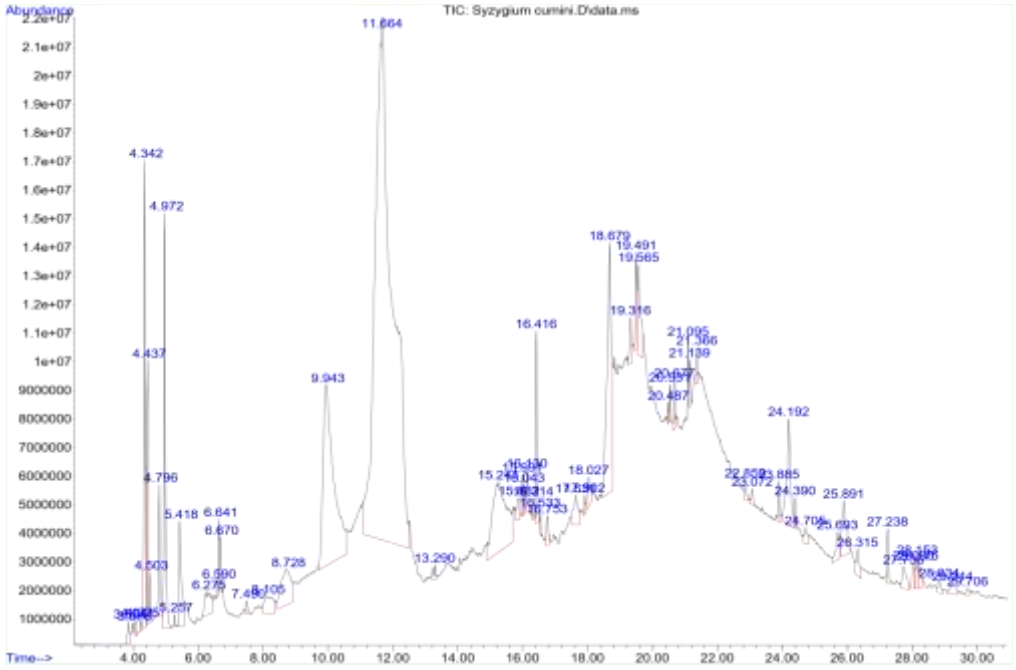

Fig. 2: GC-MS analysis of ethanolic seed extract of *Syzygium cumini*

3.5 Enzymatic antioxidant present in the ethanolic extract of *Syzygium cumini* seeds

Catalase activity has been demonstrated to be present in the ethanolic extract of Syzygium cumini seeds, with 3.6 milligrams of hydrogen per minute being extracted from the seeds. In addition, it was demonstrated that the same extract had a tremendous amount of superoxide dismutase activity, with 11.2 milligrams of hydrogen per minute being utilised.

Table. 5: Enzymatic antioxidant presence in the *Syzygium cumini* ethanolic seed extract

S. No	Antioxidant Enzymes	Crude Seed Homogenate
1	Catalase (mg of hydrogen peroxide utilized/min)	3.6
2	Superoxide Dismutase (µg of Pyrogallol autooxidation inhibition/min)	11.2

3.6 Non-enzymatic antioxidant present in the ethanolic seed extract of *Syzygium cumini*

Syzygium cumini seed ethanolic extract contains 0.86 mg tocopherol and 7.2 mg ascorbic acid per gram. Due to their reducing power (RP) and free radical scavenging potential, phenolic compounds are popular antioxidants. The ethanolic extract of Syzygium seeds contains antioxidant and antimutagenic polyphenolics. Plants rich in phenolic compounds and antioxidants are useful. Several studies have linked total phenolic content to plant antioxidant activity. When diluted with alcohol, phenolic chemicals extract better [8].

Table. 6: Non-enzymatic antioxidant present in the *Syzygium cumini* ethanolic seed extract

S. No	Non-enzymatic Antioxidant	Crude Seed Homogenate
1	Tocopherol (mg/g)	0.86
2	Ascorbic acid(mg/g)	7.2

3.7 DPPH free radical scavenging activity

The extract obtained from the seeds of Syzygium cumini exhibits a dose-dependent DPPH radical scavenging activity that spans from 250 µg to 1000 µg. As the concentration of the Syzygium cumini seed extract increases, the proportion of scavenging action also increases. Particularly noteworthy is the fact that the greatest scavenging activity (62.41%) was reported at a concentration of 1000 µg, which is significantly greater than the activity of standard ascorbic acid. [14, 15]

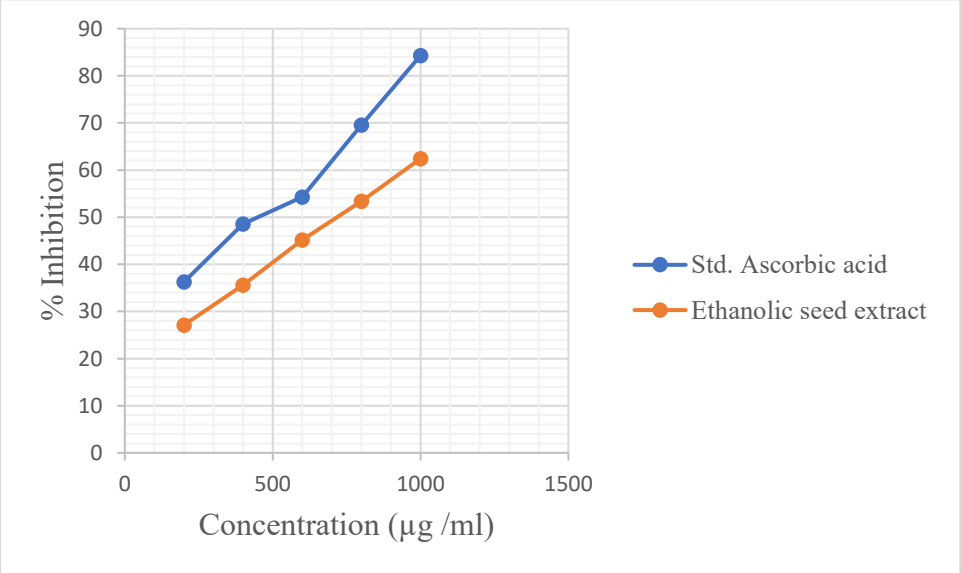

Fig. 3: In Vitro Antioxidant Activity by DPPH

3.8 Ferrous reducing power antioxidant assay

Using the Fe3+ to Fe2+ transformation assay, which is characterised by an increase in absorbance at 700 nm, it is possible to measure the antioxidant potential in a swift and uncomplicated manner. The power of the Syzygium

cumini seed extract to decrease increased with concentration, reaching its highest point at 1000 µg of extract and accounting for 56% of the absorbance. These findings were discovered. The positive control, gallic acid, showed a maximum absorbance of 84% when it was administered at the same dose, in contrast [8].

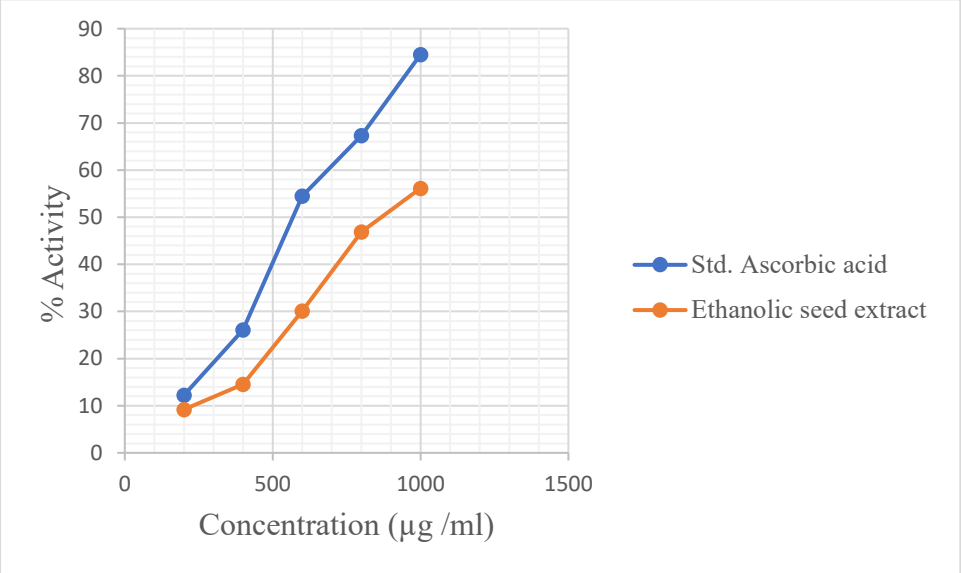

Fig. 4: Ferrous reducing power antioxidant assay

3.9 Cytotoxicity activity against colon cancer cell line (HT29)

The vitality of HT29 colon cancer cells was measured using the MTT test after 72 hours of exposure to Syzygium cumini seed extracts at doses ranging from 12.5 to 100 µg/ml. Both ethanolic and aqueous extracts decreased cell viability dose-dependently at all doses, suggesting cytotoxicity. Syzygium cumini's cytotoxicity was examined in previous GLOBOCAN 2018 studies. Syzygium cumini seed dichloromethane extracts killed HT-29 and other cancer cells. The MTT experiment showed that Syzygium cumini extract significantly suppressed HT-29 cell growth. Bax ratio expression analysis changed significantly following Syzygium cumini extract therapy. Gossypol is one of Syzygium cumini's most studied natural cytotoxic compounds. Despite poor response rates, clinical trials showed little negative effects. Researchers must study new extracts or synergistic combinations to improve efficacy [16-18].

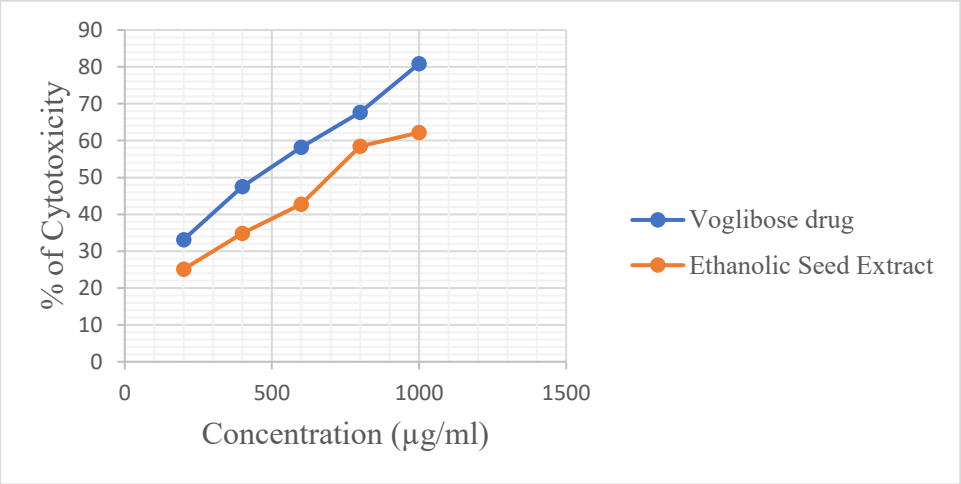

Fig. 5: Cytotoxicity activity against colon cancer cell line (HT29)

4 CONCLUSION

This study concludes that Syzygium cumini seed extracts are antioxidant, anti-diabetic, and cytotoxic. The phytochemical investigation verified alkaloids, flavonoids, tannins, terpenoids, saponins, phenols, and carbohydrates. Quantitative examination demonstrated considerable flavonoids and phenols. In antioxidant scavenging experiments, the ethanol extract outperformed ascorbic acid. Catalase and superoxide dismutase

activities showed the extract's oxidative stress-reduction potential. Its antioxidant potential was enhanced by non-enzymatic antioxidants tocopherol and ascorbic acid.
The extract also showed cytotoxic effects against HP 29 colon cancer cells, suggesting anticancer potential. These findings validate Syzygium cumini's traditional medicinal use and demonstrate its potential as a source of bioactive chemicals for diabetic and cancer medication development. Future study should isolate and characterise bioactive chemicals from Syzygium cumini seeds to understand their processes and improve their therapeutic uses.

Authors' contribution

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