INTEGRATIVE PHARMACOGNOSTICAL PROFILING & *IN VITRO* ANTIBACTERIAL ACTIVITY ASSESSMENT OF *MURRAYA KOENIGII* (L.) SPRENG

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The evolution of multi drug-resistant bacterial diseases enforces the need for new botanical anti-microbiological drugs. Curry leaf (M*urraya koenigii L. Spreng*) is often used in traditional medicine; however the pharmacognosic effects and extraction efficiency need to be affirmed meticulously. The study aimed at qualitative phytochemical profiling and physicochemical standardization of *M. koenigii* leaf powder by aqueous, ethanolic and methanolic solvent systems, conducted for the first time. MICs were measured by microdilution to determine antibacterial activities against a panel of clinically relevant pathogens; the disc diffusion method was used for microbiological analysis. Tukey's post-hoc test was used after one-way ANOVA to examine statistical differences. Physicochemical examination showed a total ash value of 15.4% ± 0.3% w/w and a low moisture content of 4.2% ± 0.3% w/w. We detected bioactive secondary metabolites, including as tannins, alkaloids, and, flavonoids were validated by phytochemical screening, with alcoholic solvents showing higher extraction yields than water. The ethanolic extract showed strong antibacterial activity against *Proteus mirabilis* (21.0 mm) and *Staphylococcus aureus* (21.0 mm) in disc diffusion experiments, but the aqueous extract was totally inactive (0.0, $p < 0.001$). *Bacillus* sp. and *Streptococcus mutans* showed the lowest MIC values for the ethanolic extract (0.156 mg/mL). These results validate *M. koenigii's* potential development as a natural therapeutic agent by establishing pharmacognostic quality markers for the plant and showing that its ethanolic extract contains extremely powerful antibacterial agents, especially effective against Gram-positive clinical isolates.

Keywords: *Murraya koenigii*, antibacterial activity, pharmacognosic standardization, phytochemical screening, minimal inhibition

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КОМПЛЕКСНЫЙ ФАРМАКОГНОСТИЧЕСКИЙ АНАЛИЗ И ОЦЕНКА АНТИБАКТЕРИАЛЬНОЙ АКТИВНОСТИ *MURRAYA KOENIGII* (L.) SPRENG *IN VITRO*

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Эволюция вызываемых бактериями с множественной лекарственной устойчивостью заболеваний обусловливает потребность в новых противомикробных препаратах на основе растительного сырья. Листья карри (*Murraya koenigii* L. Spreng.) часто используют в традиционной медицине, однако необходимо подтвердить их фармакологическое действие и эффективность экстракции. Цель исследования: провести качественное фитохимическое профилирование и физико-химическую стандартизацию порошка листьев карри с применением систем с водой, этанолом и метанолом в качестве растворителей. МПК измеряли методом микроразведений, чтобы определить наличие антибактериальной активности в отношении панели клинически значимых патогенов. Для микробиологического анализа использовали диско-диффузионный метод. Для оценки статистических различий после однофакторного дисперсионного анализа использовали апостериорный тест Тьюки. При проведении физико-химического анализа установлены содержание золы общей 15,4% ± 0,3% (по массе) и низкое содержание влаги, составившее 4,2% ± 0,3% (по массе). Выявлены биологически активные вторичные метаболиты, в том числе танины, алкалоиды и флавоноиды, наличие которых подтверждено при проведении фитохимического скрининга. Применение спиртовых растворителей продемонстрировало большую эффективность экстракции по сравнению с водой. Этанольный экстракт показал высокую антибактериальную активность в отношении *Proteus mirabilis* (21,0 мм) и *Staphylococcus aureus* (21,0 мм) в экспериментах с применением диско-диффузионного метода, а водный экстракт продемонстрировал полное отсутствие активности (0,0; $p < 0,001$). У *Bacillus* sp. и *Streptococcus mutans* были самые низкие значения МПК при применении этанольного экстракта (0,156 мг/мл). Полученные результаты подтверждают возможность разработки *M. koenigii* как природного лекарственного средства путем определения фармакогностических маркеров качества растения и демонстрации факта, что его этанольный экстракт содержит чрезвычайно мощные антибактериальные вещества, особенно эффективные против клинических изолятов грамположительных бактерий.

Ключевые слова: *Murraya koenigii*, антибактериальная активность, фармакогностическая стандартизация, фитохимический скрининг, минимальное ингибирование

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Multidrug-resistant (MDR) microorganisms are becoming more common, which is making the worldwide burden of bacterial illnesses worse. Growing bacterial resistance highlights the need for improved surveillance and novel therapies since it presents significant global health challenges [1].

Natural products made from plants have shown promise in resolving this issue. Compared to single-target synthetic medications, medicinal plants offer a rich supply of structurally diverse bioactive chemicals with numerous modes of action, which may lower the risk of resistance development Herbal

remedies are particularly popular in developing countries, where almost eighty percent of the people receives primary care from traditional natural plant medicines [2].

Murraya koenigii (L.) Spreng. is a tropical to subtropical tree that belongs to the Rutaceae family and is indigenous to Asia, particularly India and Sri Lanka [3]. Commonly referred to as curry leaf plant or kari patta, this evergreen tree is grown for its aromatic leaves that are a vital ingredient in South Asian cooking. Apart from its use in culinary practices, *M. koenigii* has been extensively reported as a traditional remedy for the management of many conditions in systems such as Ayurveda and Unani [4]. Historically the leaves were utilized internally as a tonic, stomachic, carminative, antihelminthic and for skin eruptions as well as vomiting [5].

The potential pharmacological properties of *M. koenigii* are attributed to its high phytochemical profile. Many secondary metabolites that produced by the plant include carbazole alkaloids, terpenoids, flavonoids, essential oils and polyphenols [6]. Koenigine, mahanimbine, and murrayanine belong to the class of alkaloids with carbazole skeletons, this composition being responsible for their diverse biological activities such as antioxidant, antibacterial, and anticancer abilities [7]. Recent scientific research has confirmed many conventional claims. Studies have demonstrated *M. koenigii's* significant anti-inflammatory, antibacterial, antioxidant, antidiabetic, and cytotoxic qualities. Scientific research has recently proved a great many old wives' tales. *M. koenigii* has been shown to possess considerable anti-inflammatory, antibacterial, antioxidant, antidiabetic and cytotoxic properties [8]. Additionally, the plant has shown potential for treating biofilm-associated infections [9]. Which is a crucial component of important antibiotic-resistant chronic bacterial disease. Pharmacognostic standardization is essential to guarantee the identity, quality and purity of herbal medicines [10].

The WHO states that defining pharmacognostic parameters — such as macroscopic and microscopic traits, physicochemical constants, and phytochemical profiles — is essential for assessing the quality of medicinal plants [11]. There are still few comprehensive investigations on the leaves of *M. koenigii*, however previous pharmacognostic research has focused on the stems and roots [12, 13].

The same is the case with the content of essential oil of *M. koenigii* which has been one subject for number of studies which indicates considerable variation based on the plant geographical origin. β-caryophyllene (16.6 to 26.6%) and α-humulene (15.2 to 26.7%) were the two primary volatile metabolites detected in Malaysian specimens [13]. Headspace analysis revealed that 27.4% of the constituents were β-caryophyllene and 37.5% were α-pinene which were the most common compounds in previous studies [14]. Linalool (32.83%), elemol (7.44%), geranyl acetate (6.18%), and myrcene (6.12%) were the primary components of the essential oil from southern India [15]. We aimed to analyze *Murraya koenigii* leaves comprehensively towards an integrated pharmacognostic approach for evaluation of antibacterial activity: MIC determination and in vitroantibacterial efficacy against clinically relevant pathogens.

METHODS

Authentication and Collection of the Plant Material

The leaves of *Murraya koenigii* were collected from the Sulaymaniyah Botanical Garden, Sulaymaniyah Iraq, in April 2025 and dried in the shade. The The plant material was identified and authenticated by a taxonomist by the earlier reported method [16]. The dried leaves were ground to a particle size of 250–500 μm. Extraction was performed using 70% ethanol at a raw material-to-solvent ratio of 1 : 10 (w/v) via maceration for 24 hours at room temperature. The evaporation process was then used to filter the extracts and concentrate [14].

Pharmacognostic Assessment

Parameters of Physicochemistry

Physicochemical analysis was performed on powdered leaf material in accordance with WHO recommendations for herbal medication quality control. Among the parameters established were: Examining foreign organic stuff visually and separating unnecessary materials. Determine the moisture content (loss on drying) by drying the powder at 105°C until its weight remains constant [11].

Acid-insoluble, water-soluble and total ash were measured by burning at 450°C in a muffle furnace [10].

Screening for Phytochemicals

To find saponins, tannins, steroids, terpenoids, flavonoids, cardiac glycosides, alkaloids, resins, amino acids, & other secondary metabolites, both the aqueous and solvent extracts underwent qualitative phytochemical screening using conventional techniques [17].

Antibacterial Action Assesment

Strains of Bacteria

A panel of Gram-positive and Gram-negative bacteria obtained from microbiology department, Thi Qar university, was used to evaluate the antibacterial actions against: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Proteus mirabilis*, and *Enterobacter aerogenes*.

Method of Disc Diffusion

The agar well diffusion method [18] was first used to screen the extracts for antibacterial actions. Mueller–Hinton agar plates were covered with bacterial cultures that had been cultivated overnight and adjusted to the 0.5 McFarland standard. The infected agar surface was covered with wells or discs that had been impregnated with different amounts of test samples. Positive controls were standard antibiotic discs. The zone of inhibition's diameter was determined in millimeters after that we incubated the plates for 24 hrs at 37 °C [19, 20]. Three replicates' means are used to calculate the values.

Determining the MIC

We performed the broth microdilution (two-fold) method to calculate the extracts' MIC. The test samples were serially diluted two times in microtiter 96-well plates. A bacterial suspension (about 1×10^2 CFU/mL) was added to each well. For a whole day, we incubated the plates at 37 °C. The minimum concentration exhibiting no discernible growth of bacteria was identified as the minimum inhibitory concentration (MIC) [21].

Table 1. The Physicochemical parameters of *M. koenigii* leaf

Parameter	Value (% w/w)
Foreign organic matter	< 1%
Moisture content	4.2 ± 0.3
The total ash	15.4 ± 0.3
The acid-insoluble ash	4.7 ± 0.2

Statistics Analysis

Every experiment was carried out in triplicate, and the mean ± standard deviation (SD) was used to express the results. One-way analysis of variance (ANOVA) and Tukey's post-hoc test for multiple comparisons were used in the statistical study. Statistical significance was defined as a *p*-value of less than 0.05. Each table lists the statistical tests that were applied [2, 19].

RESULTS

Physicochemical Parameters

Table 1 displays the determined physicochemical properties of the powdered leaf material.

The medicine was devoid of additional plant parts, molds, insects, and other animal contaminants because the foreign organic matter was less than 1% w/w. The dried leaf samples had a moisture level of roughly 4.2% w/w. The drug's dampness promotes the growth of common bacteria and mold, and inadequate drying results in the enzymatic breakdown of active ingredients. Therefore, the sample's low moisture level suggests that the leaves were properly dried to make storage easier.

The obtained total ash value was approximately 15.4% w/w. The low ash levels suggest that the plant medication has a low inorganic content. The crude medicine was less contaminated by inorganic elements like sand or soil, as indicated by the acid-insoluble ash value of roughly 4.7% w/w.

Screening for Phytochemicals

Several types of bioactive chemicals were found in the aqueous and solvent extracts using qualitative phytochemical screening (Table 2). Standard phytochemical tests were used for qualitative analysis. The mean of three separate experiments is shown in the results.

Tannins, saponins, steroids, terpenoids, and cardiac glycosides were found in the aqueous leaf extract according to phytochemical study. A more comprehensive profile with additional resin detection was displayed by the methanolic leaf extract.

Antibacterial Activity

Disc Diffusion Results

Table 3 and Figure show the antibacterial actions of *Murraya koenigii* ethanol and aqueous extracts of leaves against eight clinically relevant bacterial infections. Zones of inhibition are shown as mean ± standard deviation. While no activity (0.0 mm) was detected regarding the aqueous extract against any of the test organisms, the ethanol extract displyed strong antibacterial action against all the tested organisms, where inhibition zones ranged from 13 mm against Klebsiella pneumoniae to 21 mm against Staphylococcus aureus and Proteus mirabilis. The F-values ranged beyween 881.25 (*S. aureus*) and 433.50 (*K. pneumoniae*) which showed significant differences among the examined treatment groups for all the used bacterial strains.

We detected comparable activities between the positive control group regarding *B. subtilis* (20 mm/20 mm) and *S. aureus* (21 mm / 20 mm) In contrary, we detected significantly inferior action against Gram-negative organisms such as *K. pneumonia*, *E. coli*, *S. typhi*, *P. aeruginosa*, *P. mirabilis*, and *E. aerogenes* ($p < 0.05$).

The MIC values of the extracts against various test pathogens are presented in Table 4. *Bacillus sp*. and *S. mutans* showed the highest activity for the ethanolic extract, where MICs were 0.156 mg/mL, for both strains. MIC readings of higher concentration (0.625 mg/mL) were detected for *S. typhi*, E. coli, and *S. aureus*, without any efficacy of aqueous extract.

DISCUSSION

The physicochemical factors of our study provide reliable markers for recognizing adulteration & confirming batch-to-batch constancy in herbal medicinal mixes. According to our results, the foreign organic matter amount was < 1% w/w, which indicates that the medication didn't contain contamination from molds, insects, other animal elements or other plant parts [11]. This is a vital criterion of quality because using foreign ingredients may compromise the herbal and safety efficacy.

A content of moisture of 4% w/w in our results is crucial for the herbal remedy's stability. The high moisture levels may

Table 2. Phytochemical constituents detected in *M. koenigii* leaf extracts

Phytochemical Class	Aqueous Extract	Methanolic Extract	Ethanol Extract
Tannins	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Resins	–	+	+
Amino acids	+	+	+

Note: + — present, – — absent

Table 3. The antibacterial zones of inhibition of *Murraya koenigii* ethanol and aqueous extracts of leaves against tested bacterial

Test Organism	Ethanol Extract (mm)	Aqueous Extract (mm)	Positive Control Gentamicin 10 µg (mm)	*F*	*p*
Staphylococcus aureus	21	0	20	881.25	< 0.001
Escherichia coli	14	0	16	456	< 0.001
Pseudomonas aeruginosa	17	0	19	625.33	< 0.001
Salmonella typhi	16	0	18	522	< 0.001
Bacillus subtilis	20	0	20	600	< 0.001
Klebsiella pneumoniae	13	0	17	433.5	< 0.001
Proteus mirabilis	21	0	23	738	< 0.001
Enterobacter aerogenes	14	0	20	526	< 0.001

boost the appearance of common molds and bacteria, and active breakdown of substances. The low content of moisture in our sample shows that adequate drying were performed to enable storage and protect bioactive components [11].

The total ash value (15.4% w/w), offers an increase mineral content of the drug. The adequate ash levels exhibit that the inorganic material of the plant is not high, which is a good sign. The acid insoluble ash concentration (4.8% w/w) shows insignificant contamination of the crude product by sand or soil [10].

These pharmacognostic variables are consistent with studies conducted in different regions. Gujarati studies found that the moisture content was 1.12% ± 0.03% w/w and 1.31% ± 0.12% w/w, the acid-insoluble ash content was 0.59% ± 0.01% w/w and 0.20% ± 0.02% w/w, and the total ash content was 15.66% ± 0.32% w/w (Anand) and 12.29% ± 0.08% w/w [12]. The little differences that can be attributed to geographic factors, soil composition, and agricultural practices emphasize the need for regional pharmacognostic criteria.

According to earlier research, phytochemical screening was used to identify a variety of bioactive compounds [17]. These compounds are recognized for their antimicrobial properties and contribute to the plant's overall antibacterial activity.

Tannins have a remarkable bactericidal effect. Because they can bind to proteins and stop the formation of bacterial cell walls, tannins are polyphenolic compounds with bacteriostatic & bactericidal qualities. They can hinder the formation of biofilms by creating complexes with bacterial adhesins, according to numerous studies [22]. Saponins can cause cell lysis and compromise the integrity of bacterial cell membranes due to their amphiphilic properties [23]. Steroids and terpenoids are well known for their membrane-active properties and ability to inhibit bacterial enzymes [24].

Also in accordance with our results other studies showed that the antibacterial activity of *M. koenigii* extracts shown significant efficacy against a variety of therapeutically relevant pathogens, including both Gram-positive and Gram-negative bacteria [2, 25, 26]. The qualitative phytochemical screening of *Murraya koenigii* leaf extracts revealed that while resins were only present in the organic solvent extracts saponins, tannins, terpenoids, steroids, flavonoids, cardiac glycosides, amino acids, and alkaloids were present in all three extracts (aqueous, methanolic, and ethanolic). These different phytochemical groups are consistent with previous reports on *M. koenigii* that identified terpenoids, tannins, flavonoids and carbazole alkaloids, as unique components of this medicinal plant [27].

The complete absence of resins in the aqueous extract and their presence in both methanolic and ethanolic extracts show the superior extraction efficiency of organic solvents for non-polar bioactive compounds, supporting the notion that methanol and ethanol are better solvents for extracting a wider range of secondary metabolites than water [28]. In consistence with

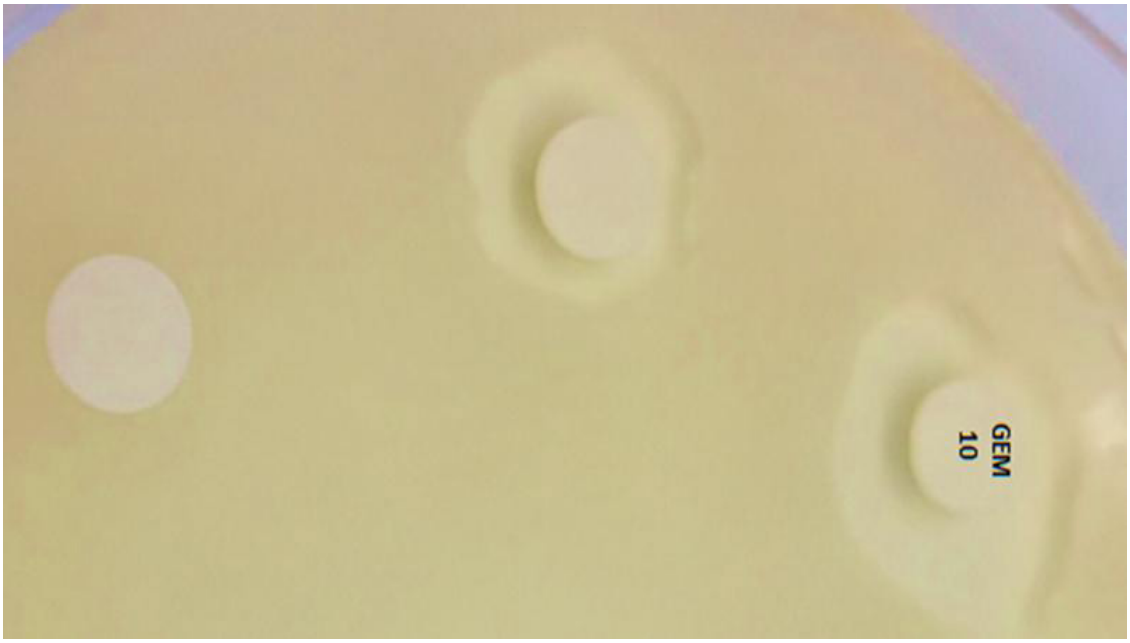

Fig. Showing different inhibition zones of aqueous, ethanolic, and positive control

Table 4. MIC values of *M. koenigii* extracts against test pathogens

Test Organism	Ethanol Extract	Aqueous Extract
S. mutans	0.156	–
S. aureus	0.625	–
E. coli	0.625	–
Salmonella typhi	0.625	–
Salmonella sp.	0.312	–
Bacillus sp.	0.156	–

previous studies, this study tested the phytochemical character and antibacterial action of *Murraya koenigii* (curry leaf) extracts. Flavonoids, alkaloids, phenols, steroids, terpenoids, glycosides, tannins, and saponins were the detected bioactive ingredients discovered in the ethanol extract, giving to phytochemical study. High alkaloid components were discovered a upon comparing to other therapeutic plants [27], which is consistent with our profile [27].

Disc diffusion and MIC techniques were used to evaluate the antibacterial effectiveness against 8 bacterial species. With inhibition zones ranged from 21.0 mm to 13.0 mm, the ethanol extract presented variable antibacterial activity. Stimulatingly, none of the tested bacteria were influenced by the aqueous extract. This may be due to water's weak ability to absorb non polar bioactive materials with antibacterial potentials, such as terpenoids, carbazole alkaloids, and flavonoids.

The ethanol extract's effecay against Gram-positive infections was established by the fact that its efficiency against *Bacillus subtilis* and *S. aureus* was equivalent to that of the positive control results. The earlier reported results [27] are consistent with our work. The thick peptidoglycan layer cell walls of Gram-positive bacteria, which endorses compound penetration in contrary to the outer membrane permeability barrier of Gram-negative bacteria, clarifies the detected difference in susceptibility, with Gram-positive bacteria being more susceptible.

The plant extract exhibited *Pseudomonas aeruginosa* (17.0 mm inhibition diameters), a common cause of hospital-acquired infections because of its resistance to antibiotic and biofilm production, was one of the clinically challenging bacteria against which the used extract had distinguished action. A detailed review (2024) was maintained by the presence of moderate activity against *S. typhi* and *E. coli*. Also, activity against *P. mirabilis* and *Enterobacter aerogenes* indicated the extract potential of broad-spectrum activity confirming the previous findings that the organic solvent extracts are better than aqueous ones [29].

Strong action was detected against *Bacillus* sp. and *Streptococcus mutans* (MIC = 0.156 mg/mL), with the influence against *S. mutans* being particularly notable owing to its contribution in dental caries. Higher MIC values were establish for Gram-negative and Gram-positive bacteria, such as *E. coli* and *S. typhi* and *S. aureus* (0.625 mg/mL), which is consistent with previous research. The traditional usage of curry leaves for gastrointestinal troubles is supported by the MIC against *Salmonella* sp. (0.312 mg/mL).

CONCLUSIONS

The combination of numerous phytochemicals, such as terpenoids, alkaloids, tannins, and flavonoids which disturb the bacterial cell membranes and enzymes, is accountable for the antibacterial action. The study supports *M. koenigii's* traditional usage by efficiently establishing pharmacognosic standards for the plant and viewing that its ethanol extract has strong, and broad-spectrum antibacterial action equivalent with other antibiotics. Although more *in vivo* and clinical studies is required, the results support the development of *M. koenigii*-based natural antibacterial products and emphasize the superiority of ethanol extraction solvent.

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