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# Isolation of a phenolic component from Syzygium cumini under bioassay direction: Evaluation of antidiabetic potential.

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## ABSTRACT

Syzygium cumini, popularly known as Java plum or black jamun, is a natural plant in tropical and subtropical climates that has several medicinal uses. All components of the plant, such as the seeds, leaves, and wood, contain several beneficial phytoconstituents, such as glucosides, anthocyanins, steroids, phenols, flavonoids, and terpenoids. This study employed an in vitro α-amylase assay-guided technique to isolate a phenolic compound from the seed extract of S. cumini. After the extract was screened for phytochemicals, its total phenolic content (TPC) and total flavonoid content (TFC) were assessed. We investigated the extract's fractions F1–F9 to see if they might stop α-amylase. We employed FT-IR, 1H-NMR, 13C-NMR, and mass spectrometry to describe the pure molecule (called compound 1) that we got by recrystallizing the fraction from water with the lowest $IC_{50}$ (the most α-amylase inhibition). After that, rats with diabetes caused by streptozotocin were utilized to see if the isolated chemical had any antidiabetic properties. Fractions F2, F5, and F7 all had $IC_{50}$ values of 75.28, 131.25, and 192.76 µg/mL, respectively. They all showed substantial α-amylase inhibition and were seen as good choices for isolating the active ingredient. We used Fraction F2 to separate compound 1 because it had the lowest $IC_{50}$. During days 7 to 28 of the trial, the treatment of either compound 1 or glibenclamide significantly lowered blood glucose levels. In conclusion, this work demonstrates that the compound isolated from S. cumini seeds had the potential for application as an antidiabetic agent, particularly in the management of type 2 diabetes.

***Keywords:*** *fraction, Syzygium cumini, ferulic acid, α-amylase, antidiabetic, and streptozotocin*

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## 1. INTRODUCTION

Syzygium cumini (L.) Skeels, often known as Java plum or black jamun, is an important medicinal plant that grows in tropical and subtropical areas of the world. It can live for more than a hundred years and grow fast to a height of 30 meters or more. S. cumini is seen to be important for the economy because it has a lot of medical and commercial value in various fields. People use the seeds, leaves, and wood of this plant because it has a lot of phytochemicals and is a good source of antioxidants. Some of the important phytoconstituents in S. cumini are glucosides, anthocyanins, steroids, phenols, flavonoids, and terpenoids. The fruit is also rich in vitamins, minerals, and carbs. Its pulp has a lot of manganese, calcium, potassium, iron, zinc, and salt. S. cumini has a lot of pharmacological activity, including chemoprotective, analgesic, hypoglycemic, anti-inflammatory, anti-allergic, antioxidant, antihyperglycemic, antiplaque, astringent, antimicrobial, gastroprotective, antidiarrheal, and antibacterial activities. The seeds in the middle of the jamun fruit are 1 to 2 cm long and have a slightly bitter taste. The nutritional and phytochemical composition of jamun fruit may vary based on its maturity, origin, agricultural methods, and post-harvest processing. S. cumini has attracted considerable research interest owing to its therapeutic significance, particularly in conventional antidiabetic therapies. Nevertheless, the specific bioactive constituents responsible for its antidiabetic effects must be identified and extracted. This work aimed to find a phenolic

compound from S. cumini seeds using a bioassay-guided approach and evaluate its potential for diabetes treatment. [4, 5, 6]

## 2. MATERIALS AND METHODS

### Total Phenolic Content, Phytochemical Screening, and Extraction

The Syzygium cumini seeds came from DKC Agrotech Pvt. Ltd. and were checked at BSI-Prayagraj. The dried seeds were roughly powdered after being defatted with petroleum ether. Then, they were extracted with water, ethanol, chloroform, and petroleum ether in that sequence of polarity. The extraction yields for petroleum ether, chloroform, ethanol, and water were 3.04%, 1.18%, 10.22%, and 7.37%, respectively. To identify the primary classes of compounds, each extract underwent an initial phytochemical screening. The petroleum ether and chloroform extracts showed that alkaloids, phenolics, and sterols were present. The ethanol and aqueous extracts, on the other hand, worked well for alkaloids, phenolics, glycosides, and flavonoids. We assessed the total flavonoid content (TFC) and total phenolic content (TPC) of each extract. The aluminum chloride colorimetric method was used to find TFC, which was then expressed as quercetin equivalents (QE) per gram. The Folin–Ciocalteu reagent was used to find TPC, which was then expressed as gallic acid equivalents (GAE) per gram of extract. The ethanol extract had the highest TPC and TFC, with values of 197.8 mg GAE/g and 118.5 mg QE/g, respectively. Figure 1 shows that all of the other extracts had lower TPC and TFC values than the ethanolic extract.

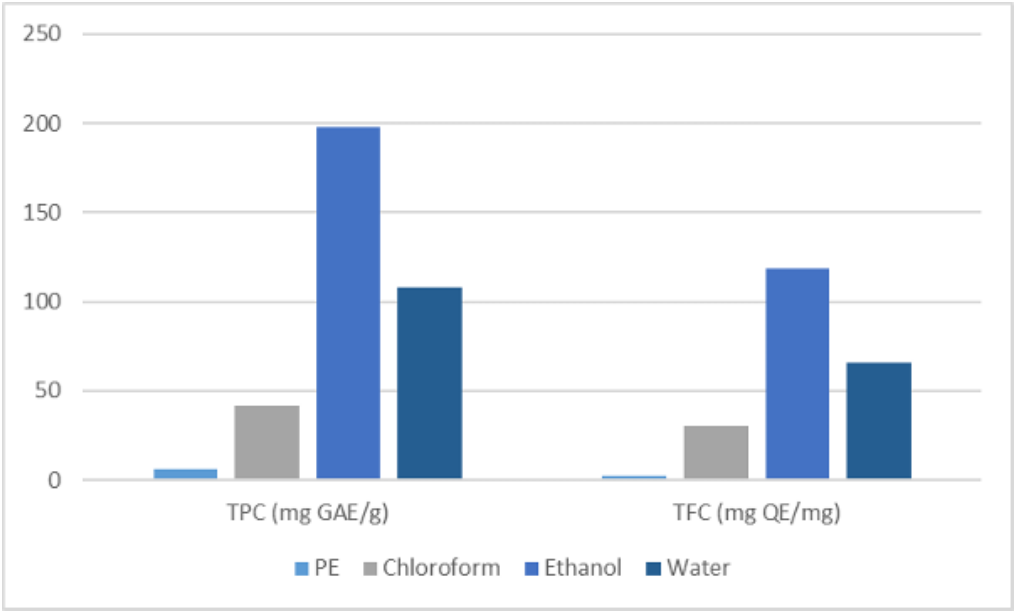


***Figure 1.*** **Displays the concentrations of phenolics and flavonoids in S. cumini extracts.**

### Fractionation and Isolation of Bioactives

We used a bioassay to separate the active component, which was the ethanolic extract with the most phenolic and flavonoid concentration. Preliminary thin-layer chromatography (TLC) of the ethanol extract in chloroform:methanol (9:1) revealed three distinct components. We used column chromatography on silica gel (60–120 mesh) to separate the extract into nine fractions (F1–F9). We did this by starting with 100% chloroform and then adding 5% methanol at a time. To identify the bioactive fraction, all fractions were concentrated and assessed for α-amylase inhibitory activity in vitro (as elaborated below). [12, 14] [6]

### Experimental Design

Prior to the experiment, the healthy adult male Wistar albino rats (190-200 g,) underwent a one-week acclimatization period at the animal facility of BHABHA University, Bhopal. The study was approved by the Institutional Animal Ethics Committee (BPRI/CPCSEA/24/26), dated (15/04/2024) and all procedures adhered to CPCSEA guidelines. One intraperitoneal dose of streptozotocin (STZ; 55 mg/kg in cold 0.1 M citrate buffer, pH 4.5) made the person diabetic. Rats were classified as diabetic and included in the study if their fasting blood glucose levels exceeded 250 mg/dL after seven days. There were five groups of rats, each with six rats: Group I had normal control (0.9%), diabetic control (STZ only), diabetic + compound 1 (100 mg/kg), diabetic + compound 1 (200 mg/kg), and diabetic + glibenclamide (2.5 mg/kg). For 28 days, participants orally ingested Compound 1 and glibenclamide daily, suspended in warm 0.9% saline (vehicle) [5, 9]

**Statistical Analysis**

The data are shown as the mean ± SEM. We utilized GraphPad Prism software to do statistical comparisons with the Student's t-test and one-way ANOVA. Differences were considered significant at $P < 0.05$.

## 3. RESULTS

After removing the fat with petroleum ether, the S. cumini seed powder was extracted using solvents with higher polarity. The yields for petroleum ether, water extracts, ethanol, and chloroform were 3.04%, 1.18%, 10.22%, and 7.37%, respectively. Phytochemical screening showed that the petroleum ether and chloroform extracts contained alkaloids, phenolics, and sterols. The ethanol extract, on the other hand, contained alkaloids, phenolics, glycosides, and flavonoids. The aqueous extract contained sterols, glycosides, phenolics, and alkaloids. Quantitative analysis showed that the ethanol extract had the highest total phenolic and flavonoid contents (197.8 mg GAE/g and 118.5 mg QE/g, respectively). The TPC and TFC values of the other extracts were lower. As a result, the ethanol extract was chosen for further separation (Figure 1).

**Separation and isolation:** Bioassay-guided fractionation was used to isolate the active antidiabetic part from the ethanol extract. We tested the α-amylase inhibitory activity of fractions F1–F9, which were made via column chromatography, in vitro. Figure 2 shows how much each fraction at 1 mg/mL stops α-amylase from working. The $IC_{50}$ values for fractions F2, F5, and F7 were 75.28, 131.25, and 192.76 µg/mL, respectively. This means that the enzymes were strongly inhibited. Consequently, these fractions were demonstrated to be effective for isolating active components. We chose Fraction F2 for more processing since it had the highest activity (lowest $IC_{50}$) of all the fractions. To characterize it, a pure phenolic compound (compound 1) was made by recrystallizing fraction F2 from water.

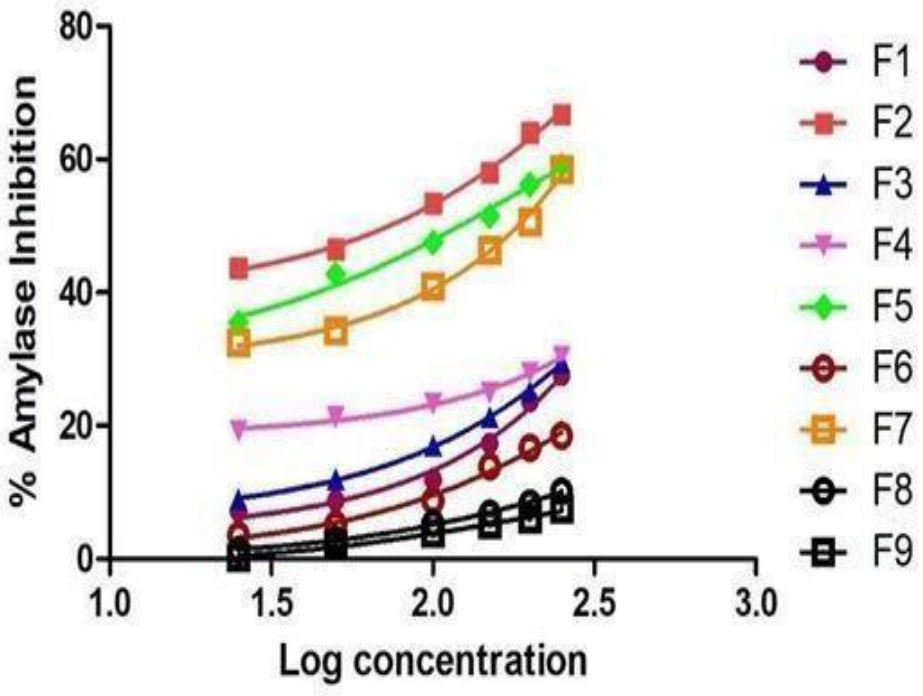


***Figure 2.*** **depicts the percentage of α-amylase inhibition induced by S. cumini fractions F1–F9 at a concentration of 1 mg/mL.**

The antidiabetic study used doses of 100 mg/kg and 200 mg/kg of compound 1 because acute toxicity tests indicated no deaths or other bad effects up to 2000 mg/kg. Both 100 and 200 mg/kg of Compound 1 and 2.5 mg/kg of glibenclamide effectively lowered blood glucose levels in streptozotocin-diabetic rats compared to the diabetic control. The hypoglycemic effect was evident by day seven of treatment and persisted through days fourteen, twenty-one, and twenty-eight (Figure 3).

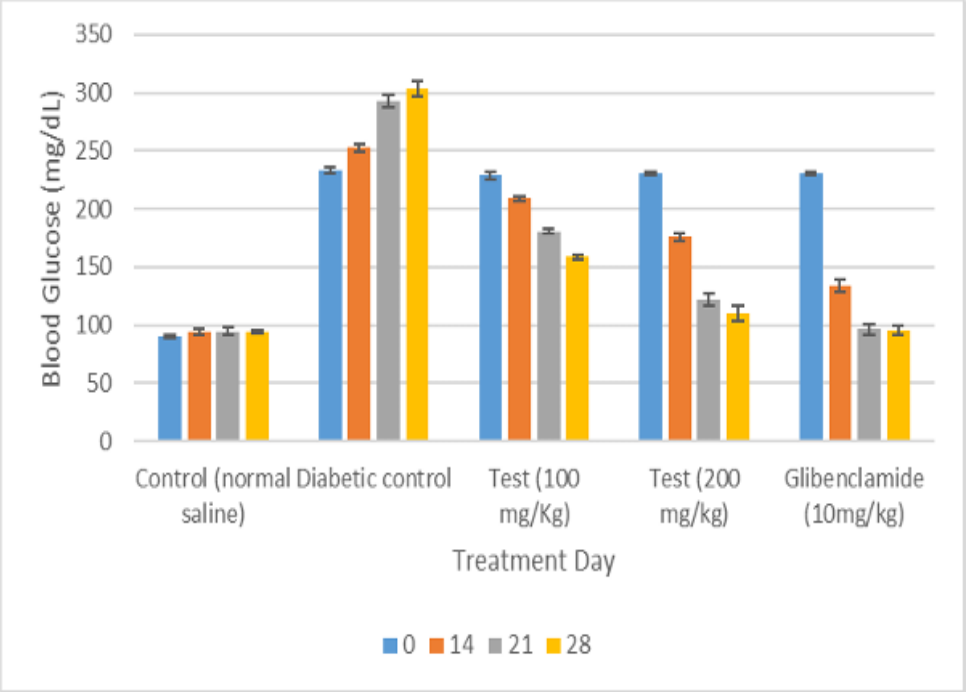


***Figure 3.*** **The effect of Compound 1 on blood glucose levels in streptozotocin-induced diabetic rats over 28 days. The mean ± SEM is the same for each group of six values.**

## 4. DISCUSSION

Alternative medicine systems have been more important in the last few years for treating long-term ailments like diabetes. Flavonoids are a type of naturally occurring polyphenolic chemical that is abundant in the human diet. They have many biological effects, including being antioxidants, anti-inflammatory, antidiabetic, and antibacterial. Due to these benefits, flavonoids and other phenolics may be good choices for treating diabetes. Given this, the present study aimed to isolate a bioactive phenolic compound from S. cumini seeds and evaluate its potential as an antidiabetic agent. As expected, the ethanolic seed extract of S. cumini had the highest levels of phenolics and flavonoids among the solvent extracts. This aligns with previous studies indicating that phenolic compounds significantly contribute to the antidiabetic effects of plant extracts. Using a bioassay-guided fractionation method, we found an active fraction (F2) with significant α-amylase inhibitory activity. From this fraction, we were able to extract a bioactive molecule.

09 cm^−1 (C=O stretch), 1604 cm^−1 (aromatic C=C stretch), and 1512 cm^−1 (aromatic ring vibration). The fact that these spectroscopic properties are the same as those of ferulic acid proves that chemical 1 is a derivative of ferulic acid. In vitro α-amylase inhibition is a well-known way to test for diabetes since it lowers blood sugar levels after meals. Our in vivo results demonstrated that compound 1 had efficacy comparable to the widely used medicine glibenclamide, significantly reducing blood glucose levels in diabetic rats. This research indicates that the phenolic compound derived from S. cumini seeds contributes to the plant's antidiabetic properties, presumably by inhibiting enzymes responsible for carbohydrate degradation, hence enhancing glycemic control. The therapeutic potential of jamun seed constituents is substantiated by the significant parallels in enzyme inhibitory effects observed in both in vitro and in vivo studies of S. cumini extracts in prior research.

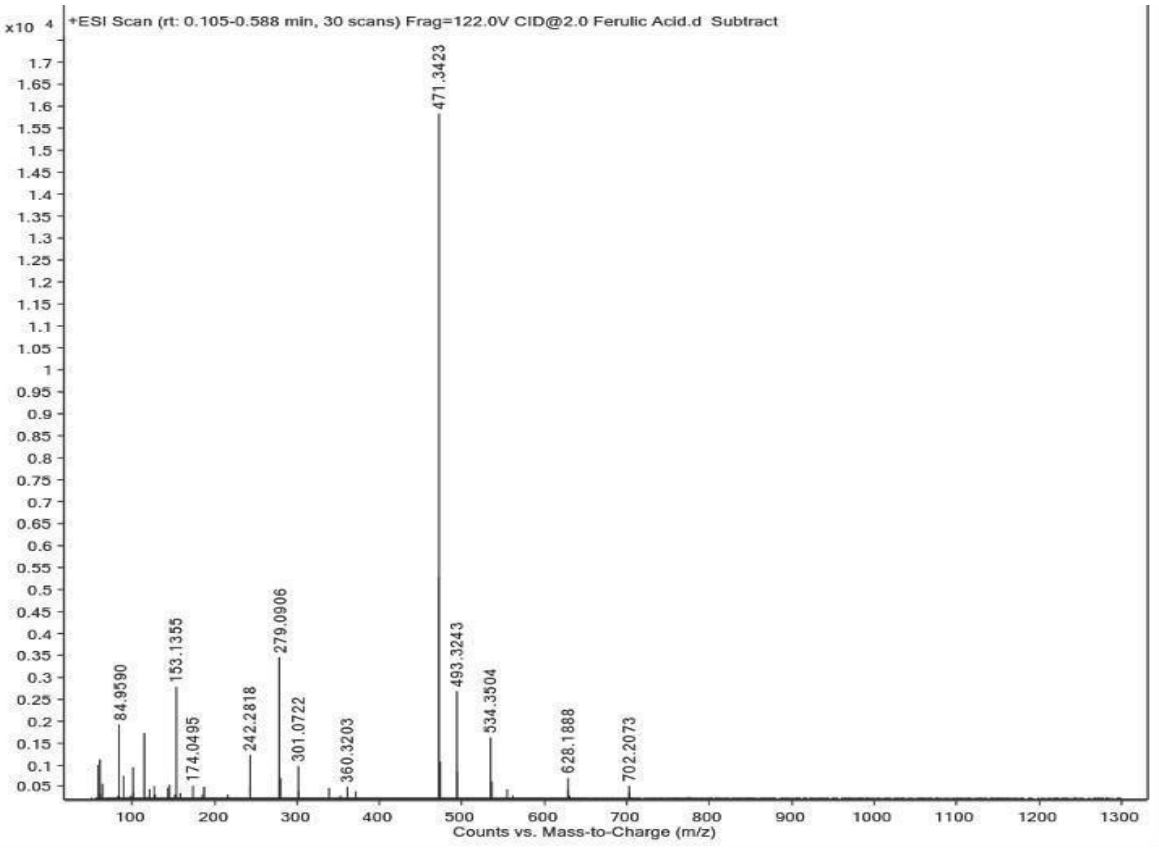


***Figure 4.*** **Compound 1's mass spectrum (isolated from S. cumini seeds) is illustrated**

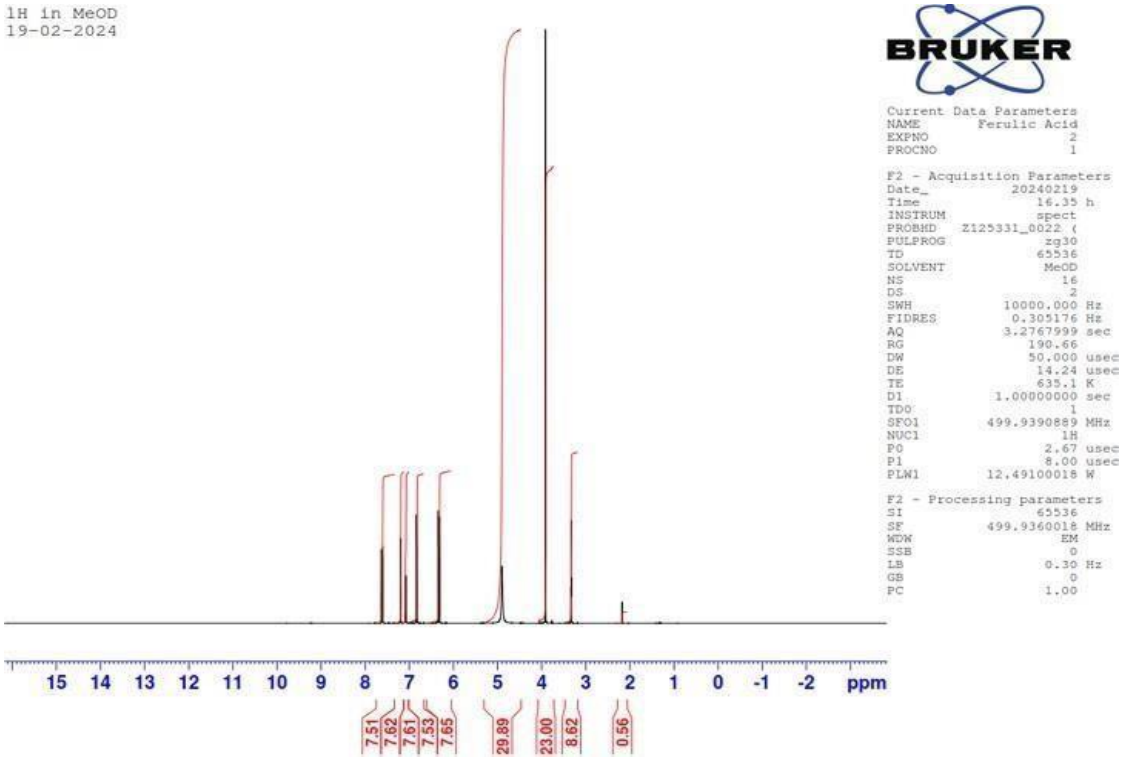


***Figure 5.*** **shows the 1H-NMR spectra of Compound 1.**

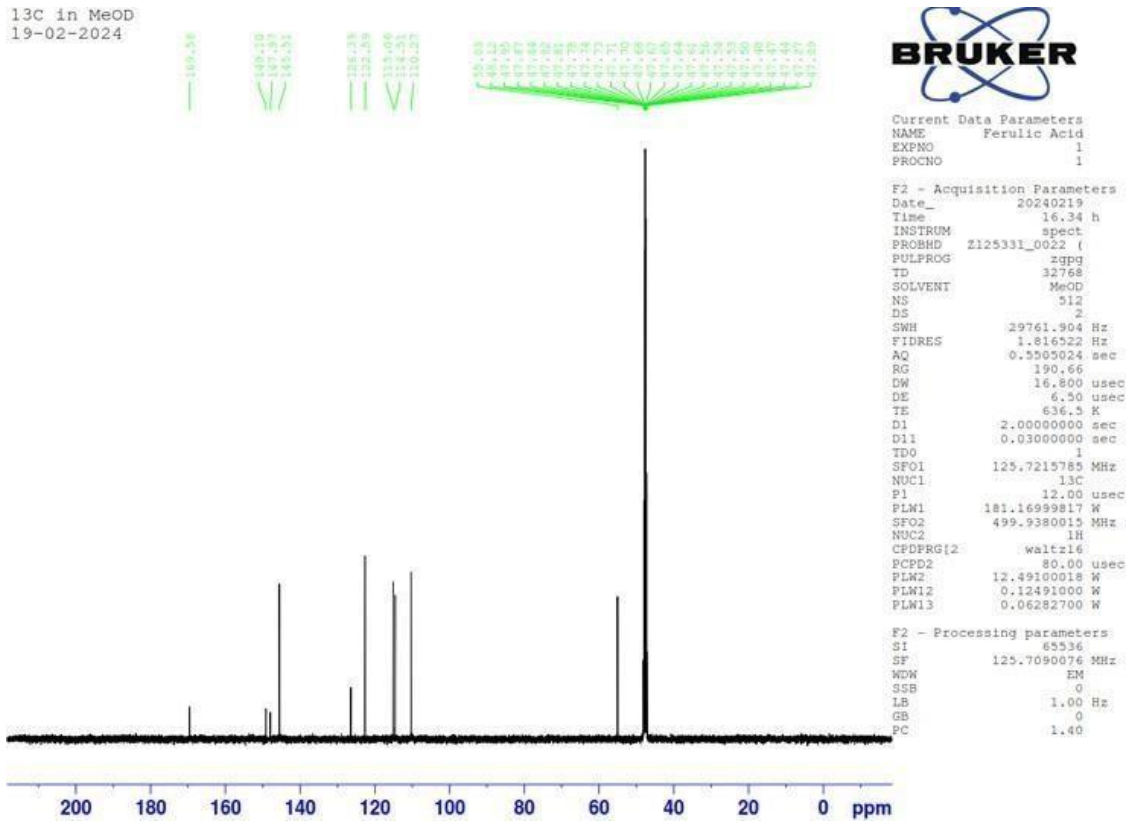


***Figure 6.*** **The ^13C-NMR spectra of Compound 1.**

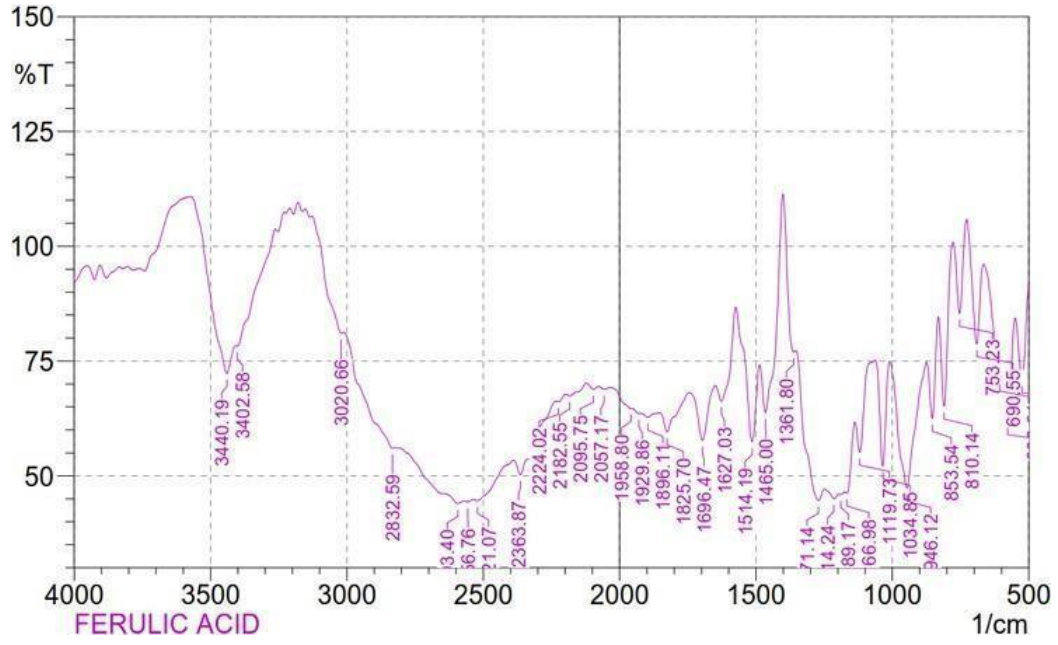


***Figure 7.*** **shows the FTIR spectrum of Compound 1.**

## 5. CONCLUSION

Using a bioassay-guided methodology, we were able to successfully remove a ferulic acid derivative (compound 1) from

the ethanolic extract of S. cumini seeds in this work and examined its potential as an antidiabetic. The results show that the isolated chemical has a strong antidiabetic impact since it is a derivative of ferulic acid. The findings indicate that S. cumini seeds possess bioactive phenolic compounds that warrant further investigation for potential application as therapeutic agents in diabetes management..

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