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Exploration of Phytochemical, Antioxidant and Anticancer Potential of *Syzygium cumini* Seeds

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ABSTRACT

The phytochemical analysis, antioxidant capacity, and anticancer potential of an extract made from *Syzygium cumini* seeds through green extraction technique are all examined in this work. The existence of bioactive substances with a variety of medicinal uses, including as flavonoids, saponins, steroids, tannins, and alkaloids, was verified by phytochemical screening. According to the DPPH test, which was used to evaluate the antioxidant activity, the extract demonstrated high free radical scavenging activity with an IC50 value of 25.8 µg/ml, in contrast to ascorbic acid's 8.5 µg/ml. Hep3B liver cancer cell lines were used to evaluate the anticancer potential over 24, 48, and 72-hour treatments; the results demonstrated a dose- and time-dependent decrease of cell viability. The extract's IC_{50} values, which showed increased potency over time, were 77.4 µg/ml, 73.05 µg/ml, and 71 µg/ml, respectively. These results imply that the extract from *Syzygium cumini* seeds have strong anticancer and antioxidant qualities, which may be related to its diverse phytochemical composition. In addition to recommending more research to identify active chemicals, clarify molecular processes, and evaluate clinical application for cancer therapy, the study validates *Syzygium cumini* traditional medicinal usefulness.

INTRODUCTION

Herbal remedies have grown in popularity as a result of their low toxicity and lack of side effects, which are common in allopathic treatments. To study the efficacy and safety of herbal medication, a proper analysis is necessary.(Chitnis et al., 2012).The medical history of Syzygium cumini is extensive, and it is used extensively in folk and traditional medicine. Problems with digestion, liver health, and blood sugar balance are just a few of the many areas it tackles. The seeds, both dried and powdered, have widespread application in the treatment of diabetes.
(Rizvi et al., 2022).Among its many names, Syzygium cumini (Myrtaceae family) is also known as Eugenia cumini and Syzygium jambolanum. Some other names for this fruit are Jamun, Indian Blackberry, Black Plum, Java Plum, and Jambul (El-Nashar, et al 2021). With their abundance of beneficial nutrients, including fibre, vitamins, and minerals, fruits should be a staple in any balanced diet. They have several medicinal benefits due to the phytochemicals, antioxidants, and phenolic compounds found in them (Akonor 2020).Syzygium cumini seeds are rich in bioactive compounds such as flavonoids, phenolic acids, saponins, and tannins(Kumar et al., 2022). The antioxidant activity of Syzygium cumini seeds has been demonstrated through assays like DPPH, showing significant inhibition percentages(Kavital & Hiremath, 2023). Ethyl acetate extracts exhibit the highest antioxidant activity compared to other extracts, indicating their potential in combating oxidative stress. Extracts from Syzygium cumini seeds have shown promising results against breast cancer cell lines (MCF-7), highlighting their potential as anticancer agents (Ruthurusamy et al., 2015).

Despite its evergreen and large occurrence in varied climatic situations in South Asia, the critically important indigenous plant *Syzygium cumini*, which originates from Indonesia and India and is a member of the Myrtaceae family. Ripe fruits contain purple-black anthocyanins, which have several antioxidant and health effects. Tannic acid, gallic acid, oxalic acid, quercetin, ferulic acid, guaiacol, catechin, epicatechin, tannic acid, and certain alkaloids are the components of S. cumini seeds that have antioxidant capabilities, which are utilised in traditional medicine (Das et al., 2023).

The presence of these compounds demonstrates a range of pharmacological actions that impact human health and metabolism. These activities include protecting the liver, reducing inflammation, preventing Cancer, and fighting germs. (Tak et al. 2022). Because of its anthocyanin and ellagic acid content, jamun has an anticancer effect by retaining its capacity to inhibit the ROS production. The adjuvant treatment of cancer is the most prevalent usage of jamun. (Singh et al. 2019).

Various studies have investigated the bioactive components and antioxidant properties of *S. cumini.* However, none of the aforementioned research examined the pulp and seed of *S. cumini* independently for bioactive chemicals and antioxidant activity, and we are unaware of any HPLC profiling of the two. This is why we set out to methanolic extract the pulp (the edible portion, pulp with peel) and seed of *S. cumini* and look into its bioactive chemicals, antioxidant capabilities, and specific phenolic components. (Benherlal & Arumughan, 2007; Balyan & Sarkar, 2017 and Singh et al, 2016). *Syzygium cumini* has a long and storied history of use in folk and traditional medicine. Problems with digestion, liver health, and blood sugar balance are just a few of the many areas it tackles. The seeds, both dried and powdered, have widespread application in the treatment of cancer (Rizvi et al., 2022).

The antioxidant and anticancer effects of *S. cumini* have been studied, but so far, no one has been able to pinpoint the bioactive components, particularly in the commercially important fruit portions, and examine the processes that lead to these effects. The objective of this work was to determine the most effective land race and its commercially important fruit by GC-MS profiling, pathway enrichment analysis, and the identification of new bioactive components linked to antioxidant and anticancer potentials.

Objectives: The main objectives of the present study were:

• To investigate the phytochemical of *Syzygium cumini* seeds extract.

• To explore the antioxidant and anticancer activities of *Syzygium cumini* seeds extract.

Materials And Methods

The current research set out to characterize the phytochemical, antioxidant, and anticancer properties of an extract from *Syzygium cumini* seeds. This study involved carrying out the following procedures:

• Extracts from *Syzygium cumini* seeds were prepared using green technology, followed by a cell culture assay, statistical analysis, and phytochemical testing.

Materials: Here is a list of all the materials that were considered for this study:

Chemicals, and Apparatus: Here is a list of the substances and equipment that were used in the study: The following ingredients are needed: the following ingredients: glucose, nutritional agar, sulphuric acid, methanol, ethanol, ferric chloride, sodium hydroxide, gentamycin, dimethyl sulfoxide, DPPH, (3-(4,5-Dimethylthiazol-2-yl)-2,5 Diphenyltetrazolium bromide), and distant water. The following items are required: a balance, a vortex, a microwave oven, an incubator, test tubes, vials, a micropipette, aluminum foil, falcon tubes, forceps, Eppendorf tubes, beakers, test tubes racks, and cotton plugs and swabs.

Sample collection and preparation of extract: The seeds of *Syzygium cumini* were gathered from Pakistan's Punjab and Khyber Pakhtunkhwa plains and lower hills. A fine powder was made from ground *Syzygium cumini* seeds, which were then preserved in jars that could not be opened. Two hundred grammes of the powdered substance were mixed with 600 ML of 80% ethanol in a flask, heated for six minutes, filtered, and then repeated three times. Following filtration, the crude extracts and rotational dilution were lyophilized for 2-3 days and subsequently kept at 4oC for future use.

Phytochemical screening: Having bioactive substances such tannins, flavonoids, steroids, alkaloids, saponins, and saponins-like compounds was determined by phytochemical analysis of *Syzygium cumini* seeds (da Silva, 2017) We used the flavonoid test to screen for flavonoids; to this, we added a few drops of diluted NaOH and took around 20 mg of each extract. The presence of flavonoids in the extract is confirmed when the yellow color becomes colorless or disappears after adding a few drops of diluted sulfuric acid. About 20 milligrams of extract was mixed with 20 milliliters of distilled water for the saponin screening. The next step was a 15-minute hand-shaking of the test tube. The presence of saponins in the extract was shown by the foam pattern that formed on top of the test tube. Twenty milligrams of the extract was dissolved in a few drops of one percent picric acid in an Eppendorf tube for the purpose of alkaloids screening. If the extract produces precipitates of a yellowish hue, it means that alkaloids are present. To conduct the steroid screening, about 20 mg of extract was added to 2 ml of concentrated sulfuric acid via the test tubes' side walls in an Eppendorf tube. The presence of steroids in the *Syzygium cumini* seeds extract was verified by the dark reddish green color that developed. Twenty milligrams of extract, dissolved in forty-five percent ethanol, was used for tannin screening

in individual Eppendorf tubes. The next step was to boil the test tube for 5 minutes before adding 2 milliliters of a 15% ferric chloride solution.

Anti-oxidant activity: For antioxidant activity we followed the protocol. For this 4 mg of sample was taken in Eppendorf. 1 ml of methanol was added in 4 mg sample and vortex it. Then this solution was placed in falcon tube and in that tube 9 ml of methanol also added to make final concentration 400 µg/ml. After that, 62.5µl was transferred from the falcon tube to one Eppendorf, 125 µl was transferred to the second Eppendorf, 250 µl was transferred to the third Eppendorf, 500 µl was transferred to the fourth, and 1 ml was transferred to the fifth. To create total concentrations of 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, and 400 µg/ml, the following volumes were added from the falcon tube: 937.5 µl in an Eppendorf of 62.5 µl, 875 µl in an Eppendorf of 125 µl, 750 µl in an Eppendorf of 250 µl, and 500 µl in an Eppendorf of 500 µl. We removed 340 µl from each Eppendorf to get 660 µl volume and added 1ml DPPH solution in each Eppendorf.

Similar concentrations for standard were also prepared from ascorbic acid. Then tubes were covered with aluminum foil and placed in dark place. After then, a spectrophotometer was used to measure the solution's absorbance at 517 nm. The reference solution was DPPH, and the control was methanol. Every reagent, with the exception of the plant extract, was present in a reference solution. The antioxidant activity percentage was determined using the following formula. Antioxidant activity %age = (Ao-A1) / Ao×100

Anti-Cancer Assay: The research begins with obtaining Hep3B cells from the American Type Culture Collection in Virginia, USA. In DMEM, combined with 10% FBS and 20 µl/ml streptomycin, these cells were cultured. During the incubation period, the cultures were maintained at 37°C in a humidified chamber with 5% CO2. We started by using a 96-well plate and growing 1×106 Hep3B cells in it. After letting the cells grow in an incubator overnight, we treated them with extract from *Syzygium cumini* seeds at varying doses. The positive control group received Taxol, whereas the negative control group received unpolished media.

Three independent wells were dug in order to acquire precise results. After 100 µl of DMSO was applied, absorbance at 540 nm was measured using an ELISA reader. *Syzygium cumini* seeds were tested for their anticancer effects by comparing the absorbance of control and treated cells. In order to compare the means and standard deviations, the data was subjected to a one-way analysis of variance (ANOVA) using the statistical package for the social sciences (SPSS).

RESULTS AND DISCUSSION

Phytochemical study to determine bioactive components and anticancer potential were among the several activities of the Syzygium cumini seeds extract that were tested.

Phytochemical Analysis:

Phytochemical screening assays were performed to determine if the extract of *Syzygium cumini* seeds contained a variety of beneficial chemicals. The presence of flavonoids, alkaloids, steroids, tannins, and saponins—all of which are involved in preserving various cellular pathways in organisms—was well demonstrated by every test. Flavonoids, also known as bioflavonoids, are secondary metabolites that have a structure consisting of 15 carbon rings. They are abundant in microorganisms, plants, and animals. Flavonoids are substances that function as physiological regulators, chemical messengers, and cell cycle inhibitors. They are also engaged in several other metabolic cycles.

Flavonoids have also been found in *Syzygium cumini* seeds, much like in a number of other plants and fruits. The extract from *Syzygium cumini* seeds was analyzed to see whether flavonoids were present. The extract from *Syzygium cumini* seeds showed favorable flavonoid findings by becoming yellow in the test tube, which vanished when a few drops of sulphuric acid were added. Animals and plants both contain large amounts of a family of chemicals called saponins. These amphipathic glycosides are distinguished by the soap-like froth that forms on the utensils' surface when they are shaken in an aqueous solution. These hydrophilic moieties are structurally paired with lipophilic triterpenes or steroid derivatives.

Distilled water was used to dilute the extract from *Syzygium cumini* seeds, and the tube was agitated for approximately 15 minutes. The presence of saponins in the *Syzygium cumini* seed extract was verified by the formation of a distinct foam layer in the test tube. The substances that are physiologically active are called steroids. The four ringed chains that make up steroids are organized in a certain way. The two primary roles of steroids in the cell are as a signaling molecule and as a crucial part of the cell membrane. Animals are the primary source of steroids, while some are also obtained from plants and mushrooms.

Samples were subjected to a steroid detection test in order to identify steroids, and the dark reddish hue confirmed that the required group of phytochemicals was present in the extract from *Syzygium cumini* seeds. The family of biomolecules known as tannins combines with proteins and other substances, including amino acids, to generate precipitates. The primary source of tannins is living things. In the kingdom Plantae, tannins are found in a variety of species. for the detection of tannins in the extract of *Syzygium cumini* seeds. After dissolving the extract from *Syzygium cumini* seeds in 45% methanol and boiling them for five minutes, the color became dark green when 1 ml of FeCl3 was added, confirming the presence of tannins.

Table 1: Qualitative phytochemical screening of *Syzygium cumini* seeds extract

extract	Flavonoids	Saponins	Steroids	Tannins	Alkaloids
Syzygium cumini seeds extract by using ethanol as solvent	+	+	+	+	+

Naturally occurring compounds known as alkaloids frequently include a heterocyclic nitrogen atom. Most alkaloids are nonvolatile, crystalline, and colourless. It has been determined that alkaloids are the last product of nitrogen metabolism. The alkaloid-containing plant Syzygium cumini is only one of several. The extract from *Syzygium cumini* seeds produced yellowish precipitates in the test tube, indicating the presence of alkaloids.

Antioxidant assay:

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) test was used to assess the antioxidant capacity of Syzygium cumini seeds extract obtained by green extraction method. As a free radical, DPPH can react with different substances to release one atom of hydrogen. The ability of different antioxidants to reduce DPPH free radicals is assessed using this activity. Various concentrations, including 25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml, and 400µg/ml, were used to conduct the antioxidant activity. The DPPH free radical scavenging activity of the Syzygium cumini seeds extract was shown to be dosage dependant when compared to that of normal ascorbic acid. Syzygium cumini seeds extract has an IC50 value of 25.8µg/ml against DPPH radical, whereas the positive control, ascorbic acid, has an IC50 value of 8.5µg/ml. The qualitative estimation of antioxidants in the extract which was estimated by the IC_{50} standards, low IC_{50} shows powerful antioxidant potentials.

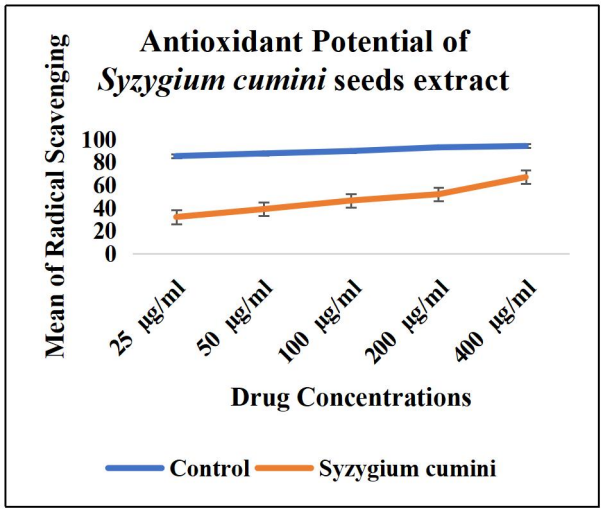

Figure 1: Antioxidant activity exhibited by *Syzygium cumini* seeds extract with reference to standard ascorbic acid.

Anti-Cancer Assay: The anticancer impact of an extract from Syzygium cumini seeds was assessed in this study. Hep3B cells are inhibited by an extract from Syzygium cumini seeds. The antitumor action against Hep3B cells was demonstrated by the extract of Syzygium cumini seeds. The Hep3B cells were treated with several doses of Syzygium cumini seeds extract (3.125, 6.25, 12.5, 25, 50, 100 µg/ml) for 24 hours to study the effect of the extract on cell growth that is dose-dependent. The extract of Syzygium cumini seeds was shown to have an inhibitory concentration IC50 of 77.4 µg/ml against Hep3B cells, as evaluated in comparison to the untreated control. Syzygium cumini seeds percentage of cell viability at a concentration of 100 µg/ml.

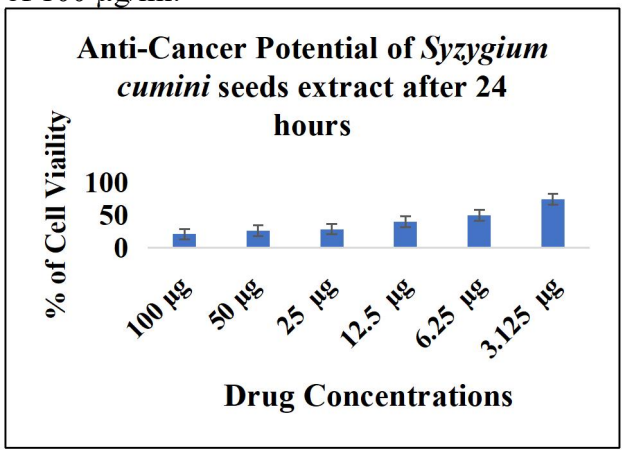

Figure 2: Anti-Cancer Potential of *Syzygium cumini* seeds extract against Hep3B cells after 24 hours treatment.

The antitumor action against Hep3B cells was demonstrated by the extract of Syzygium cumini seeds. For 48 hours, Hep3B cells were treated with several quantities of Syzygium cumini seeds extract (3.125, 6.25, 12.5, 25, 50, 100 µg/ml) to study its influence on cell growth that was dose-dependent. The extract from Syzygium cumini seeds was shown to have an inhibitory concentration IC50 of 73.05 µg/ml against Hep3B cells, as evaluated in comparison to the untreated control. This indicates that the compound inhibits cell growth. Syzygium cumini seeds percentage of cell viability at a concentration of 100 µg/ml.

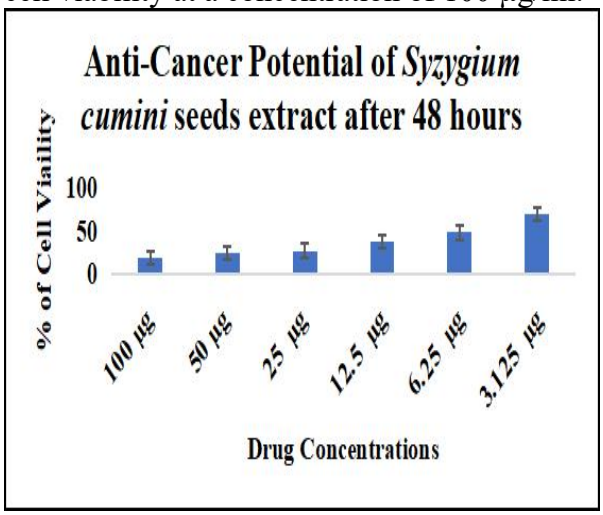

Figure 3: Anti-Cancer Potential of *Syzygium cumini* seeds extract against Hep3B cells after 48 hours treatment.

The antitumor action against Hep3B cells was demonstrated by the extract of Syzygium cumini seeds. Over the course of 72 hours, Hep3B cells were treated with varying quantities of Syzygium cumini seeds extract (3.125, 6.25, 12.5, 25, 50, 100 µg/ml) to study its influence on cell proliferation that was dependent on dosage. We assessed the inhibition in cell growth relative to the untreated control and found that the extract from Syzygium cumini seeds had an inhibitory concentration IC50 of 71 µg/ml against Hep3B cells. Syzygium cumini seeds

percentage of cell viability at a concentration of 100 µg/ml.

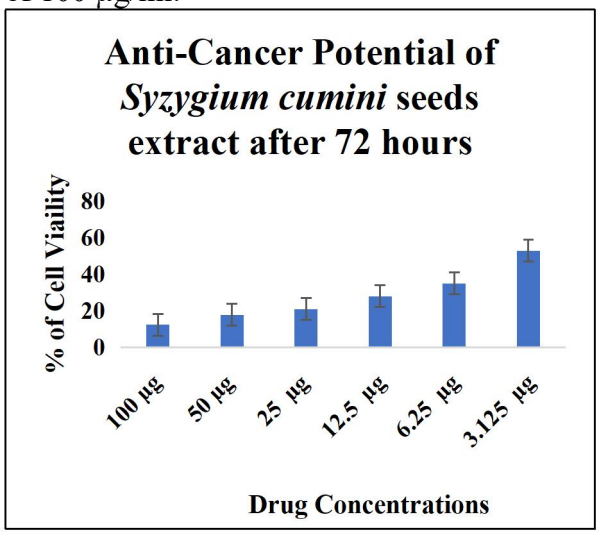

Figure 4: Anti-Cancer Potential of *Syzygium cumini* seeds extract against Hep3B cells after 72 hours treatment.

The Cell Culture experiment revealed the percentage of Hep3B cells that were viable following treatment with extract from Syzygium cumini seeds at doses of 3.125, 6.25, 12.5, 25, 50, and 100 µg/ml for 24, 48, and 72 hours. At 24, 48, and 72 hours, the inhibitory concentration IC50 of the Syzygium cumini seeds extract against Hep3B cell lines was determined to be 77.4, 73.05, and 71. µg/ml, respectively, when compared to the untreated control group.

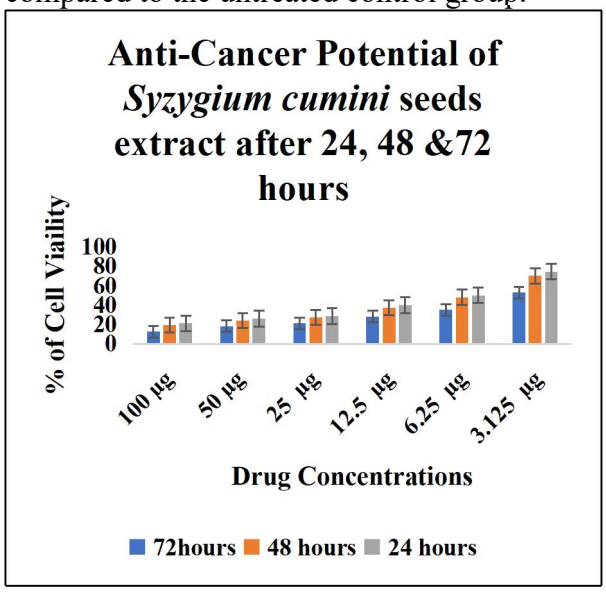

Figure 5: Comparison of Anti-**Cancer Potential of *Syzygium cumini* seeds extract** against Hep3B cells at different concentrations **after 24, 48 &72 hours treatment.**

Conclusion:

This study concluded that the extract from *Syzygium cumini* seeds has a rich profile of bioactive substances, such as tannins, alkaloids, flavonoids, saponins, and steroids. These phytochemicals support a number of biological processes and are well-known for their potential as medicines. In a dose-and time-dependent manner, the extract demonstrated strong anticancer activity against Hep3B liver cancer cells. With longer exposure times, the IC_{50} values gradually dropped, suggesting that longer therapy was more effective. The findings point to the potential of *Syzygium cumini* seeds as a natural anticancer drug by indicating that their extract may successfully lower cancer cell viability. To properly confirm its therapeutic potential, more research on the mechanisms of action and in vivo effectiveness is advised.

Future Recommendations:

Future studies should concentrate on identifying and separating the precise bioactive substances that give *Syzygium cumini* seed extract its anticancer properties. To comprehend the molecular processes underlying its anticancer effect, in-depth mechanistic research is required. Clinical trials and in vivo research are also necessary to confirm its effectiveness and safety in people. Its therapeutic potential might be further increased by investigating synergistic effects with currently available chemotherapeutic drugs. Researching other cancer cell lines might possibly uncover more extensive anticancer uses.

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