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Research Article

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Proximate, Techno-functional Properties and Characterization of *Murraya koenigii* Linn Leaf Powder

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Abstract

Curry leaves (*Murraya koenigii*) are renowned for their culinary and health benefits. This study explores their health advantages and conducts a comprehensive analysis, encompassing proximate composition, technofunctional properties, and lipid digestion inhibition. Proximate analysis unveiled moisture (2.10 ± 0.72%), ash (2.99 ± 0.24%), fiber (6.62 ± 0.21%), fat (1.92 ± 0.17%), and protein (9.49 ± 0.6%), while technofunctional attributes included tap density (0.34 ± 0.72 gm/cm^3), bulk density (0.453 ± 0.24 gm/cm^3), Hausner's ratio (1.28 ± 0.42), angle of repose (37.26 ± 0.24°), and Carr's ratio (16.90 ± 0.10). Inhibition assays demonstrated concentration-dependent effects against α-amylase (IC_{50}: 22.43 ± 0.15 µg/ml) and glucose (IC_{50}: 10.13 ± 0.42 µg/ml). Additionally, curry leaf powder was characterized using scanning electron microscope (SEM), Fourier transform infrared (FTIR) spectroscopy, high performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) techniques, yielding insights into its structural and chemical properties. This study provides a comprehensive understanding of *M. koenigii*, spotlighting its multifaceted health benefits, composition, and functional attributes.

Keywords

α-Amylase inhibition, Glucose inhibition, IC_{50}, *In vitro* lipid digestion inhibition, *Murraya koenigii*, Techno-functional properties

Introduction

Curry leaf, scientifically known as *M. koenigii* (chromosome number 2n=18), belongs to the Rutaceae family, which comprises of 150 genera and 1600 species [1]. The specific epithet honours the botanist, Johann Konig, while the genus name, Murraya, commemorates the Swedish physician and botanist Johann Andreas Murraya, who passed away in 1791. This botanical name belongs to the Rutaceae family [2]. *M. koenigii* originates from East and South India, Pakistan, Sri Lanka, China, and Hainan, but is widely cultivated in Southeast Asia, the United States, and Australia. It grows throughout India up to 1500-1655 meters above sea level, including the Andaman Islands. It is also found in moist forests at elevations of 500-1600 meters in Guangdong, Shainan, S Yunnan (Xishuangbanna), Bhutan, Laos, Nepal, Pakistan, Sri Lanka, Thailand, and Vietnam. Curry leaves have spread to Malaysia, South Africa, and Reunion Island through South Indian immigrants [3, 4].

In India, there are two known species of the Murraya genus, *M. koenigii* (Spreng) and *M. paniculata* (Jack) [5]. *M. koenigii* can grow in full sun or light shade and is distributed from South and East Asia to Australia [6]. The utiliza-

tion of *M. koenigii* can be traced back to the 1st and 4th centuries AD, as described in Tamil and Kannada literature, where it was referred to as Kari and used as a flavouring agent [7]. India, the leading producer and consumer of curry leaves, dominates the market despite its classification as a minor spice crop. This herb is commercially cultivated in select Indian states and holds significant trade value due to its high demand both domestically and internationally. Curry leaf is particularly sought after in Gulf, European, and African countries. In 2016-17, India exported 600 tons of curry leaves worth 37 lakhs, with the European Union accounting for 20% of the export value [8]. Due to its fragrance and aroma, it is considered an important ingredient in South Asian cuisine [9]. *M. koenigii* is a plant species known for its rich content of organic compounds, including alkaloids, flavonoids, carbohydrates, and sterols.

Curry leaves exhibit a diverse chemical composition, including flavonoids, alkaloids, antioxidants, sterols, resin, and crystalline glycosides [10]. These compounds are present in various solvents such as ethanol, ethyl acetate, water, chloroform, and petroleum ether. The leaves are rich in proteins, carbohydrates, minerals, vitamin C, vitamin B complex, carotene, fibres, nicotinic acid, oxalic acid, carbazole, and koenigin. Aromatic compounds like O-phellandrene, P-elemene, P-caryophyllene, and P-gurjunene contribute to its distinctive aroma. α-pinene, β-pinene, β-caryophyllene, and β-phellandrene are also present, potentially aiding in controlling food spoilage. Major phytoconstituents such as koenidine, koenimbine, koenigine, koenine, coumarine, girinimbin, phebalosin, mahanimbicine, bicyclomahanimbicine, and isomahanimbin are found in different parts of the plant, including roots, leaves, and bark [11].

Triterpenoid alkaloids like tetrahydromahanimbine and cyclomahanimbine are present in the leaves [12]. The bark contains carbazole alkaloids like girinimbine, koenioline, xynthyletin, murrayacine, murrayazoline, and murrayazolidine. The fruit pulp primarily consists of soluble sugars, ascorbic acid, tannic acid, and minerals [13]. Seeds contain isobyakangelicol, xanthotoxin, and furocoumarins. The essential oil extracted from fresh leaves is a rich source of calcium and vitamin A, containing caryophyllene, dipentene, di-α-phellandrene, Dα-pinene, D-α-terpinol, and D-sabinene. The leaves of Murraya plant contain six main alkaloids, namely koenimbine, bispyrayafoline, bismahanine, isomahanine, O-methyl murrayamine, and O-methyl mahanine [14]. Dried Murraya leaves yield glycozoline and 1-formyl-3-methoxy-6-methyl carbazole, while the stem bark contains koenigine-quinone A and koenigine-quinone B [10]. These structures correspond to 7-methoxy-3-methyl carbazole-1,4-quinone and 6,7-dimethoxy-3-methyl carbazole-1,4-quinone, respectively. Additionally, 9-carbethoxy-3-methyl carbazole and 9-formyl-3-methyl carbazole have been identified. Stem bark extracts yielded 2-methoxy carbazole-3-carboxylate and 1-hydroxy-3-methyl carbazole [15].

Other compounds isolated from the stem bark include mukonal, murrayazolinol, mahanimbinol, murrayazolidine, murrayacinine, mukonidine, murrayazolinine, murrayanine, girinimibine, mahanimbine, girinimbinol, and mahanimbilol. These compounds may serve as potential precursors or intermediates in the biosynthesis of girinimbine and mahanimbine. From the root extract of Murraya plant, several compounds were isolated, including mukoline, girinimbine, marmesin-1"-O-rutinoside, mukolidine, murrayanol, and murrayagetin. Additionally, three monomeric and five binary carbazole alkaloids, along with Koenoline (1-methoxy-3-hydroxy methyl carbazole), were derived from the roots and stem bark of Murraya species [16, 17]. In the chromatographic analysis of the seeds' petroleum ether extract, compounds such as koenimbine, girinimbine, mahanimbine, mahanine, isomahanine, and 2-methoxy-3-methyl carbazole were identified [10]. The structural confirmation of bioactive carbazole alkaloids like koenimbine, koenine, and kurryam was achieved through 2D nuclear magnetic resonance spectra. These carbazole alkaloids, along with murrayazolidine and murrayanol, were found in the petroleum extract of the fruit pulp [18].

The essential oil obtained from fresh leaves of *M. koenigii* was primarily composed of apinene (51.7%), along with constituents such as sabinene, β-pinene, β-caryophyllene, limonene, bornyl acetate, terpinen-4-ol, α-humulene, and γ-terpinene. The essential oil from flowers contained 43.9% sesquiterpenoids and 34.4% monoterpenoids, with major constituents including β-caryophyllene, α-caryophyllene, selinene, cardinene, β-thujene, trans-ocimene, β-phellendrene, gujunene, linalool, α-pinene, and β-elemene. The volatile oil obtained from fruits through hydrodistillation had a composition including caryophyllene oxide, β-caryophyllene, tridecanoic acid, terpinen-4-ol, dehydroaromadendrene, α-cadinol, and (Z, E)-farnesol, among others. Murraya leaves have therapeutic properties attributed to their diverse chemical composition, including carbazole alkaloids, flavonoids, antioxidants, and other compounds. These components contribute to their ability to scavenge free radicals and make them beneficial for various biological processes. Murraya leaf extracts have been used in traditional herbal medicine due to their antimicrobial, antibacterial, and anticancer activities [10, 19-21]. The multifaceted potential of *M. koenigii*, specifically its essential oil, alcoholic and acetone leaf extracts, has been extensively studied. These extracts have shown antibacterial effects against *Staphylococcus aureus* and *Bacillus subtilis*, as well as mosquitocidal properties [22].

The presence of alkaloids contributes to their anticancer properties and cytotoxic effects against tumour cell lines [23]. Murraya leaf extracts also possess radical-scavenging properties and exhibit antifungal activity against *Candida albicans* and *C. tropicalis* [24]. Furthermore, Murraya leaves demonstrate potent antihelmintic properties, larvicidal and mosquitocidal activity against mosquitoes, and potential cognitive enhancement and neuroprotection [25]. In addition, Murraya, in combination with succinic anhydride, acts as an angiotensin-converting enzyme inhibitor, beneficial for hypertension management [10]. The ethanolic extract of Murraya leaves has shown favourable effects on body weight, cholesterol levels, triglycerides, blood sugar levels, and body fat deposition [26]. Moreover, Murraya leaf extracts possess hypoglycemic agents that reduce glucose levels by increasing insulin secretion and inhibiting gluconeogenic enzymes. The presence of flavonoids contributes to its hypoglycemic effects while increasing cal-

cium availability and exhibiting a positive inotropic effect, thereby demonstrating antihypertensive activity [27].

Materials and Methods

Materials

Plant material and chemicals

The leaves of *M. koenigii* were obtained from the market and all the analytical grade chemicals utilized in the experiments were obtained from the Department of Food Science and Technology at Lovely Professional University, located in Phagwara, Punjab.

Methods

Herbal powder preparation

The curry leaves were carefully inspected to remove any foreign particles. Afterwards, they were subjected to drying in a tray drier (B. S. exports) at a controlled temperature of 50 °C ± 2.0 °C for 24 h. Once dried, the leaves were finely powdered using a grinder before being stored in a ziplock bag at room temperature.

Determination of total yield of curry leaf powder

To determine the yield, a modified sieving analysis method [28] was utilized. The sample was placed on a sieve shaker (Harrison) and vibrated at an amplitude of 60 for 20 min, using a test sieve with a mesh size of 250 µm. The weight of the sieved sample was measured, and the yield was calculated using the following equation:

$$\text{Total yield (\%)} = \frac{\text{Weight of sieved powder (g)}}{\text{The initial weight of the sample (g)}} \times 100$$

Moisture content (%)

Adhering to a methodology delineated by Haryanto et al. [29], a sample weighing about 10.0 ± 0.5 g was positioned within a crucible. The moisture content of the dehydrated powder was ascertained using the oven dry procedure within a hot air oven (Vidya Udyog), set at a temperature of 105 ± 2.0 °C. The final weight was noted once a stable weight was achieved. The moisture content of the curry leaf powder was calculated employing the subsequent equation:

$$\text{Moisture content (\%)} = \frac{\text{Weight of the crucible before drying (g)} - \text{Weight of the crucible after drying (g)}}{\text{Weight of the crucible before drying (g)}} \times 100$$

Ash content (%)

The methodology and procedure utilized in this analysis were detailed by Bhardwaj et al. [30]. To begin, a crucible was used to weigh 2.0 ± 0.5 g of curry leaf powder. The powder was then subjected to incineration in a muffle furnace (Narang Scientific work) at a temperature of 560 °C for a period of 16 h until it was fully transformed into ash. Subsequently, the crucible was cooled in a desiccator, and the final weight of the crucible was measured. The ash content was determined by applying the following equation:

$$\text{Ash content (\%)} = \frac{\text{Weight of the crucible before drying (g)} - \text{Weight of the crucible after drying (g)}}{\text{Weight of the crucible before drying (g)}} \times 100$$

Fat content (%)

5.0 ± 0.5 g of *M. koenigii* powder is meticulously transferred into a thimble. The flask with a rounded bottom is accurately weighed, and the experimental arrangement is established, utilizing hexane as the selected extracting solvent. The extraction process is conducted over a span of 6 h to ensure a comprehensive extraction of lipids, in accordance with the approach outlined by Khassetia and Affrina [31]. Following the extraction phase, the solvent is eliminated, and the resultant lipid extract is concentrated using a rotary evaporator or a comparable device. The concentrated lipid extract is subsequently subjected to drying within a desiccator or a drying equipment to eliminate any lingering solvent residues. The quantification of the lipid content is achieved through the application of the provided equation.

$$\text{Fat content (\%)} = \frac{\text{Weight of the round bottom flask after extraction (g)}}{\text{Weight of the sample (g)}} \times 100$$

Protein estimation

The protein estimation was conducted using the Kjeldahl method described Sharma and Dhuria [32]. A nutritional evaluation of rosemary leaf powder and black cumin seed powder as phytogenic feed additives [32]. A weighed amount of 0.5 to 1 g of curry leaves powder was transferred into a digestion vessel. The sample was then mixed with concentrated sulfuric acid (H_2SO_4) and a digestion catalyst, such as copper sulfate ($CuSO_4$), for the digestion process. Gradual heating of the digestion vessel using a Bunsen burner or heating mantle was performed until the contents became clear or showed a pale-yellow color, indicating the completion of digestion. After cooling, the contents were diluted with distilled water and transferred into a distillation apparatus. Ammonia released during the distillation process was collected in an acidic solution, while the resulting distillate was titrated with standardized sulfuric acid. The protein content was subsequently evaluated and calculated using the provided formula.

$$\text{Protein estimation (\%)} = \frac{\text{N x F x 6.25}}{\text{Weight of the sample (g)}}$$

The variables in this context are designated as follows: 'N' represents the amount of ammonia nitrogen obtained from the titration, measured in milligrams (mg). The variable 'F' represents the factor indicating the concentration of the sulfuric acid solution used for titration. Additionally, the numerical value of 6.25 is employed as a conversion factor to convert nitrogen content into protein content. This conversion factor assumes an average protein nitrogen content of 16%.

Fiber estimation

The AOAC, 2005 method as described by Akter et al. [33] was employed to estimate the crude fiber content of the defatted dry sample. Approximately 2.5 g of the defatted dry sample was used for the fiber analysis using Fibraplus FES06E. The sample was subjected to boiling with a 1.25% dilute H_2SO_4 solution, followed by washing with water. Subsequently, it was boiled again with a 1.25% dilute NaOH solution. The remaining residue after digestion was collected as the crude fibrate

residue, which was then placed in a furnace and digested at a temperature of 600 ˚C. The crude fiber content was determined using the following formula.

$$\text{Crude fiber } (\%) = \left[\left(\text{Loss in weight on ignition} \div \text{Weight of the sample}\right) \text{ x } 100\right]$$

Tapped density and bulk density

The measurement of bulk density (ρbulk) involves determining the total volume occupied by both the solid particles and the void spaces in a divided powder. The calculation of bulk density for curry leaf powder was performed following the method described by Mulani et al. [34] and Etti et al. [35]. In this method, the powder is filled into a 50 ml measuring cylinder up to the 50 ml mark. The cylinder is then dropped from a height of 1 inch onto a hard wooden surface at 2-second intervals, and the resulting volume of the powder is carefully measured.

On the other hand, tap density (ρtab) is a different type of bulk density that is obtained by subjecting the container to tapping or vibrating in a specific manner. This tapping or vibrating action helps to achieve a more efficient arrangement of particles, leading to increased particle packing density. To determine tap density, the initial volume or mass of the powder is recorded. Then, the measuring cylinder or vessel containing the powder is mechanically tapped 500 times. Periodic readings of the volume or mass are taken until minimal or no further changes in volume or mass are observed. Tapped density provides an indication of the compactness of the powder after mechanical tapping. Consequently, tap density is typically higher than bulk density.

$$\rho_{bulk} = \frac{W_t}{V_{bulk}}$$

The determination of tapped density (ρtab) for powders involves utilizing an equation that incorporates the weight of the powder (Wt) and the volume of the powder obtained from a tarred graduated cylinder without any tapping (Vbulk).

$$\rho_{tab} = \frac{W_t}{V_{tab}}$$

The provided equation utilizes the weight of the powder (Wt) and the volume of the powder bed after 500 taps (Vtab).

Hausner's ratio and Carrs index

Carr index and Hausner ratio are metrics utilized to evaluate the flow characteristics of a powder which is performed as highlighted by Mochahary et al. [36]. The Carr index is determined by dividing the difference between the tapped density and bulk density by the tapped density. According to Carr's flowability index, powders with a Carr index ranging from 5% to 15% are considered to have excellent flowability, whereas a Carr index exceeding 25% generally indicates poor flowability.

$$Carrs\ Index = \frac{\rho_{tab} - \rho_{bulk}}{\rho_{tab}}$$

Hausner ratio is a parameter employed to assess the flowability of a powder. It is calculated by dividing the tapped density by the bulk density. A Hausner ratio value ranging from 1.0 to 1.1 indicates a powder that flows freely, while Hausner ratio values between 1.1 and 1.25 suggest a powder with moderate flowability. If the Hausner ratio exceeds 1.25 and goes up to 1.4, it signifies a powder that is challenging to flow, and a Hausner ratio exceeding 1.4 indicates a powder that exhibits very poor flowability.

$$Hausner\ Ratio = \frac{\rho_{tab}}{\rho_{bulk}}$$

Angle of repose

The protocol to determine the angle of repose (θ) for *M. koenigii* powder was according to Kundan et al. [37]. The desired quality of the dried powder is carefully taken and placed in a funnel positioned 6 cm above a horizontal base. The powder is then allowed to flow freely, forming a heap over a sheet of paper on the horizontal plane. During this process, the height and radius of the powder heap are meticulously measured and recorded. The angle of repose is calculated from the given equation

$$Angle\ of\ repose\ (^{\circ}) = tan^{-1}\left(\frac{h}{r}\right)$$

The pile's height is represented by 'h' while its radius is denoted by 'r'.

The angle of repose serves as an important indicator of the flow characteristics of the powder. Generally, an angle of repose of around 30 degrees is indicative of a material that flows freely, suggesting good flowability. Conversely, angles of repose approaching 40 degrees typically indicate a material that flows poorly, highlighting reduced flowability.

Plant extract preparation

A modified extraction method described by Kumar et al. [38] was used to obtain the acetone extracts. To create these extracts from the herbs, 1 g of powdered herb material was dissolved in 10 ml of various extraction solutions such as methanol, chloroform, ethanol, hexane, and acetone, at a temperature of 35 ˚C. This mixture was then placed in an orbital shaker and incubated at 150 rpm for 48 h. After the incubation, the tubes were centrifuged at 4000 rpm for 5 min. The resulting extracts were left to evaporate at a refrigerated temperature range of 4 - 7 ˚C. The weight of each extract was measured to calculate the yield as a percentage. The extract with the highest yield was selected and stored in airtight glass vials at -18 ˚C for further analysis.

Total phenolic content determination

The determination of total phenolic content in the methanol extracts was performed with some modifications based on the method described by Alara et al. [39]. In a test tube, 300 µl of the ethanol extract solution was combined with 1 ml of methanol, and 200 µl of Folin-Ciocalteu reagent. After an incubation period of 5 min, 600 µl of a 0.02 mM sodium carbonate solution was added, followed by a 2-hour incubation at 40 ˚C. A calibration was prepared by creating a blank with methanol instead of the extract and constructing a gallic acid standard curve. The absorbance of the resulting solutions was measured at 765 nm using a UV-spectrophotometer (Visible Spectrophotometer 168). Total phenol content was calculated

using the equation y = 4.61x + 0.0848 (R^2 = 0.9703), where y represents the absorbance and x represents the concentration of gallic acid in micrograms per milliliter (µg/ml). Thereafter, total phenolic content in the *M. koenigii* leaf extracts was determined using the equation below and the result was expressed as milligram gallic acid equivalents per gram of dried weight sample (mg GAE/g d.w.).

$$Total\ phenolic\ content = \frac{Concentration\ \left(\frac{mg}{ml}\right) \times Volume\ of\ solvent\ (mL)}{Weight\ of\ dried\ sample\ used\ (g)}$$

Total flavonoid content estimation

The quantification of total flavonoid content in the methanol extracts of all samples followed a modified procedure based on the method developed by Alara et al. [39]. Each plant extract was dissolved in methanol at a concentration of 500 µg/ml. In a test tube, 300 µl of the extract was mixed with 3.4 ml of an aqueous methanol solution (30% v/v). Then, solutions of sodium nitrite (0.5 M) and aluminum chloride (0.3 M) were added to the mixture. After a 5-minute incubation period, sodium hydroxide solution (1 M) was introduced, and the mixture was thoroughly mixed before measuring the absorbance at 506 nm against a blank using Visible Spectrophotometer 168. A calibration curve using a quercetin standard was established for accurate determination. The total flavonoid content was calculated using the equation y = 0.005312x + 0.01153 (R^2 = 0.9861), where y represents the absorbance at 506 nm and x represents the amount of rutin in micrograms of quercetin equivalents (RE) per milliliter of extract. The results were reported as micrograms of quercetin equivalents (RE) per milligram of extract.

DPPH estimation

DPPH radicals were created by dissolving approximately 0.004 g of DPPH reagent in 100 ml of methanol. For the assay, 20 µl of the extract was mixed with 180 µl of DPPH. The mixture was left in the dark for 15 min, and the absorbance at 517 nm was measured using a Visible Spectrophotometer 168. Blank samples containing only methanol and standard samples with ascorbic acid in methanol were included in the analysis. The extracts were tested at different concentrations to determine the IC_{50}, which represents the concentration at which the DPPH absorbance is reduced by 50%. This methodology closely follows that of previous researchers [39, 40]. The degree of scavenging was calculated using the following equation:

$$DPPH\ estimation\ (\%) = \frac{Absorbance\ of\ blank - Absorbance\ of\ the\ sample}{Absorbance\ of\ the\ blank} \times 100$$

Amylase inhibition assay

The procedure for evaluating the inhibitory potential of amylase activity was carried out following the protocol delineated by Kumar et al. [38]. A substrate solution was formulated by dissolving 500 mg of soluble starch in 25 ml of 0.4 M NaOH, subsequently subjecting it to a temperature of 100 °C for a duration of 5 min. The resultant solution's pH was titrated to 7.0 using HCl, with the final volume adjusted to 100 ml utilizing distilled water. Diverse concentrations of plant extract solutions were prepared using acetate buffer of pH 6.5 as the medium. Within a microplate well, a concoction comprising 20 µl of the specimen and 40 µl of the substrate solution was amalgamated. Sequentially, 20 µl of α-amylase solution (50 µg/ml) was introduced into the mixture. A brief incubation period of 15 min at a temperature of 25 °C ensued. Termination of the enzymatic reaction was accomplished by adding 80 µl of 0.1 M HCl, succeeded by an augmentation of 200 µl of 1 mM iodine solution. Subsequently, measurement of the optical density was performed at 650 nm utilizing a visible spectrophotometer (Visible Spectrophotometer 168). The quantification of amylase inhibitory activity was executed through the implementation of the ensuing formula:

$$Amylase\ inhibition\ assay\ (\%) = 1 - \frac{(OD2 - OD1)}{(OD4 - OD3)} \times 100$$

The amylase inhibition activity was determined by comparing the optical densities of different solutions. OD1 represented the optical density of the solution containing plant extract, starch, and amylase. OD2 indicated the optical density of the solution containing plant extract and starch. OD3 denoted the optical density of the solution containing starch and amylase. Finally, OD4 represented the optical density of the solution containing only starch.

Glucose uptake assays using dialysis bag

The glucose uptake assay was conducted using a dialysis membrane with a pore size of 2000 KDa (6 cm × 15 mm), following the methodology described by Kumar et al. [38]. The dialysis membrane was soaked in distilled water to allow for expansion. Then, one end of the dialysis tube was sealed, and 1 mg of herbal extract was added to 10 ml of the dialysis membrane. Subsequently, 15 ml of a 0.22 mM glucose solution was added and thoroughly mixed. The system was incubated at 37 °C for 4 h. After the incubation period, centrifugation was performed at 4800 rpm for 20 min. A centrifugal tube containing 45 ml of 0.15 M NaCl was prepared. The movement of glucose from the dialysis membrane to the outer solution was measured at different time intervals using a glucometer (Control D Glucometer).

FTIR analysis

Following the method outlined in the existing literature by Asema et al. [40], the FTIR analysis was executed. Approximately 5 mg of each specimen was blended with 5 mg of KBr to facilitate qualitative assessment. Subsequently, the desiccated and powdered form of each sample underwent evaluation using a PERKIN ELMER FTIR spectrometer featuring a KBr beam splitter. The acquired spectra of these samples were examined according to the guidelines provided by Stuart. The FTIR spectrophotometer operated at a resolution of 0.5 cm^{-1}, and the software employed was Spectrum 10 software. The analyzed spectral range encompassed values spanning from 400 cm^{-1} to 4000 cm^{-1}.

HPLC analysis

The utilized Agilent HPLC system comprised a Model 1100 pump with a multi-solvent delivery arrangement, alongside an L-7400 (Hitachi) ultraviolet (UV) detector. The system was outfitted with an Agilent C18 column (5 µm, diameter not specified). The mobile phase was composed of two

components: (A) 0.03 M phosphoric acid in water and (B) methanol. Prior to utilization, the mobile phase was subjected to vacuum filtration through a 0.45 µm membrane filter. The flow rate was upheld at 1 ml/min, and UV absorbance measurements were performed within the 260 – 360 nm range. The operational temperature was maintained at ambient conditions. Compound identification was accomplished by matching retention times with standards, UV spectra, and UV absorbance ratios subsequent to co-injection of both samples and standards. This methodology draws inspiration from prior research Ghasemzadeh et al. [41].

GC-MS analysis

Utilizing a SHIMADZU system and gas chromatograph, we performed GC-MS analysis on this extract, employing the methodology highlighted by Viswanathan et al. [42]. The system was coupled with a mass spectrometer (GC-MS) equipped with an Elite-1 fused silica capillary column (30 m × 0.25 mm ID. × 1 µMdf), constructed entirely from dimethyl poly siloxane. The GC-MS detection employed an electron ionization system with an ionization energy of 70 eV. Helium gas (99.999%) was utilized as the carrier gas, maintaining a consistent flow rate of 1 ml/min., while an injection volume of 2 µl was introduced (using a split ratio of 10:1). The injector temperature was set at 250 °C, and the ion-source temperature at 280 °C. The temperature of the oven was programmed to start at 110 °C (isothermal for 2 min), followed by an incremental rise of 10 °C/min to 200 °C, and subsequently, a 5 °C/min increase to 280 °C. The final stage comprised a 9-minute isothermal period at 280 °C. Mass spectra were recorded at 70 eV, with a scan interval of 0.5 s and fragment analysis spanning from 45 to 450 Da. The comprehensive GC running time spanned 36 min. The relative percentage composition of each component was determined by comparing its average peak area against the total areas. The software used to manage mass spectra and chromatograms was the GCMSsolution™ software.

SEM analysis

The morphological characterization of dried ethonolic extract of curry leaf was performed by SEM using a JEOL microscope. Samples were visualized at the 100, 200, 500, 1,000, and 2,000x magnification as described in the literature by Fernandes et al. [43].

Statistical analysis

The study conducted experiments three times and presented the results as the average ± standard deviation. To assess significant differences between the means, they employed a statistical analysis known as one-way analysis of variance and followed it up with the Duncan post hoc test at a significance level of $p < 0.05$.

Results and Discussion

Proximate analysis

The curry leaves were dehydrated using a tray dryer set at a temperature of 40 ± 3 °C until the moisture content reached 2.10%, resulting in a low moisture level in the dried plant leaves. This process was carried out to enhance the preservation quality and extend the shelf life of the leaves. The proximate analysis of curry leaf analysis has been summarized in table 1. The ash content was measured at 2.99 ± 0.24%, which falls within a similar range as the 3.43% reported in the study conducted by Malik et al. [44]. Furthermore, the total fat content was notably low, making the curry leaves a potential dietary supplement suitable not only for weight loss but also for individuals with diabetes and cardiovascular issues, who are advised to consume low-fat diets. The fat content was determined to be 1.92 ± 0.17%, similar to the 2.43 gm/100 gm reported in the research by Parnami and Varma [45].

The substantial amount of dietary fiber present in the leaves makes them an excellent component for the diets of individuals with diabetes and heart conditions. The fiber content was measured at 6.62 ± 0.21, aligning closely with the value of 5.22 gm/100 gm documented by Parnami and Varma [45], as well as the value of 4.13 reported by Malik et al. [44] in 2021. The protein content was found to be 9.49 ± 0.6%, matching the 10.33% reported by Lal and Kaur [46], who conducted research on curry leaves dried using a hot air oven. This is also in agreement with the findings of Lal and Kaur [46], where the protein content was reported as 10.33% for the dried leaves.

Table 1: Results for the proximate analysis for *M. koenigii* powder.

Proximate analysis of *M. koenigii*	
Moisture content (%)	2.10 ± 0.72
Ash content (%)	2.99 ± 0.24
Fiber content (%)	6.62 ± 0.21
Fat content (%)	1.92 ± 0.17
Protein estimation (%)	9.49 ± 0.6

Techno-functional properties

The micrometric characteristics of the desiccated curry leaf closely resemble the investigations conducted by Asema et al. [40] and Aftab and Pandey [47]. The tapped density of the powder, measured at 0.34 gm/cm^3, aligns with the range of 0.33 gm/cm^3 found by Aftab and Pandey [47]. Similarly, the bulk density of 0.453 ± 0.24 gm/cm^3 is in proximity to the value reported in Asema et al. [40]. The Hausner's ratio stands at 1.280, mirroring the outcomes of both Asema et al. [40] and Aftab and Pandey [47], who recorded values of 1.2 and 1.38 respectively. The angle of repose is measured at 37.26°, akin to the 34.176° attained by Aftab and Pandey [47], indicating favorable flowability and reduced cohesion. Moreover, the Carr's ratio of 16.90 closely resembles the 16.6 obtained by Aftab and Pandey [47], which signifies relatively satisfactory flowability. The results for techno-functional properties has been summarized in table 2. Overall, the combined findings indicate strong flowability, fine particle size and substantial compressibility observed in the curry leaf powder.

Phytochemical analysis

A summary of the phytochemical analysis is depicted in table 3. The total phenolic content is quantified as a percentage of gallic acid equivalents. The phenolic content in the ethanolic extract was determined to be 99.47 mg GAE/g, akin to the

Table 2: Results for the techno-functional properties for *M. koenigii* powder.

Techno-functional properties	
Tap density (gm/cm^3)	0.34 ± 0.72
Bulk density (gm/cm^3)	0.453 ± 0.24
Hausner's ratio	1.28 ± 0.42
Angle of repose (°)	37.26 ± 0.24
Carr's ratio	16.90 ± 0.10

Table 3: Results for the phytochemical analysis for *M. koenigii* powder.

Phytochemical analysis for *M. koenigii* powder	
Total phenolic content (as percent gallic acid equivalents (GAE))	99.47 ± 0.72
Total flavonoid content (as percent as percentage of quercetin)	8.35 ± 0.24
DPPH (% inhibition)	6.20 ± 0.42

findings of Sepahpour et al. [48] when utilizing 80% ethanol. The total flavonoid content, expressed as a percentage of quercetin, was measured at 8.35 mg GAE/g, resembling the results from both *M. koenigii* and *M. minutum*, where the values were 9.75 mg GAE/g and 9.16 mg GAE/g, respectively, as investigated by Abeysinghe et al. [17]. Regarding the DPPH scavenging activity, the antioxidants present in the *M. koenigii* leaf extracts exhibited the capability to diminish the stable DPPH radical, resulting in a yellow-colored diphenyl picryl hydrazine compound. The ethanol leaf extract of *M. koenigii* demonstrated concentration-dependent DPPH scavenging activity. The IC_{50} value for the percentage inhibition of DPPH by the ethanol extract was determined as 6.20%, aligning with the value of 1.556% reported by Sablania et al. [49].

In vitro lipid digestion activity

The outcomes of the assays measuring α-amylase inhibition, lipase inhibition, and glucose inhibition for the *M. koenigii* extracts, accompanied by their respective IC_{50} values, are presented in the provided table 4. The IC_{50} value for the α-amylase inhibition assay was determined to be 22.43%, falling within a comparable range to the findings of Ghosh et al. [50] where the reported IC_{50} value stood at 36.72. Similarly, the IC_{50} value for the glucose inhibition assay was observed to be 10.13%, akin to the study conducted by Xie et al. [51] which investigated the impact of curry leaf (*M. koenigii* Spreng.) on glucose levels in ob/ob Mice (Figure 1).

FTIR analysis

The FTIR technique's characteristic narrow peaks were leveraged to establish correlations between vibrations and specific chemical bonds or individual functional groups within the molecule. Consequently, the FTIR spectroscopy of *M. koenigii* played a pivotal role in illuminating its chemical composition by unveiling characteristic peaks linked to discrete functional groups. The sharp intensity of peaks in the FTIR spectrum of *M. koenigii*, was found at 3283.39, 2919.52, 1728.78, 1606.42, 1374.14, 1317.98, 1242.85 and 1027.01 cm^{-1}, provided significant insights into its intricate molecular composition (Table 5 and figure 2). Notably, the analysis revealed the presence of a

Table 4: Results for the *in vitro* lipid digestion assay for *M. koenigii* powder.

In vitro lipid digestion assay			
Inhibition assay			
	Concentration (µg/ml)	**% of inhibition**	**IC_{50}**
α- amylase inhibition assay	20	41.11 ± 0.13	22.43 ± 0.15
	40	77.49 ± 0.485	
	60	79.94 ± 0.49	
	80	86.65 ± 0.21	
	100	95.93 ± 0.61	
Lipase inhibition assay			
	Time (min)	**Glucose (mg/ml)**	**IC_{50}**
Glucose inhibition assay	15	0.35 ± 0.45	10.13 ± 0.42
	30	0.65 ± 0.13	
	45	0.79 ± 0.11	
	60	1.73 ± 0.15	
	75	2.15 ± 0.04	
	90	1.93 ± 0.1	
	105	0.76 ± 0.08	

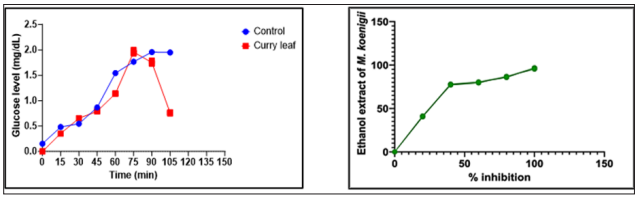


Figure 1: Glucose inhibition assay and α-amylase inhibition assay of *M. koenigii* powder.

hydroxyl group (OH) evidenced by a distinct peak at 3283.39 cm^{-1}, which aligned with a similar finding reported at 3372 cm^{-1} in the hydroalcoholic extract of curry leaf powder, as documented by Khurana et al. [52]. Similarly, a peak at 2919.52 cm^{-1} indicated the medium stretching of a -C-H alkene group, indicating the inherent C-H stretching frequency characteristic of the parent hydrocarbon chain of the compound. This observation concurred with prior investigations by Khurana et al. [52] demonstrating a peak at 2855 cm^{-1}, and by Swetha et al. [53] with a peak observed at 2915.93 cm^{-1}.

Moreover, a pronounced peak at 1728.78 cm^{-1} corresponded to the robust stretching of a -C=O δ-lactone group.

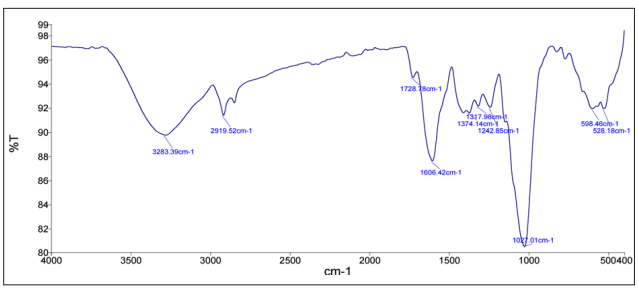


Figure 2: FTIR spectra for *M. koenigii* powder.

Table 5: FTIR data for *M. koenigii* powder.

FTIR						
Peak	**Peak (cm^{-1})**	**Bond**	**Bond strength**	**Bond vibration**	**Functional group**	**Ref.**
1	3283.39	O-H	Strong	Stretching	Alcohol	[52]
2	2919.52	C-H	Medium	Stretching	Alkene	[52, 53]
3	1728.78	C=O	Strong	Stretching	δ-lactone	[53, 54]
4	1606.42	C=C	Strong	Stretching	α, β-unsaturated ketone	[55]
5	1374.14	$C-H_3$	Medium	Stretching	Phenol	[53, 56]
6	1317.98	C-N	Strong	bending	Alcohol and the alkyl group	[57]
7	1242.85	C-O	Strong	Stretching	-	[59]
8	1027.01	C-N	Strong	Stretching	Aliphatic	[58]

This alignment was corroborated by analogous findings in the leaf extract of *Capparis divaricata* Lam., which displayed a similar peak at 1732.93 cm^{-1} [53]. These analogous peaks were also identified in the leaves, stem, flower, and fruit of *A. albicans* and *Tephrosia tinctoria*, as documented by Kumar and Prasad [54]. At 1606.42 cm^{-1}, a peak was attributed to the C–O stretching vibration of primary and secondary alcohols in cellulose, a result consistent with studies involving *Paederia scandens* and *Ocimum sanctum* L. [55]. Similarly, a peak at 1374.14 cm^{-1} indicated medium stretched $C-H_3$ phenol bonding, akin to *Acacia catechu* ethanolic seed extract [56] and the leaf extract of *C. divaricata* Lam [53].

One of the distinctive features of the FTIR spectrum was the peak at 1317.98 cm^{-1}, indicating a strong stretching -C-N aromatic amine bond. This observation finds resonance in the presence of the same peak at 1317 cm^{-1} in polyaniline/*Tectona grandis* sawdust [57]. Furthermore, a comparable peak at 1027.01 cm^{-1} was identified in modified lotus (*Nelumbo nucifera*) stem powder, affirming the presence of robust C-N stretching vibrations [58]. Consistent with the spectrum's analysis, an aliphatic group was discerned, characterized by a peak recorded at 1242 cm^{-1}. This peak corresponded to –COO- and C-N bending frequencies. Similarly, a corresponding FTIR peak was documented in cumin, as reported by Siddiqui et al. [59].

HPLC analysis

The provided HPLC data represented in both table 6 and figure 3 a comprehensive analysis of detected compounds through chromatography. Table 6 displays distinct peaks, each associated with a specific compound, delineated by their retention times. Two UV detection wavelengths, 360 nm and 260 nm, were utilized to observe the absorption characteristics of the compounds. Compound identification includes common names such as myricetin-3-galactoside, quercetin-O-pentohexoside, quercetin-3-glucoside, quercetin-3-acetylhexoside, kaempferol-O-glucoside, and quercetin. Data is referenced by Singh et al. [60], implying that identification is based on comparison with standards or prior research. It's noteworthy that some data entries lack compound names and references, indicating either unidentified compounds or missing information. Compound relationships can be inferred from the presence of similar modifications, such as quercetin derivatives. Overall, this HPLC analysis furnishes valuable insights into the composition of the sample and the specific compounds present, offering a basis for further interpretation and analysis (Figure 4).

GC-MS analysis

Components identified from curry leaf extract (Figure 4) using GC-MS are Benzenemethanol, 2-(2-aminopropoxy)-3-methyl, Glycerin (4.159), 3 3-Methylbenzyl alcohol, TBDMS derivative (7.087), α-Phellandrene (10.185), Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl) (10.656), Methyl 6,6,8,8,10,10-hexamethyl-3-oxo-2,5,7,9,11-pentaoxa-6,8,10-trisilatridecan-13-oate (10.752), Silane, cyclohexyldimethoxymethyl (12.779), 2-Butenethioic acid, 3-(ethylthio)-, S-(1-methylethyl) ester (13.460),

Table 6: HPLC data for *M. koenigii* powder at 260 nm and 360 nm.

HPLC (360 nm)			HPLC (260 nm)	
Peak	**Retention time (min)**	**Compound**	**Retention time (min)**	**Compound**
5	8.139	Myricetin-3-galactoside	6.731	-
6	9.227	Quercetin-O-pentohexoside	8.139	Myricetin-3-galactoside
7	10.4	Quercetin-3-glucoside	9.216	Quercetin-O-pentohexoside
8	11.221	Quercetin-3-acetylhexoside	10.411	Quercetin-3-glucoside
9	13.568	Kaempferol-O-glucoside	11.2	Quercetin-3-acetylhexoside
10	16.299	Quercetin	13.536	Kaempferol-O-glucoside
11	-	-	16.373	Quercetin

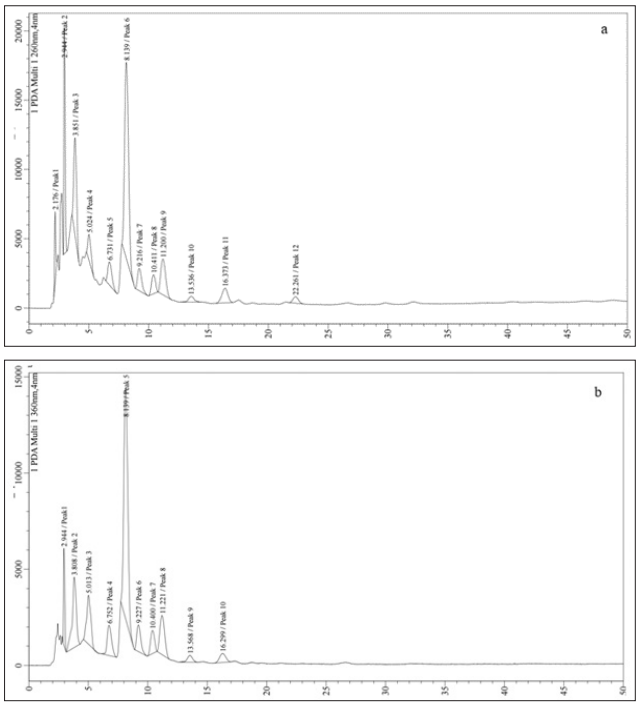


Figure 3: HPLC spectra for *M. koenigii* powder at (**a**) 260 nm and (**b**) 360 nm.

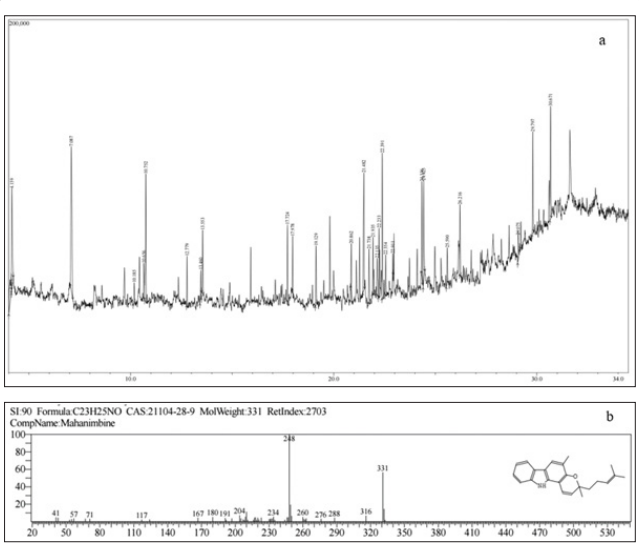


Figure 4: GC-MS spectra for *M. koenigii* powder at (**a**) 260 nm and (**b**) 360 nm.

2,2'-Bis-trimethylsilylbenzhydryl methyl ether (13.553) Phenol, 3,5-bis(1,1-dimethylethyl) (17.724), Mollugin, trimethylsilyl ether (17.978), 2,5-Cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene (19.129), Butane, 1,4-bis(9,10-dihydro-9-methylanthracen-10-yl) (20.862), Neophytadiene (21.482), E-11(13-Methyl)tetradecen-1-ol (21.738), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (21.935), 2,4-Imidazolidinedione, 5-(2-methylpropyl)-, (S) (22.135), Benzeneethanamine, 4-methoxy-N-[(pentafluorophenyl) methylene]-3-[(trimethylsilyl)oxy] (22.233), Hexadecanoic acid, methyl ester (22.391), Methylphosphonic acid, 2TMS derivative (22.554), 2,3,8-Trimethyl-1H,9H-pyrrolo[3,2-H] quinolin-6-one (22.911), Methyl stearate (24.336), l-Norvaline, N-(2-methoxyethoxycarbonyl)-, hexyl ester (24.425), Methyl 6,6,8,8-tetramethyl-3-oxo-2,5,7,9-tetraoxa-6, 8-disilaundecan-11-oate (25.590), 1-Cyclohexyldimethylsilyloxy-3, 5-dimethylbenzene (26.216), Naphthalene, 1,2,3,4-tetrahydro-6-methoxy-2-(4-pentylcyclohexyl)-, trans (29.075), Mahanimbine (29.797) and 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicos (30.671) the components are similar to Arivoli et al. [61].

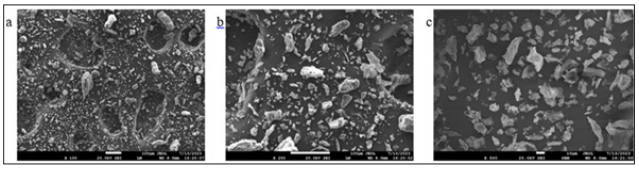


Figure 5: SEM images for *M. koenigii* powder at different magnification. (**a**) ×100 magnification, (**b**) ×200 magnification, and (**c**) ×500 magnification.

SEM analysis

The surface morphology of dehydrated curry leaf powder was studied using SEM (Figure 5). The particle seen at ×500 magnification shows irregular pattern of the molecule of approx. 4.53 µm the results are like curcumin powder performed by Zhao et al. [62].

Conclusion

This paper highlights the substantial health benefits and applications of *M. koenigii* leaves. Valuable insights into their nutritional composition and functional attributes contribute to understanding their role in human health. The analysis shows that *M. koenigii* leaf powder is a commendable source of health-enhancing components. Its high fiber content and low-fat levels align with dietary recommendations for combatting chronic diseases. The elevated phenolic content further emphasizes its potential as a natural antioxidant, crucial for neutralizing free radicals and reducing oxidative stress-related damage. The distinction between the high phenolic content and lower flavonoid levels provides insights into *M. koenigii*'s phytochemical composition. This difference can guide future research into the specific health effects of these compounds. The study's focus on qualitative and quantitative analyses, including advanced techniques like HPLC, SEM, FTIR, and GC-MS, offers a comprehensive approach to characterizing *M. koenigii* leaf powder. This multi-faceted exploration enhances understanding of its composition and potential uses.

Further, this research establishes a strong foundation for investigating the potential health benefits of *M. koenigii*. Insights from this study could be utilized to develop functional foods, supplements, or therapeutic agents harnessing the nutritional and bioactive potential of these leaves. As the nutraceutical and natural remedy field expands, these findings hold promising implications for innovative interventions that contribute to global health and wellbeing.

Acknowledgements

None.

Conflict of Interest

None.

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