RESEARCH PAPER

Comprehensive Phytochemical Profiling and Chromatographic Standardization of Sequential Solvent Extracts of *Syzygium cumini* Fruits

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ABSTRACT

The given work was done to perform an analytical review and phytochemical standardization of the solvent-based and chromatographic Syzygium cumini fruit extracts. The extraction of fruits using different solvents of varying polarity was repeated in order to get different extract fractions. To measure the changes in phytochemical distribution, extracts were analysed in terms of percentage yield, the preliminary phytochemical composition, total phenolic content (TPC) and total flavonoid content (TFC). Quantitative estimation indicated that polar extracts and more so ethanol and aqueous portions had very high contents of phenolics and flavonoids, thus this showed that polyphenolic constituents were extracted successfully. It was observed that phenolics, flavonoids, tannins or saponins were the most abundant phytochemicals in polar extracts with little or no phytochemicals in non-polar extracts. The HPTLC/HPLC-based chromatographic fingerprinting was able to produce repeatable chemical profiles and prepare key marker compounds identifiable and quantifiable. Major phenolic markers included gallic acid, ellagic acid and anthocyanins where anthocyanins was the most abundant in the quantified compounds. Combination of solvent-based extraction, spectrophotometric estimation, and chromatographic fingerprinting has provided a dependable analysis system of quality analysis of Syzygium cumini fruit extracts. Altogether, the results emphasize the significance of the standardization of phytochemicals through the usage of markers to guarantee the quality, consistency, and reproducibility of herbal raw materials and formulations.

Keywords: *Syzygium cumini; phytochemical standardization; total phenolic content; total flavonoid content; chromatographic fingerprinting*

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1. INTRODUCTION

1.1 Importance of Phytochemical Standardization

The standardization of phytochemicals is an essential requirement to the assurance of batch-to-batch consistency in herbal preparation. It helps in the preservation of consistent therapeutic efficacy and safety in the plant-based products. Standardization helps decrease the variations caused by geographical origin, harvesting time, and processing conditions through the ability of the profiling and measurement of active phytoconstituents of the bioactivity. Standardization is becoming one of the most important requirements of the quality assurance of herbal drugs by regulatory bodies. Without standardization, pharmacological outcomes are questionable when it comes to their reproducibility. The scientific validation of the traditional medicaments rests upon the analytical profiling; therefore, phytochemical standardization will play a very important nexus role between the conventional knowledge and the modern evidence-based practice [1-3].

1.2 Dilemma of Herbal Reproducibility

Reproducing herbal preparations is hampered by natural differences in the composition of plants. Climate, soil features, altitudes, and seasonal variations are also variables that have a significant effect on phytochemical profiles. Also, the variation of the solvent polarity and protocols of extraction also alters the extract composition. Lack of clearly defined chemical markers leads to discrepancies in terms of biological activity whereas variation in authentication of raw-materials can lead to adulteration or substitution. The conventional formulas often do not have specific quality-control parameters, which is a hindrance to clinical translation and regulatory support of herbal products. In turn, this is why it is

mandatory to consider the reproducibility to enable the acceptance of herbal therapeutics worldwide [4-6].

1.3 Significance of *Syzygium cumini* Fruits

Syzygium cumini fruit is a good source of polyphenolic compounds, flavonoids and anthocyanins. These fruits are also historically eaten and used in the treatment of the metabolism disorders. The antioxidant and antidiabetic capabilities of these fruits and their enzyme-inhibiting effects have been reported as a result of empirical studies. The extracts obtained after the fruit act on alpha-amylase and alpha-glucosidase enzymes. The fruits have better safety profiles and dietary compatibility as compared to seeds and bark. Their potential roles in the development of nutraceutical and functional food are based on the bioactive composition. It is important to note that fruits have specific water-soluble phytochemicals, which prove to be highly bioavailability. Syzygium cumini fruits therefore contain therapeutic and nutritional value [7-9].

1.4 The requirement of using Marker-based fingerprinting is as follows

Proper identification of herbal extracts is impossible without the use of marker-based fingerprinting. The method is useful in qualitative and quantitative analysis of key bioactive constituents. Chemical profiles of plant materials using chromatographic methods are reproducible. Fingerprinting can help to match phytochemical composition and seen bioactivity. It also helps in the detection of adulteration as well as protecting the genuineness of herbal preparations. The marker compounds are used as the ultimate quality indicators in standardization procedures. Therefore, reproducibility is improved in pharmacological and clinical research through such profiling. To this end, quality control of herbal formulations cannot be done without marker-based fingerprinting [10-12].

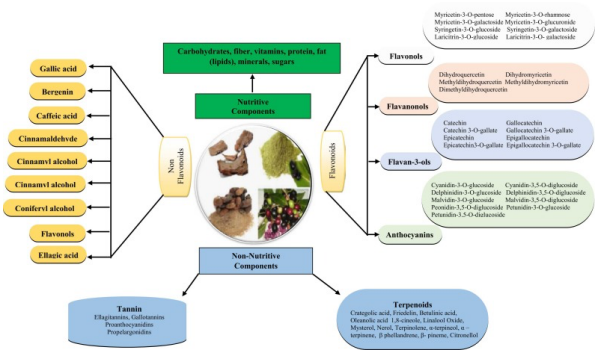

Figure 1: Conceptual Overview of Phytochemical Standardization and Marker-Based Fingerprinting in Syzygium cumini Fruits

2. MATERIALS AND METHODS

2.1 Materials

The plant material to be used in the current analytical study was fresh fruits of Syzygium cumini. The solvents used in the extraction and subsequent analyses, namely petroleum ether, chloroform, ethyl acetate, ethanol and distilled water were of analytical reagent (AR) quality and obtained out of credible chemical suppliers. In the phytochemical screening, reagents that were used were: Dragendorff reagent, Mayer reagent, ferric chloride, lead acetate, sodium hydroxide, concentrated acids and all were of analytical grade. The determination of total phenolic content was chosen based on FolinCiocalteu reagent and sodium carbonate and used gallic acid as the reference standard. The amount of total flavonoid was determined by using aluminum chloride and potassium acetate as the standard sample is quercetin. The chromatographic analyses were carried out using precoated silica gel 60 F254 HPTLC plates to develop the fingerprints and HPLC-grade solvents were utilized in the preparation of mobile phase. The chromatographic identification and quantification were done using reference marker compounds of known purity. Calibration The analytical measurements were done with calibrated instruments, in this case, a UV -Visible spectrophotometer, and an HPTLC system with a densitometric scanner and an HPLC system with an appropriate detector. The entire study was done using distilled water, and all glass wares were cleaned and dried first before use.

2.2 Successive Solvent Extraction

Fruits of Syzygium cumini were shade-dried and coarsely powdered then subjected to a series of successive solvent extraction with stronger polarity solvents, petroleum ether, chloroform, ethyl acetate, ethanol and distilled water. They have been extracted using a Soxhlet apparatus and constant reflux was maintained until exhaustive extraction occurred in each solvent. After the completion of every extraction cycle, the solvent was evaporated at low pressure. The dried extracts were weighed to measure the percent yield and put in airtight containers storing at 4 o C until further measure was carried out [13,14].

2.3 Preliminary Phytochemical Screening

The initial qualitative phytochemical screening of all solvent extracts was conducted by use of standard chemical tests to indicate major classes of secondary metabolites. The extracts were subjected to test of carbohydrates, alkaloids, flavonoid, phenolic compounds tannins, saponins, glycosides and terpenoids. The observations were made in terms of

characteristic colour changes or formation of a precipitate, which showed the presence or absence of certain phytoconstituents in each extract [15-17].

2.4 Estimation of Total Phenolic Content (TPC)

The FolinCiocalteu colourimetric Folin method was used to determine the total phenolic content. An aliquot of both extracts was combined with Folin Ciocalteu reagent, after which sodium carbonate solution was put. The mixture of the two reactants was left to react with each other at room temperature during a certain time period and then the absorbance was recorded by a UV–Visible spectrophotometer. As the reference standard, gallic acid was selected and the findings were calculated in terms of milligrams of the gallic acid equivalence per gram of extract [18-20].

2.5 Estimation of Total Flavonoid Content (TFC)

The aluminium chloride colourimetric method was used in determining the total flavonoid content. Aluminium chloride reagent and potassium acetate were added to extract solutions after which they were incubated under monitored conditions. The absorbance was measured spectrophotometrically at a given wavelength. Quercetin was taken as the reference compound and the total flavonoid content was calculated in milligrams of quercetin equivalents per gram of extract [21-23].

2.6 Development of HPTLC/HPLC Method.

To establish a reliable fingerprint profile of fruit extracts of *Syzygium cumini* a chromatographic analysis was done. In the case of HPTLC analysis, the mobile phase composition was optimised in order to have a good separation of phytoconstituents. Samples were transferred on the silica gel as bands on pre-stained silica plates and the plate was then developed by placing it in a chamber. The densitometric scanning was performed under optimisation. In the HPLC analysis, the chromatographic parameters such as mobile phase composition, flow rate, detection wavelength and run time were optimised to achieve well-resolved and reproducible chromatograms [24-26].

2.7 Marker Compound Quantification

The selected marker compounds were quantified by using validated HPTLC/HPLC. Standard solutions of the marker compounds were made and the calibration curves were plotted using linear regression analysis. Sample extracts were also determined using optimised chromatography conditions and useful markers contents were determined using peak area of sample extracts against the standard calibration plots. The findings were presented in terms of milligrams of marker compound per gram of extract [27-29].

2.8 Statistical Analysis

All the experiments of analysis have been carried out thrice, mean and standard deviation were used to present the results. Linear regression analysis was used to estimate the calibration curves. The phytochemical content was statistically correlated with the analytical parameters with the help of the corresponding statistical software. Whenever the difference was less than 0.05, it was considered to be significant statistically [30,31].

3. RESULTS AND DISCUSSION

The results of the repeated solvent extractions indicated the existence of a clear effect of solvent polarity on extractive yield. Table 3.1 shows that non-polar petroleum ether obtained the least amount of extract, which polar solvents i.e. ethanol and water obtained significantly higher amounts, thus showing that polar phytoconstituents were predominant in the fruits of Syzygium cumini.

The qualitative phytochemical profile highlighted the importance of the difference in the distribution of the secondary metabolites in the various extracts. Table 3.2 shows that the ethanol and aqueous extracts contained phenolics, flavonoids, tannins, and saponins, which are the predominant phytochemicals, whereas the non-polar extracts had low traces of phytochemicals. Such distribution justifies the high extraction efficiency of polar solvents.

These observations were supported by quantitative analyses. The best total phenolic content (TPC) and total flavonoid content (TFC) were observed in the ethanol extract, aqueous extract and ethyl acetate extract respectively as shown in Tables 3.3 and 3.4. The high values of TPC and TFC therefore suggest that the correlation between solvent polarity and bioactive polyphenolic compounds extraction is strong.

Chromatographic fingerprinting produced a good chemical profiling of the fruit extracts. Gallocic acid, ellagic acid, and anthocyanins were detected in Table 3.5, which affirmed the richness of phenolic compounds of the Syzygium cumini fruits. Table 3.6 indicated that anthocyanins were the major marker compounds, thus showing that they play an important role in the bioactive potential of the extracts.

3.1 Percentage Yield of Extracts

Table 3.1: Percentage Yield of *Syzygium cumini* Fruit Extracts Obtained by Successive Soxhlet Extraction

Sr. No.	Solvent Used	Nature of Solvent	Weight of Extract (g)	Percentage Yield (% w/w)
1	Petroleum ether	Non-polar	2.14	2.14 ± 0.12
2	Chloroform	Low polarity	3.26	3.26 ± 0.18
3	Ethyl acetate	Medium polarity	5.48	5.48 ± 0.21
4	Ethanol	Polar	11.92	11.92 ± 0.35
5	Water	Highly polar	9.64	9.64 ± 0.29

3.2 Phytochemical Screening Profile

Table 3.2: Preliminary Qualitative Phytochemical Screening of *Syzygium cumini* Fruit Extracts

Phytoconstituent	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous
Alkaloids	–	+	+	+++	++
Flavonoids	–	–	++	+++	++
Phenolics	–	+	++	+++	+++
Tannins	–	–	+	+++	++
Saponins	–	–	–	+	+++
Glycosides	–	+	+	++	++

Key: (–) Absent, (+) Present, (++) Moderate, (+++) Abundant

3.3 TPC and TFC Variation Among Extracts

Table 3.3: Total Phenolic Content (TPC) of *Syzygium cumini* Fruit Extracts

Sr. No.	Extract Type	TPC (mg GAE/g extract)
1	Petroleum ether	18.4 ± 1.2
2	Chloroform	32.6 ± 1.8
3	Ethyl acetate	76.3 ± 2.9
4	Ethanol	128.7 ± 4.1
5	Aqueous	94.5 ± 3.6

Table 3.4: Total Flavonoid Content (TFC) of *Syzygium cumini* Fruit Extracts

Sr. No.	Extract Type	TFC (mg QE/g extract)
1	Petroleum ether	9.2 ± 0.7
2	Chloroform	21.5 ± 1.3
3	Ethyl acetate	64.9 ± 2.5
4	Ethanol	112.6 ± 3.8
5	Aqueous	78.3 ± 3.1

3.5 Chromatographic Fingerprinting

Table 3.5: Chromatographic Fingerprint Profile of *Syzygium cumini* Fruit Extract

Marker Compound	Retention Time (min)	Content (mg/g extract)
Gallic acid	3.21	18.4 ± 0.9
Ellagic acid	6.78	11.6 ± 0.6
Anthocyanins	9.34	26.8 ± 1.4

3.6 Marker Compound Quantification

Table 3.6: Quantification of Marker Compounds in *Syzygium cumini* Fruit Extract

Marker Compound	Retention Time (min)	Quantity (mg/g extract)
Gallic acid	3.21	18.4 ± 0.9
Ellagic acid	6.78	11.6 ± 0.6
Anthocyanins	9.34	26.8 ± 1.4

4. CONCLUSION

The analytical study of the fruit extract of Syzygium cumini revealed that solvent polarity has a considerable effect on extractive yield and phytochemical composition. Polar solvents particularly ethanol and water were found to be the most effective in extracting bioactive constituents in terms of high yields and increase in total phenolic and flavonoid contents. Early phytochemical screening supported the presence of major concentration of phenolics, flavonoids, tannins and the saponins in polar extracts, thus highlighting the high phytochemical content of the extracts. The use of chromatographic fingerprinting and quantification of marker compounds provided reproducible chemical profiles where gallic acid, ellagic acid, and anthocyanins were identified as the primary marker compounds that provide the basis of the bioactivity of the extracts. Accordingly, the paper highlights the necessity of marker-based standardization of analysis based on analytical to guarantee the quality, consistency and reproducibility of Syzygium cumini fruit extracts.

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