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Phytochemical Profiling and Bioactivity of Murraya Koenigii Shoots: Antimicrobial, Antioxidant, and Anticancer Potentials of Solvent Extracts

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Abstract

Medicinal plants have long been integral to both traditional and modern medicines because of their wide range of bioactive chemicals. Murraya koenigii (Curry leaf) is also one of these well-known plants and is notable for its therapeutic properties. In the current study, five solvents of varying polarity were used to prepare solvent-specific extracts of M. koenigii shoots to evaluate the effect of the extraction medium on bioactivity. The antimicrobial, antioxidant, and anticancer properties were assessed systematically. Different bioactivity profiles exhibited that the yield and potency of bioactive components are significantly influenced by solvent polarity. Extracts with greater antibacterial activity, antioxidant capacity, and anticancer potential were regularly generated by polar solvents, especially methanol and ethanol. Gallic acid, quercetin, ferulic acid, and sinapic acid were among the rich phenolic profiles assessed by HPLC, and the methanol extract demonstrated the best DPPH radical scavenging activity and total phenolic content. Additionally, ethanol extract demonstrated potent antibacterial and antifungal action, particularly against Candida albicans and Klebsiella pneumoniae. It's interesting to note that, while being typically less active, the non-polar hexane extract exhibited the best $ABTS^{+}$ radical inhibition. This surprising result highlights the selective extraction of non-polar antioxidant chemicals. Methanol extract demonstrated the highest anticancer impact (lowest cell survival at 416 µg/mL), while all extracts showed dose-dependent cytotoxicity against HeLa cancer cells. Overall, these findings showed that the biochemical landscape and bioactivity of M. koenigii extracts are shaped by solvent polarity, underscoring the strategic significance of solvent selection in optimizing therapeutic potential for pharmaceutical and nutraceutical applications.

Keywords: Murraya koenigii; Phytochemicals; Antimicrobial; Cytotoxicity; HPLC

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Introduction

Medicinal plants have long been used to treat various human diseases due to their therapeutic compounds, and today, nearly 88% of the global population relies on plant-based remedies (Chaachouay and Ziadane 2024). Many modern drugs, like quinine and artemisinin, originated from traditional herbal practices. These plants offer a wealth of biologically active molecules effective against infections, oxidative stress, and chronic conditions like cancer (Ceravolo et al. 2021). Overuse of synthetic drugs and their side effects, along with rising antibiotic and antifungal resistance, has increased interest in natural alternatives (Muteeb et al. 2023). Fungal and bacterial pathogens, such as *Candida albicans*, *E. coli*, and *Staphylococcus aureus*, continue to pose health risks (Carolus et al. 2019). Oxidative stress caused by free radicals contributes to numerous diseases, including cancer, prompting the search for effective natural antioxidants (Chandimali et al. 2025). Synthetic antioxidants, once common in foods, are now being phased out due to safety concerns, shifting focus to safer plant-derived compounds (Yildiz et al. 2025). Medicinal plants are valued for their antimicrobial, nutraceutical, and pharmaceutical potential, being rich in secondary metabolites like tannins, alkaloids, and flavonoids. Traditional healers often claim these treatments are more affordable, effective, and have fewer side effects compared to synthetic drugs (El-Saadony et al. 2025). With rising interest in plant-derived substances, the World Health Organization supports the validation and integration of phytotherapy into public health programs, recognizing that many essential drugs have plant origins and encouraging ongoing research to combat antimicrobial resistance (Iduh et al. 2024). Among these, *Murraya koenigii* (curry leaf) is notable for its antioxidant, anti-inflammatory, and anticancer properties, traditionally used for ailments ranging from digestive issues to skin infections (Balakrishnan et al. 2020). The current study is designed to determine the bioactivity of *M. koenigii* shoots dependent on solvent polarity, and the goal is to identify the therapeutic potential of each fraction by systematically correlating the phytochemical profile of successive extracts with their corresponding antimicrobial, antioxidant, and anticancer potencies.

Materials and Methods

Procurement of samples and preparation of extracts

Murraya koenigii plant samples were collected from Soon Sakesar Valley, Chakwal, Pakistan, and taxonomically verified with a voucher specimen (DACB 34359) stored at the National Herbarium, Dhaka, Bangladesh. Shoots were cleaned, shade-dried, and powdered. 100g of the powdered sample was extracted using solvents (methanol, ethanol, acetone, n-hexane, dichloromethane (DCM)) with agitation for 6 hours at 200 rpm on an orbital shaker. Filtration was done using Whatman No. 1 filter paper, and solvents were evaporated under reduced pressure at 40°C using a rotary evaporator.

Evaluation of Antimicrobial Potential

Test microorganisms and culture media preparation.

Strains tested included *E. coli*, *S. aureus*, *B. cereus*, *P. aeruginosa*, *S. typhi*, *K. pneumoniae*, and *Candida albicans* were obtained from the Microbiology lab of the University of Lahore. Nutrient agar and Sabouraud's Dextrose agar were prepared, autoclaved and poured into sterilized petri plates to solidify. Petri plates were inoculated with bacterial or fungal cultures to prepare a microbial lawn for testing.

Antimicrobial Assay

The disc diffusion method was used. A volume of 30 µl of extract (1700 µg/ml) was loaded on sterile discs and incubated. Tetracycline and Dimethyl sulfoxide (DMSO) served as positive and negative controls. Results were recorded based on zones of inhibition (mm), and experiments were repeated for accuracy.

Antioxidant Potential

$ABTS^+$ Scavenging Assay and DPPH Scavenging Activity

1. 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^+$) radicals were generated and reacted with extracts. Absorbance at 734 nm was used to calculate % inhibition.
2. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution was mixed with extracts. After incubation, absorbance at 517 nm was measured to determine radical scavenging activity.

Anticancer Activity

Anticancer activity was evaluated by MTT Assay. HeLa cancer cells were cultured under standard conditions. Cells were treated with various concentrations of extracts (26–416 µg). After 24 hours, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) reagent was added. Optical density was measured at 570 nm to calculate % cell viability.

Quantification of TPC

Total Phenolic Content (TPC) was assessed using the Folin-Ciocalteu method. Extracts were reacted with the reagent and sodium carbonate, and absorbance was measured at 735 nm. Results were compared with standard antioxidants Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT) and Ascorbic acid.

HPLC-Based Phenolic Quantification

Sample Preparation and HPLC Conditions

Crude extracts were hydrolyzed with HCl and methanol, incubated at 90°C, and filtered for high-performance liquid chromatography (HPLC) analysis. Separation was performed using a C18 column and a mobile phase of tetrahydrofuran/acetonitrile/phosphoric acid. Detection was at 280 nm with a flow rate of 1 ml/min and an injection volume of 10 µL.

Statistical Analysis

The experiments were repeated in triplicate to obtain reliable results, and mean values were used for further analysis. Data are presented as mean ± SD of three independent replicates (n=3) and were analyzed by one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ considered significant.

Results

Antibacterial and Antifungal Activity of **Various Organic** Extracts of ***Murraya koenigii*** **Shoots.**

Murraya koenigii shoot solvent extracts were tested for antimicrobial efficacy, and the results of the zone of inhibition (ZOI) for each extract are summarized in Table 1. All of the extracts showed different levels of inhibition. The DCM extract's n-hexane extract had the maximum activity against Bacillus cereus (14.5 mm) and the lowest activity against Staphylococcus aureus (11 mm). Overall, the results point to a considerable influence of solvent type on Murraya koenigii shoots' antimicrobial capability.

Table 1: ZOI for six different organic extracts of Murraya koenigii shoots against six pathogenic microbial species.

Microorganisms	Mean ZOI (mm) ± SD				
	Methanol extract	Acetone extract	Ethanol extract	n-Hexane extract	DCM extract
Escherichia coli	12.75 ±0.5	12.5± 0.5	12.6± 0.5	13.0± 0.5	12.16 ±0.5

Staphylococcus aureus	11.5± 0.5	13.33 ±0.5	13.0± 0.5	12.5± 0.5	12.33 ±0.5
Pseudomonas aeruginosa	12.83 ±0.5	13.66 ±0.5	14.0± 0.5	12.5± 0.5	12.5± 0.5
Salmonella typhi	12.83 ±0.5	12.25 ±0.5	12.75 ±0.5	14.0± 0.5	11.0± 0.5
Bacillus cereus	14.0± 0.5	13.5± 0.5	13.66 ±0.5	14.5± 0.5	14.0± 0.5
Klebsiella pneumoniae	12.25 ±0.5	12.5± 0.5	14.0± 0.5	14.0± 0.5	11.75 ±0.5
Candida albicans	12.5± 0.5	11.75 ±0.5	13.75 ±0.5	13.25 ±0.5	13.5± 0.5

Methanol extract of *Murraya koenigii* shoots exhibited notable antibacterial activity against six bacterial strains. The inhibition zones were: *E. coli* (12.75 mm), *S. aureus* (11.5 mm), *P. aeruginosa* (12.83 mm), *S. typhi* (12.83 mm), *B. cereus* (14 mm), and *K. pneumoniae* (12.25 mm). Maximum activity was recorded against *B. cereus* (14 mm), and the lowest against *S. aureus* (11.5 mm).

The acetone extract showed moderate antibacterial activity, with inhibition zones against *E. coli* (12.5 mm), *S. aureus* (13.33 mm), *P. aeruginosa* (13.66 mm), *S. typhi* (12.25 mm), *B. cereus* (13.5 mm), and *K. pneumoniae* (12.5 mm). The highest inhibition was observed for *P. aeruginosa* (13.66 mm), while the least was for *S. typhi* (12.25 mm).

Ethanol extract displayed good antibacterial activity. Zones of inhibition were: *E. coli* (12.6 mm), *S. aureus* (13 mm), *P. aeruginosa* (14 mm), *S. typhi* (12.75 mm), *B. cereus* (13.66 mm), and *K. pneumoniae* (14 mm). Maximum inhibition was against *P. aeruginosa* and *K. pneumoniae* (14 mm).

N-Hexane extract demonstrated strong antibacterial potential with inhibition zones of: *E. coli* (13 mm), *S. aureus* (12.5 mm), *P. aeruginosa* (12.5 mm), *S. typhi* (14 mm), *B. cereus* (14.5 mm), and *K. pneumoniae* (14 mm). The highest activity was against *B. cereus* (14.5 mm).

The DCM extract showed inhibition zones of: *E. coli* (12.16 mm), *S. aureus* (12.33 mm), *P. aeruginosa* (12.5 mm), *S. typhi* (11 mm), *B. cereus* (14 mm), and *K. pneumoniae* (11.75 mm). Maximum activity was against *B. cereus* (14 mm), while the least was against *S. typhi* (11 mm).

Five extracts (acetone, DCM, ethanol, n-hexane, and methanol) showed antifungal activity against *Candida albicans*. The zone of inhibition ranged from 11.75 mm (acetone) to 13.75 mm (ethanol). Ethanol extract exhibited the highest antifungal effect.

Antioxidant Activity of Various Extracts

DPPH Free Radical Scavenging Activity

Methanol extract showed the highest DPPH scavenging activity (12%), followed by acetone (9.5%), DCM (6.5%), ethanol (6%), and hexane (2.8%). All were significantly lower than standards (Ascorbic acid – 89%, BHA – 76.6%, BHT – 75.05%) *(Figure 1).*

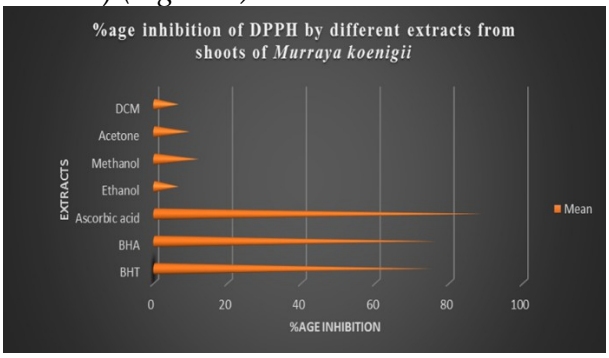

Figure 1: Bar graph representation of the comparison of the percentage inhibition of DPPH free radical scavenging potential of different solvent extracts of *Murraya koenigii* shoots.

ABTS$^+$ Radical Scavenging Activity

The ABTS$^+$ scavenging activity was: hexane (91.85%), DCM (90.15%), acetone (89.15%), methanol (88.6%), and ethanol (81.2%). Hexane extract demonstrated the highest antioxidant potential, even exceeding BHT (91.4%) *(Figure 2).*

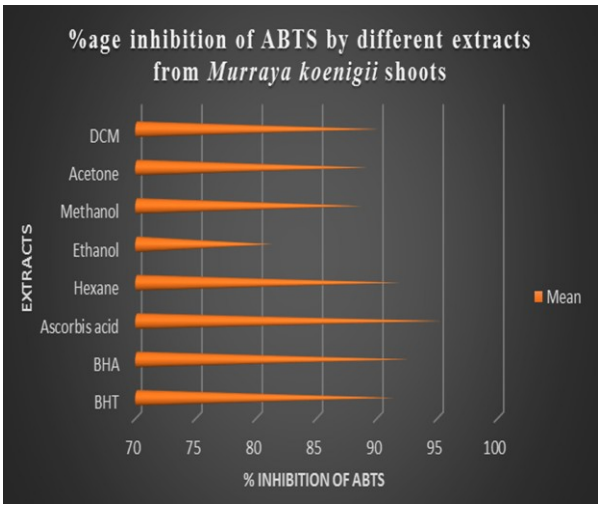

Figure 2: Represents the comparison of the percentage inhibition of ABTS+ free radical scavenging potential of different solvent extracts of *Murraya koenigii* shoots.

Anticancer Activity Against HeLa Cell Line (MTT Assay)

A range of different doses (26µg/ml, 52µg/ml, 104µg/ml, 208µg/ml and 416µg/ml) of five different solvent extractions of *Murraya koenigii* shoots (Acetone, DCM, Ethanol, n-hexane, Methanol) were used. All extracts showed dose-dependent anticancer activity against HeLa cells. Cellular viability with acetone extract ranged from 97.24% (26 µg/mL) to 68.64% (416 µg/mL). *(Figure 3)*

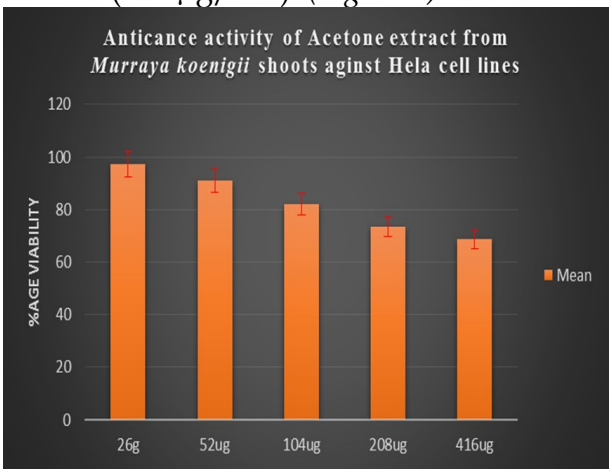

Figure 3: Anticancer activity of Acetone extract from *Murraya koenigii* shoots against the *HeLa* cell line

DCM extract showed good anticancer activity with a variable trend. Figure 4 exhibited anticancer activity of DCM extract against the *HeLa* cell line. According to the results, Viability decreased from 90.2% to 69.09%.

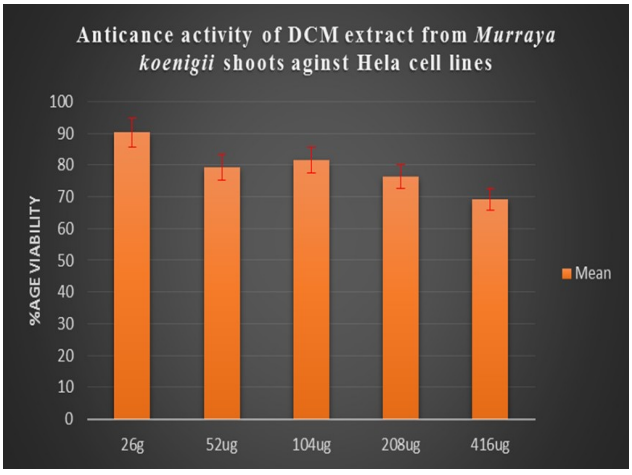

Figure 4: Anticancer activity of DCM extract from *Murraya koenigii* shoots against the *HeLa* cell line.

In Figure 5, the bar graph shows anticancer activity of ethanol extract of *Murraya koenigii* shoots against cancerous *HeLa* cell line with a variable trend**. The cellular v**iability was reduced from 98.37% to 72.46%.

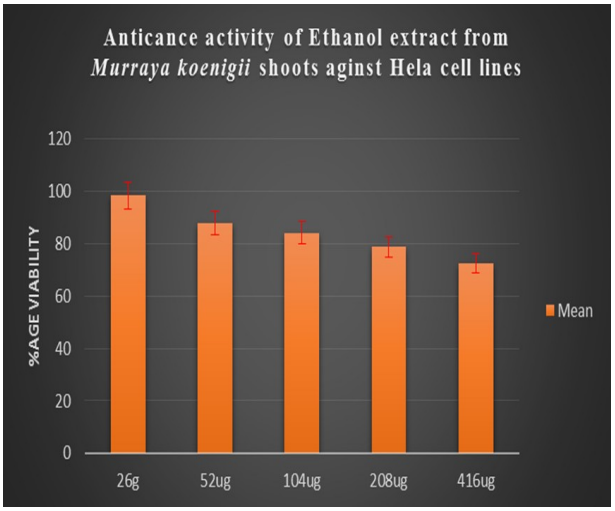

Figure 5: Anticancer activity of Ethanol extract from *Murraya koenigii* shoots against the *HeLa* cell line.

According to Figure 6, variations between values of %age cell viability were observed by the n-hexane crude extract. **The cellular** Viability ranged between 90.4%- 66.77%.

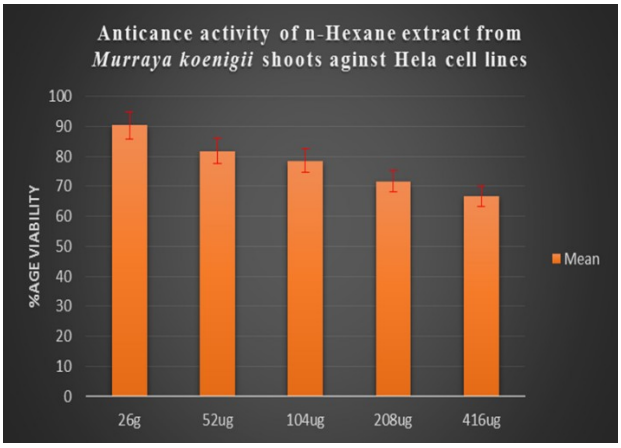

Figure 6: Anticancer activity of n-hexane extract from *Murraya koenigii shoots* against *the HeLa* cell line.

The bar graph in the figure. Figure 7 shows the anticancer activity of the methanol extract against the *HeLa* cell line. According to the results described in the pictorial view, the changes in values of %age viability showed a tremendous pattern. The **methanol extract** showed the strongest anticancer activity, with viability dropping from 85.36% to 59%.

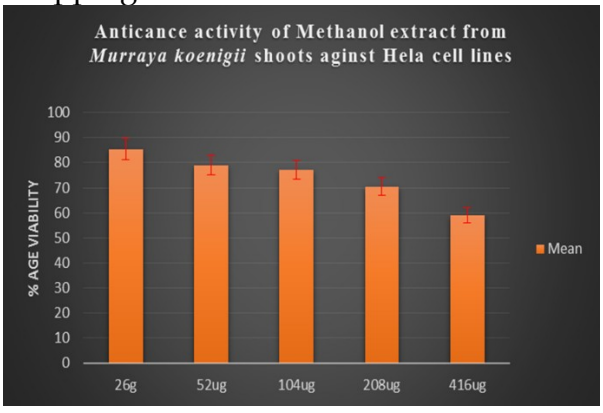

Figure 7: Anticancer activity of the Methanol extract from *Murraya koenigii* shoots against the *HeLa* cell line.

Total Phenolic Content

Total phenolic content (mg GAE/g) was highest in methanol extract (187.66), followed by ethanol (174.07), acetone (80.88), DCM (67.29), and hexane (40.71) *(Figure 8). This could be directly linked to anticancer activity patterns of these extracts as described above.*

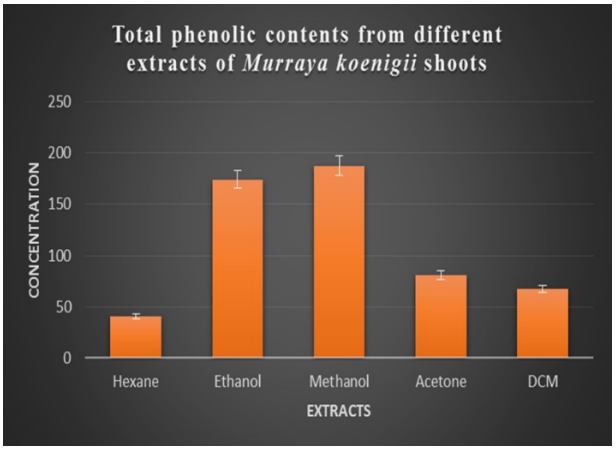

Figure 8: Total phenolic contents from different solvent extracts of *Murraya koenigii* shoots.

HPLC Analysis of Phenolic Compounds

Twelve phenolic compounds were identified and quantified via HPLC (Table 2). Notable findings are

- **Ferulic acid** was highest in ethanol (11.16 ppm).
- **Gallic acid** was also abundant in ethanol (5.32 ppm).
- **Trans-4-hydroxy-3-methoxy cinnamic acid** was dominant in DCM (5.7 ppm).
- **Methanol and hexane extracts** contained diverse phenolic profiles.

Table 2: Phenolic compounds in Murraya koenigii shoot extract by HPLC

Phenolics	Hexane extract (ppm)	Methanol extract (ppm)	Acetone (ppm)	DCM (ppm)	Ethanol (ppm)
Quercetin	1.78	0.15	0.18	0.19	1.007
Gallic acid	0.54	0.86	1.18	0.82	5.32
Chlorogenic acid	o.66	1.71	-	-	-
Ferulic acid	2.54	1.74	-	-	11.16
Sinapic acid	0.47	-	-	0.43	7.48
Vanillic acid	0.99	2.35	0.93	-	-

4-hydroxy-3-methoxy benzoic acid	1.82	-	-	-	-
p-Coumeric acid	0.45	-	-	0.26	-
m-Coumeric acid	-	0.37	1.8	0.48	
Trans-4-hydroxy 3-methoxy cinnamic acid	-	0.88	0.49	5.7	-
Caffeic acid	-	0.77	-	0.81	-
Syringic acid	0.49	-	0.8	0.55	-

Discussion

Murraya koenigii, a member of the Rutaceae family, has long been used in traditional medicine for various ailments. In this study, different solvent extracts of its shoots were evaluated for antibacterial, antifungal, anticancer, and antioxidant activities. Antibacterial assays revealed inhibition zones ranging from 11.5 to 14.5 mm. The acetone extract showed the highest activity against *Pseudomonas aeruginosa* (13.66 mm), whereas the lowest was observed against *Salmonella typhi* (12.25 mm), consistent with Dewangan et al. (2010). DCM extract exhibited the strongest inhibition against *Bacillus cereus* (14 mm) but the weakest against *Klebsiella pneumoniae* (11.75 mm), corroborating the findings of Granger et al. (2009). Similarly, hexane extract was most active against *Bacillus cereus* (14.5 mm), aligning with Kamsala et al. (2015). Ethanol extract showed strong activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (14 mm each), in agreement with Muthukrishnan et al. (2014). Methanol extract was most effective against *Bacillus cereus* (14 mm), supporting Baskaran et al. (2011).

For antifungal activity, ethanol extract demonstrated the strongest inhibition against *Candida albicans* (13.75 Mm), while acetone showed the least (11.75 mm), comparable to Zeidan et al. (2013).

Anticancer assays on HeLa cells revealed dose-dependent cytotoxicity across all extracts, with the methanol extract showing the strongest effect, reducing viability from 85.3% at 26 µg to 58.8% at 416 µg. Other extracts (hexane, acetone, DCM, ethanol) showed moderate cytotoxicity, though less pronounced. These findings are supported by Ali & Ahmad (2015) and Hung et al. (2014), who also reported strong anticancer effects of methanol extracts from other plants.

Antioxidant activity, assessed via DPPH and ABTS assays, indicated methanol as the most potent radical scavenger (12% DPPH inhibition), though still lower than standards BHA, BHT, and ascorbic acid (Al-Zubairi et al., 2011). ABTS inhibition values ranged from 81.2% to 91.85%, with hexane exceeding BHT. Variations were linked to solvent-dependent extraction of polyphenols and flavonoids (Metrouh-Amira et al., 2015). Methanol extract contained the highest phenolic content (187.66 mg GAE/g), followed by ethanol (174.07 mg GAE/g), consistent with Al-Owaisi et al. (2014).

HPLC analysis identified 13 phenolic compounds, with methanol and ethanol extracts showing the greatest diversity, including quercetin, gallic acid, ferulic acid, and chlorogenic acid. These findings align with McGaw et al. (2013) and El Baz et al. (2014), confirming high phenolic concentrations in methanol and ethanol extracts. The variety and concentration of

phenolic compounds found by HPLC are directly related to the antibacterial activity of Murraya koenigii extracts. The extracts with the largest inhibitory zones were those that were high in gallic acid, quercetin, ferulic acid, and vanillic acid, especially those that were made with methanol and ethanol. These phenolics are known to interfere with microbial growth and metabolism by chelating vital metal ions, disrupting bacterial cell membranes, and inhibiting the synthesis of nucleic acids (Dewangan et al., 2010; Granger et al., 2009).

The relationship between phenolic content and bioactivity is further supported by the cytotoxic effect of M. koenigii extracts against HeLa cell lines. The most noticeable dose-dependent decrease in cell viability was observed in the methanol extract, which exhibited the highest amounts of ferulic, caffeic, and chlorogenic acids. By modifying oxidative stress, mitochondrial malfunction, and caspase activation pathways, these substances are known to trigger apoptosis (Hung et al., 2014; Ali and Ahmad, 2015).

The phenolic content of extracts and their ability to scavenge radicals were found to be strongly correlated by antioxidant assays (DPPH and ABTS). Because of their larger total phenolic content and varied phenolic acid profiles, methanol and ethanol extracts showed improved free radical quenching. Effective hydrogen and electron donors, such as gallic acid, ferulic acid, and quercetin, can stabilize reactive radicals by creating resonance-stabilized intermediates (Nouman et al., 2015; Metrouh-Amir et al., 2015).

One of the study's main mechanistic findings is the strong positive connection between total phenolic content (TPC) and ABTS radical-scavenging activity. The n-hexane extract had the strongest ABTS inhibition (91.85%) and the highest TPC (187.66 mg GAE/g), demonstrating the critical role that phenolics play in determining antioxidant strength. By donating hydrogen atoms to neutralize $ABTS^{+}$ radicals, phenolic hydroxyl groups produce stable phenoxyl species that stop oxidative chain reactions. Through metal-chelation and electron-transfer processes, flavonoid coactivity and the redox behaviour and structural variety of phenolic acids intensify this effect (Krishnamoorthy & Subramaniam, 2014; Al-Owaisi et al., 2014). This robust TPC–ABTS connection explains the downstream effects of phenolic compounds on the extract's antimicrobial and anticancer bioactivities, highlighting them as the primary contributors to M. koenigii's antioxidant capacity.

Overall, methanol and ethanol extracts of Murraya koenigii demonstrated the strongest antibacterial, antioxidant, and anticancer activities, supporting their therapeutic potential.

Conclusion

The study provides a clear guide for selecting the optimal *M. koenigii* shoot extract based on the desired biological target. *The results* demonstrated significant **antimicrobial, antioxidant, and anticancer activities**, particularly in **methanol and ethanol extracts,** due to their high phenolic content. The choice of extraction solvent significantly influences the bioactivity, with polar solvents yielding more potent phytochemicals. The findings of our study establish an obvious relationship between the phenolic composition of *M. koenigii* shoots and observed biological activities. These findings support the therapeutic potential of *M. koenigii* and provide a foundation for future drug discovery and development.

Innovation Statement

This study creatively offers a simultaneous phytochemical and multi-bioactivity profile of *M. koenigii*. The research elaborated a previously unrecognized, distinct correlation between phenolic-dependent (DPPH, antimicrobial) and phenolic-independent (ABTS) antioxidant mechanisms of this plant. This comprehensive strategy gives the scientific community a proven, evidence-based justification for choosing particular extracts for focused therapeutic uses against cancer, oxidative stress, and infectious illnesses.

Recommendations

The bioactivities observed in vitro can be confirmed through in vivo animal model studies to assess the pharmacokinetics, bioavailability, and safety profiles of the most potent extracts or compounds. Furthermore, the primary bioactive compounds of organic fractions can be analyzed to determine their chemical composition and their pro-apoptotic pathways that contribute to the cytotoxicity observed in cell lines.

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