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Syzygium cumini Seed Extraction: Its Optimization and Characterization of Ellagic Acid for Antimicrobial Activity

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Abstract

A popular traditional medicinal herb is *Syzygium cumini*. Its therapeutic qualities are a result of the plant's phytochemical components. Study materials included *S. cumini* (Magnoliopsida: Myrtaceae) seeds, ethanol, and crude methanol extracts. The extract's antimicrobial qualities against common bacterial strains were evaluated in this agar well using diffusion. Flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenes, cardiac glycosides, and tannins are among the chemical classes that have been found in seed extracts through phytochemical analysis. Clear inhibitory effects of the extracts against clinical isolates are shown. Gram-negative and Gram-positive bacteria include *Staphylococcus aureus* and *Salmonella typhi*. It was found that the methanolic extract of the seed exhibited more antibacterial activity compared to the extracts obtained from the leaves and bark. The current study supports the use of several *S. cumini* seed extract as effective antimicrobial agents for the environmentally friendly and sustainable management of a variety of bacterial strains, but more research is needed before they can be applied in the field.

Keywords

Syzygium cumini, Antibacterial activity, Seed extracts, Response surface methodology

Introduction

S. cumini is sometimes referred to as jamun, jaman, jambul, jambolan, black plum, Java plum, and Malabar plum. A tropical evergreen tree that belongs to the Myrtaceae family of flowering plants, it is prized for its fruit, lumber, and aesthetic appeal. It is native to the Indian subcontinent and South-east Asia, including Bangladesh, Myanmar, Sri Lanka, and the Andaman Islands. It can grow up to 30 meters (98 feet) in height and has a lifespan of over a century. It is a quickly spreading plant that is regarded as invasive in many global locations. Sometimes the fruit's name, black plum, is mistranslated as blackberry, which is a distinct species and order. Australia, Hong Kong, islands in the Pacific and Indian Oceans, and other places have been exposed to *S. cumini* [1].

When young, the turpentine-smelling leaves are pinkish; as they age, they turn a leathery, glossy dark green with a yellow midrib. Due of their high nutritional content, the leaves are fed to cattle [1].

Between March and April, *S. cumini* trees begin to bloom. The flowers are tiny, fragrant, and have a diameter of 5 mm (0.2 inch). The *Syzygium* species' fruit is referred to as "drupaceous" and matures around May or June, resembling huge berries. The fruit is ovoid and oblong. Green fruit is unripe fruit. As it gets older, it turns pink, then gleaming crimson red, and finally black. White fruit

is produced by a variety of the tree. The fruit tends to turn the tongue purple and has a flavor profile of sweet, somewhat acidic, and astringent [1].

Blackberry, which is a distinct fruit in a different sequence, is sometimes transcribed wrongly when the actual term for the fruit is black plum. In addition to Australia, Hong Kong, and other locations, *S. cumini* has been introduced to islands in the Pacific and Indian Oceans. Most developed and developing countries use traditional medicine, which makes use of materials derived from medicinal plants, to treat a variety of illnesses. Research was done on the plants to learn more about their properties, chemical makeup, safety, and efficacy against toxins that cause illness and disease.

To conduct pharmacological research and create new drugs, medicinal plants from various locations were studied. Common wild medicinal plants were widely employed as do-it-yourself treatments and identified as sources of components for the pharmaceutical industry.

Materials and Methods

Materials

During the research, substances of the analytical grade are used. *S. cumini* fruits were collected and then washed with water later seeds were separated and dried. The fruits were collected in Vishakhapatnam (Andhra Pradesh, India). The taxonomic taxonomy of the plant has been confirmed. Double-distilled water has been used throughout the study.

Plant processing

The plant material was washed with tap water, rinsed in distilled water, dried in the sun for two to three days, and then powdered to be used. Before being used, the powder was stored in an airtight.

Ellagic acid quantitative measurement

Ellagic acid content was estimated using a UV-Visible spectrophotometer in comparison to a standard ellagic acid solution. In a set of five 25 ml volumetric flasks, standard solutions of ellagic acid were pipetted into concentrations ranging from 5 to 30 g/ml. At 280 nm, the ellagic acid's absorbance was calculated in comparison to methanol.

Preliminary extraction studies

To choose a suitable solvent, preliminary extraction experiments are conducted using methanol, acetone, and water as test solvents. The methanol solvent was added to a conical flask along with 1 g of the prepared fruit pulp powder. After being diluted to 50 ml, the solution was filtered using Whatman No. 1 filter paper after being allowed to soak for some time. Similar steps were taken with the remaining solvents. The extraction samples were determined using a UV-Visible spectrophotometer.

Response surface methodology (RSM)

One such statistical and mathematical methodological combination is RSM. RSM can be used to concurrently build and solve multivariate equations by assessing quantitative data from analytical experiments along with their interaction terms. RSM is based on a polynomial equation that can be used to forecast and describe the ideal conditions for experiments, as well as to model the relationship between the independent and dependent elements. The Box-Behnken design (BBD) is a well-known RSM design that enhances the extraction of bioactive compounds. The BBD, which consists of rotating lower-dimensional designs, is used to estimate all two-way, quadratic, and linear interactions. It forbids diminution. There are no corners in their domain. The axial points outside the created design space box of the circular factorial design element are contained. This enables one to approximate the predicted reaction with the same variance regardless of the distance from the centre of the design area. Stat-Ease Design Expert Software version 12 was used to forecast the extract yield (response) using a 2^{nd} order polynomial equation [2-7].

Antimicrobial activity

The cold percolation was used to create a methanolic extract of the plant material. The same volume of solvent was used to soak various amounts of dry powder about 24 h at 37 °C with constant at 150 rpm.

The diffusion and dilution methods are the two ways to perform an antimicrobial susceptibility test. The agar well diffusion method is widely used to assess the antibacterial activity of plant or microbiological extracts. The entire surface of the agar plate is inoculated by spreading a quantity of microbial inoculums. The extract solution or antimicrobial agent is then added to the well after it has been aseptically pierced using a sterile cork borer or tip to create a hole punch with a diameter of 6 to 8 mm.

Depending on the test microorganism, the appropriate agar plates are subsequently incubated, and an evaluation is conducted on the inhibiting growth zones' diameter. Next, 37 °C is maintained overnight for incubation.

Results and Discussion

Extraction studies

In order to extract the bioactive components, the extraction solvent must be carefully chosen. Three different solvents, namely water, acetone, and methanol, were used in the experiments. *S. cumini* seed extract powder yields a good amount of catechin when methanol is employed as the solvent. The solvent for the subsequent extraction experiments was methanol. The trials were conducted using the optimisation method known as one-factor-at-a-time. Catechins were extracted using methanol solutions.

The trials' findings showed that with 80% ethanol, the maximum ellagic acid extraction was 44%. The highest ellagic acid extraction, of 58.32%, was reached at pH 7, according to research done to ascertain the effect of pH on extraction. Different plant powder sizes (354, 328, 250, and 205 m) were used for extraction. The highest percentage extraction of 82%

was reported at 205 m. With 80% methanol and a particle size of 205 m, and it was soaked for one, two, and three days at pH 4 and the results showed that the maximum extraction was achieved after 2 days at 94.5%. Figure 1 provides a visual representation of the extraction process.

RSM

To explore the main and interaction effects of these three independent variables on catechin extraction.

Final equation in terms of actual factors

% Extraction of ellagic acid = -52.66125 0.165875 Time 17.21 pH 1.22488 Temp 9.65E-17 Time x pH 0.000025 Time x Temp 9.13E-17 pH x Temp - 0.00139 Time^2 - 1.22875 pH^2 - 0.012263 Temp^2

For certain levels of each element, the reaction can be predicted using the equation described in terms of the real factors. In this case, the levels of every constituent should be stated in their original units. Because the intercept is not in the centre of the design space and the coefficients are scaled to account for the units of each element, this equation should not be used to calculate the relative influence of each factor.

Fit statistics

A fair agreement is shown by the difference between the adjusted R^2 of 0.9999 and the predicted R^2 of 0.9996, which is less than 0.2. Adeq precision determines the signal-to-noise ratio. The ratio ought to be more than 4. You have a sufficient signal strength with a ratio of 395.107. This model can be used to navigate the design area (Table 1).

Response surface plots and interaction of the ellagic acid extraction parameters

It was feasible to observe how several factors, such as time, pH, and methanol concentration, affected the extract yield by applying a 3D response plot to the experimental data. To optimize energy savings and extraction performance, the extraction length was adjusted in this study. Figure 2 show how longer extraction times produce a higher total yield.

Figure 1: Showing image of *S. cumini* fruits.

Furthermore, the plots showed that the overall extraction yield remained largely constant when the extraction duration was extended over a 40-h period. Figures 2 show how pH has a major effect on extraction. Solvent concentration is a crucial factor that also impacts extraction efficiency. When the percentage of methanol was assessed, a statistically significant linear effect was seen. It was found that the optimal concentration of catechin was 80% methanol. It has been shown that an 80% methanol content works well.

Optimization

The goal of this study's optimization component is to maximise the response. The components needed to attain the greatest outcomes for this objective are selected by the Design Expert software. The optimal conditions of 80% methanol, 62 h of extraction time, and 50 °C resulted in a peak ellagic acid extraction of 82%.

Effect of different solvent percentages on ellagic acid and gallic acid

Different solvents can be used to optimise the extraction of flavonoids, methanol showing high number of percentages. Different solvents are ethanol, methanol, ethyl acetate and water. The results can be seen in figure 3.

Effects of different solvent percentages on ellagic acid

It has been found that a mono-component solvent system is less efficient in extracting phenolic compounds than a mixture of methanol and various water amounts. A small

Table 1: Fit statistics.

Std. dev.	0.0082
Mean	41.47
C.V.%	0.0199
R^2	1
Adjusted R^2	0.9999
Predicted R^2	0.9996
Adeq precision	395.107

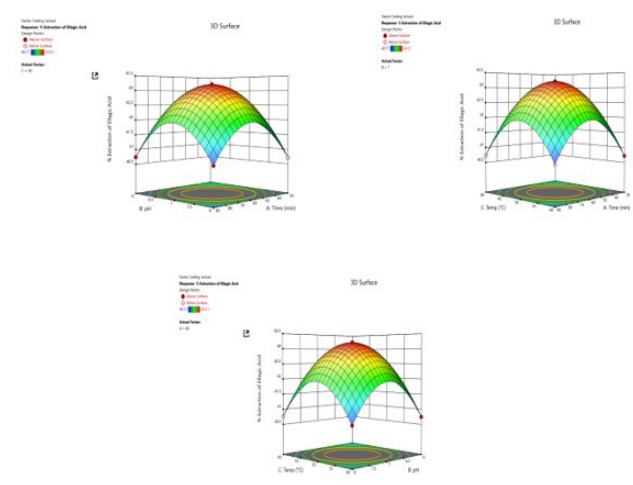

Figure 2: Images of RSM.

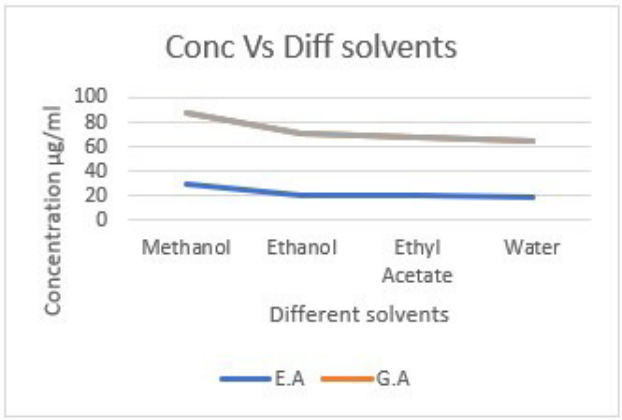

Figure 3: Effects of different solvent percentages on ellagic acid and gallic acid.

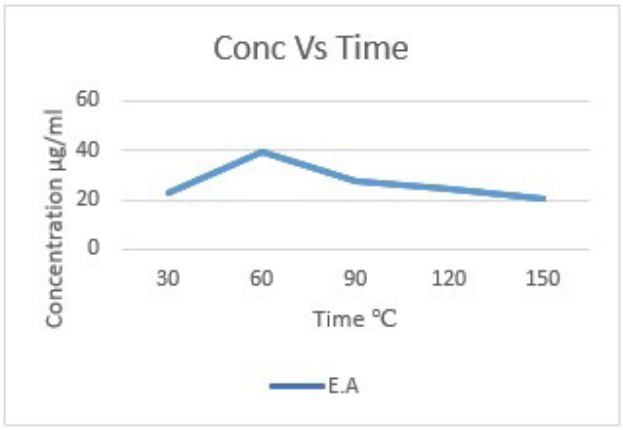

Figure 5: Extraction time.

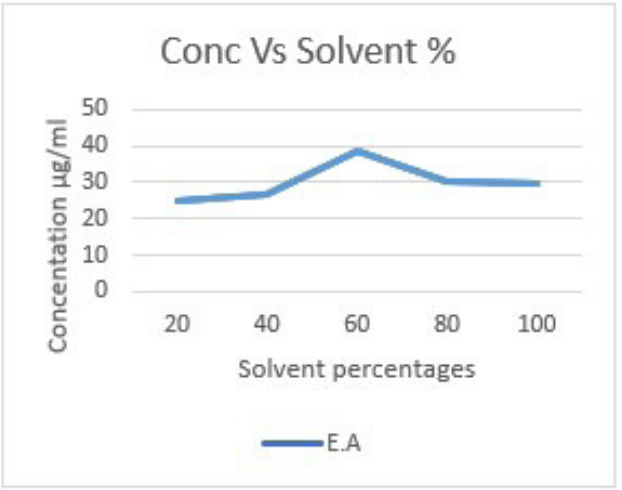

Figure 4: Effects of different solvent percentages on ellagic acid.

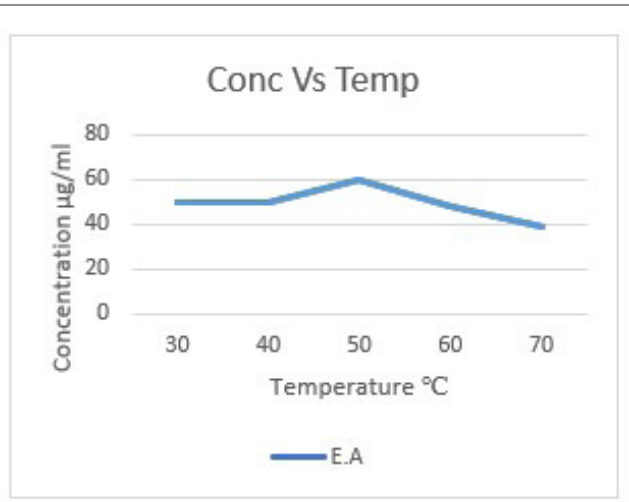

Figure 6: Effect of temperature on ellagic acid extraction.

amount of water is generally added to an organic solvent to increase its polarity. As a result, polyphenol extraction is made simpler when using methanol at different concentrations (20%, 40%, 60%, 80%, and 100%) (Figure 4).

Ellagic acid is affected by the length of soaking time

For the greatest amount of flavonoid extraction, the soaking period must be carefully evaluated. Depending on how much was extracted of the flavonoids, this variable can have a value between a few seconds and 24 h. For 30, 60, 90, 120, and 150 min, respectively, the solvent was in contact with the powder. The chart below displays an estimation of the extraction time (Figure 5).

Effect of temperature on ellagic acid extraction

Temperature has an impact on how much methanolic chemicals are extracted. The solubility and diffusion rate of the separated components both increase with temperature. It is one of several significant physiologically active compounds that are susceptible to destruction by high heat. To achieve the maximum yield, kaempferol was extracted at a variety of temperatures ranges from 30 °C to 70 °C (Figure 6).

Antimicrobial activity

Cultures of test bacteria were equally disseminated throughout the Mueller-Hinton Agar plate surface using sterile cotton swabs. Initial evaluation of the compounds under investigation's antibacterial activity was conducted using the agar well diffusion method. The reference antibiotics was streptomycin (30 µg). Compounds, antibiotics, and dimethyl sulfoxide were allowed to diffuse for one hour at room temperature. After that, lids were placed on each dish, and they were all incubated for 24 h at 37 °C. Plates were checked for a zone of bacterial growth inhibition after incubation. The antibacterial activity of the substances was expressed as the average diameter of the inhibition zone measured in mm, and the inhibition zones' sizes were evaluated. Compounds deemed non-active were those that did not display an inhibition zone, defined as an inhibition zone diameter of less than 6 mm. The inhibitory zone diameters of each chemical were measured in triplicate using two separate experiments, and the means were recorded (Figure 7 and table 2).

Conclusions

Human health has greatly improved with plant-based pharmaceuticals, and this has encouraged the creation of new therapeutic substances. The results of the present investigation suggest that ellagic acid produced from the seed of *S. cumi-*

Table 2: Antimicrobial activity.

S. No	Plant part	Zone of inhibition (mm)							
		S. aureus				*S. typhi*			
		100 µg	250 µg	500 µg	1000 µg	100 µg	250 µg	500 µg	1000 µg
1	*S. cumini*	0	0	0	8	12	19	26	34
2	Streptomycin (30 µg)	34				32			

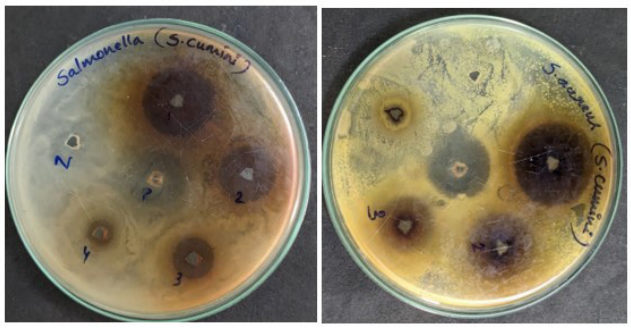

Figure 7: Antimicrobial activity.

ni seed could be used in pharmacology. The following are the study's main conclusions:

- The extraction solvent is methanol.
- Ellagic acid was produced, according to RSM-BBD research, at a temperature of 50 °C, a period of 60 h, and a methanol concentration of 80%. The optimal extract yield variables were correctly predicted in the RSM.
- Against microbes, the extract had exceptionally potent antibacterial action.

Acknowledgments

None.

Conflict of Interest

None.

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