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Biological Activities of *Murraya koenigii*

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ABSTRACT

Murraya koenigii (L.) Spreng is rich in bioactive phytochemicals. The methanol extract of the plant was evaluated for its phytochemical composition and biological activities. Our results showed the presence of alkaloids, glycosides, tannins, triterpenoids, steroids, proteins, amino acids, coumarins, and quinones. The quantitative analysis of the extract revealed the presence of phenolic and flavonoid compounds. The extract exhibited strong antioxidant activity. The extract also showed the metal-chelating and antiglycation activities. The antimicrobial evaluation of the ethyl acetate extract exhibited the antibacterial activities against *E. coli, P. aeruginosa, B. subtilis* and *S. enterica.*

Keywords: *Murraya Koenigii;* Phenolic and Flavonoids Contents; Antioxidant; Metal Chelation; Antiglycation; Antimicrobial Activities

Introduction

Medicinal plants have long been served as an indispensable source of bioactive compounds contributing to modern drug discovery and traditional healthcare systems worldwide [1]. The increasing prevalence of chronic diseases, coupled with the limitations of synthetic drugs, has renewed interest in the natural phytochemicals for safer therapeutic alternatives [2,3]. In recent years some medicinal plants are used for treatment of various cancers. These plant-derived substances have been linked to a variety of anticancer characteristics, such as proapoptotic, antiproliferative, antioxidant, antiangiogenic, antimetastatic, and anti-inflammatory effects [4]. *Murraya koenigii* (L.) Spreng locally known as curry leaf, is an aromatic plant belonging to the family Rutaceae and is native to South and Southeast Asia [5]. It is widely used both as a spice and as a traditional remedy in the Ayurvedic and Unani system of medicine for the treatment of various ailments such as diabetes, dysentery, inflammation and microbial infections [5,6]. Phytochemical studies have demonstrated that *M. koenigii* contains a number of clases of the secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, coumarins, steroids and phenolic acids [7]. Among these, carbazole alkaloids such as mahanimbine, girinimbine, and koenimbine have shown the antioxidant and antimicrobial properties [8]. The phenolic and flavonoid compounds contribute significantly to the free radical scavenging and metal-chelating activities of the plant, thus preventing oxidative stress–related cellular damage [9].

The oxidative stress is a key factor in the pathogenesis of aging, diabetes, cardiovascular and neurodegenerative diseases [10]. Hence, the identification of the potent natural antioxidants from the plant remains an active research priority. Furthermore, the metal-chelating activity of the plant is crucial in mitigating the metal-induced oxidative stress by sequestering transition metals such as iron and copper that catalyze radical formation [11]. The extract also exhibits significant antiglycation potential, where its phenolic constituents inhibit the formation of advanced glycation end products (AGEs), thereby reducing the risk of diabetic and aging-related complications [12]. Several *in vitro* studies have supported the ability of the extracts of the plant to inhibit protein glycation in a dose-dependent manner [13]. In addition to its antioxidant and antiglycation effects, the extracts of the plant have demonstrated a broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria. The ethyl acetate and methanol extracts are particularly effective due to the presence of high concentration of carbazole alkaloids and terpenoids in them that disrupt microbial cell membranes and interfere with enzyme activity [14,15]. The combination of these bioactivities have declared the plant as a promising candidate for the development of natural therapeutics and nutraceutical formulations [16].

The present study focuses on the evaluation of the methanol and ethyl acetate extracts of the leaves of the plant for determination of their phenolic and flavonoid contents besides their antioxidant, metal-chelating, antiglycation and antimicrobial activities [17].

Literature Review

Murraya koenigii (L.) Spreng locally known as curry leaf, has been extensively studied for its pharmacological potential and rich phytochemical composition over the past two decades [18]. The plant belongs to the family Rutaceae is native to India and Sri Lanka but now cultivated widely across tropical and subtropical Asia including Pakistan [19]. Its traditional use in the Ayurveda and folk medicine system for managing diabetes, inflammation, gastrointestinal disorders and infections has drawn scientific attention to exploit its therapeutic efficacy [20].

Phytochemical Compositions

Earlier reports showed the presence of a wide range of bioactive compounds, including carbazole alkaloids, flavonoids, terpenoids, tannins, phenolics and glycosides in the plant [21]. A number of alkaloids including mahanimbine, girinimbine and koenimbine have been reported from the plant which possess the cytoprotective and antimicrobial effects [22]. The carbazole derivatives with anticancer, antioxidant and anti-inflammatory properties have also been isolated from the plant [23]. The methanol extract is rich in phenolic and flavonoid compounds such as gallic acid, quercetin, rutin and kaempferol, which directly correlate with the antioxidant potential of the plant [24]. The LC-MS and GC-MS studies further revealed that these phytochemicals are key contributors to the pharmacological activities of the plant [25].

Antioxidant and Metal Chelation Activities

The extracts of the plant were subjected to the DPPH, ABTS and FRAP assays to determine their antioxidant and metal chelating activities [26]. The methanol extracts of the leaves of the plant exhibited a potent free-radical scavenging activity, often comparable to ascorbic acid having the IC_{50} values between 150–200 µg/mL [27]. The phenolic hydroxyl groups of phenolic compounds and flavonoid contribute to the hydrogen-donating and radical-quenching mechanisms [28]. The metal-chelation studies have revealed that the extract of the plant can effectively bind transition metals such as Fe^{2+} and Cu^{2+}, thereby inhibiting Fenton-type oxidative reactions [29]. The recent studies indicated up to 70 % ferric-chelating efficiency of the methanol extract of the plant thereby supporting its role as a natural antioxidant and metal chelates [30].

Antiglycation and Antidiabetic Potential

It has been reported that the chemical constituents of the plant include alkaloids and phenolic compounds which may contribute to the antiglycation and antidiabetic activities [31]. The extract of the plant demonstrated a significant inhibition of advanced glycation end products (AGEs) formation *in vitro*, comparable to aminoguanidine, the standard antiglycation agent [32]. Similar studies reported over 80 % inhibition at higher concentrations which may be attributed to the suppression of protein crosslinking and reactive carbonyl species [33]. Animal studies have further confirmed that chronic administration of the extract of the plant improves glycemic control, reduces lipid peroxidation and restores antioxidant enzyme balance [34]. These findings support the ethnopharmacological uses of the plant for the treatment of diabetes and metabolic disorders.

Antimicrobial Activities

The antimicrobial potential of the plant has been evaluated against a wide spectrum of gram-positive and gram-negative bacteria [35]. The ethyl acetate and methanol extracts exhibit the strongest antibacterial activity with the inhibition zones up to 20 mm against *E. coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* [36]. The carbazole alkaloids such as mahanimbine and girinimbine disrupt the microbial membranes and inhibit the key metabolic enzymes [37]. The essential oils and terpenoids of the plant display antifungal activity against *Candida albicans* and *Aspergillus niger* [38]. A recent report has highlighted the synergistic antibacterial effects of the extracts of the plant when combined with the conventional antibiotics [39].

Contemporary Advances

A recent study has explored nanocarrier systems and molecular docking approaches to enhance the therapeutic potential of the phytochemicals of the plant [40]. The nanoencapsulation of methanolic extracts has significantly enhanced the stability of antioxidant constituents and enabled the controlled release of bioactive phytochemicals, including carbazole alkaloids, flavonoids, and phenolic compounds. Furthermore, *in silico* studies have identified carbazole alkaloids as potent inhibitors of oxidative stress and key microbial enzymes, thereby underscoring the therapeutic potential of *Murraya koenigii* in pharmaceutical and nutraceutical applications [41]. As a wide spectrum of the biological activities of the plant, it is named as a pharmacologically versatile plant. Our investigations have further strengthen the concept.

Materials and Methods

Plant Material and Extraction Procedure

Dried leaves of *Murraya koenigii* (250 g) were purchased from a certified herbal store in Mirpur (AJK), Pakistan. The leaves were grinded to a powdered form (150 g) and macerated in an analytical-grade methanol (1 L) for 14-20 days with occasional shaking at room temperature. The extract was filtered through Whatman No. 1 filter paper and filtrate was concentrated under the reduced pressure on rotary evaporator. The concentrate was semi solid material (7.98 g) corresponds to a yield of 5.32 %. The extract was stored at 4 °C in airtight containers for further use.

Preparation of Stock Solution

A stock solution (1 mg/mL) was prepared by dissolving 50 mg of the methanol extract in 50 mL of methanol. This stock solution was used for all qualitative and quantitative bioassays.

Phytochemical Screening

Standard phytochemical tests were carried out to detect the presence of major secondary metabolites including alkaloids (Mayer's and Dragendorff's tests), flavonoids (NaOH test), glycosides (Keller–Killiani test), tannins ($FeCl_3$ test), steroids (Salkowski test), triterpenoids (copper acetate test), proteins (Xanthoproteic test) and phenolics. The methanol extract tested positive for most bioactive groups, confirming the presence of a broad range of phytoconstituents.

Determination of Total Phenolic and Flavonoid Contents

1.1.1.Total Phenolic Content (TPC): The total phenolic content was determined using the Folin–Ciocalteu method. Gallic acid (50–250 µg/mL) was used to plot a calibration curve, and absorbance was measured at 765 nm. Results were expressed as micrograms of gallic acid equivalents per milliliter (µg GAE/mL). The methanol extract showed a TPC of 155.5 ± 2.3 µg GAE/mL.

1.1.2.Total Flavonoid Content (TFC): The total flavonoid content was determined by using the aluminum chloride colorimetric method with quercetin as standard (10–50 µg/mL). The absorbance was measured at 540 nm and the extract exhibited 28.77 ± 1.1 µg QE/mL.

Antioxidant Activity

1.1.3.The DPPH Radical Scavenging Assay: Antioxidant potential was assessed by the DPPH radical scavenging assay. A 0.1 Mm DPPH solution was mixed with varying concentrations of extract (100–500 µL) and incubated in the dark for 30 min. Absorbance was measured at 517 nm, with ascorbic acid as a reference. The extract exhibited strong antioxidant activity with IC_{50} = 180 ± 5 µg/Ml.

1.1.4.The ABTS Radical Cation Decolorization Assay: The $ABTS^{\bullet+}$ radicals were generated using potassium persulfate (2.45 mM) and ABTS solution (7 mM), incubated for 16 h in the dark. Extract concentrations (100–500 µL) were added to the radical solution, and absorbance was measured at 734 nm. Rutin was used as standard. The extract showed IC_{50} = 150 ± 4 µg/mL, indicating high radical scavenging efficiency.

Metal Chelating Activity

The ferrous ion chelating capacity of the extract was measured by the ferrozine assay. Different concentrations (100–500 µL) of extract were mixed with 0.1 mM $FeSO_4$ and 0.25 mM ferrozine, and absorbance was recorded at 562 nm. The methanolic extract showed 72.11 % inhibition at 500 µL and an IC_{50} = 340 ± 6 µg/mL, reflecting good metal-binding ability.

Antiglycation Activity

The anti-glycation potential was evaluated using the BSA-glucose model system. The reaction mixture containing 10 mg/mL BSA, 5 % glucose, and varying concentrations of extract (100–500 µL) was incubated at 37 °C for 14 days. Formation of advanced glycation end-products (AGEs) was quantified by measuring absorbance at 370 nm. The extract showed dose-dependent inhibition of AGEs, with 84.21 % inhibition at 500 µL and an IC_{50} = 270 ± 7 µg/mL, compared to aminoguanidine as control.

Antimicrobial Activity

The antibacterial efficacy of the methanolic extract and its solvent fractions (n-hexane, chloroform, ethyl acetate, aqueous) was evaluated by the agar well diffusion method against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Salmonella enterica*. Each sample (1 mg/mL, 100 µL) was added to wells on inoculated agar plates and incubated for 24 h at 37 °C. Rifampicin served as positive control. The ethyl acetate fraction exhibited the highest inhibition (54.38 ± 1.8%), followed by chloroform (43.33 ± 1.2 %), n-hexane (24.19 ± 1.0 %), and aqueous fraction (16.77 ± 0.9 %).

Results and Discussion

The dried leaves (250 g) of *Murraya koenigii* were extracted in methanol. The methanol extracted was concentrated on rotary evaporator under reduced pressure. This afforded a semi solid material 7.98 (yield 5.32 %). The qualitative screening of the extract indicated the presence of alkaloids, glycosides, tannins, triterpenoids, steroids, proteins, amino acids, coumarins and quinones. The previous reports indicated the presence of carbazole alkaloids, flavonoids and phenolics compounds as major constituents of the plant.

Total Phenolic and Flavonoid Contents

The quantitative analysis of the crude methanol extract of the plant showed the presence of total phenolic and flavonoids contents as 155.5 ± 2.3 µg GAE/mL and 28.77 ± 1.1 µg QE/mL, respectively. Earlier reports indicated the total phenolic content in the range of 10-100 µg GAE/mL. The magnitude of the total phenolic content is indicative of the level of the may be linked to the antioxidant and metal-chelating activities. Differences in the values of the total phenolic contents may be attributed to plant origin, harvest time, drying, solvent polarity and extraction conditions.

Antioxidant Activity (DPPH and ABTS)

The dose response experiments showed linear relationship between concentration and antioxidant activity of the methanol extract of the plant. The DPPH inhibition ranged from 65.3 % (100 µL) to 83.6 % (500 µL) with IC_{50} = 180 ± 5 µg/mL while the ABTS inhibition ranged from 67.8 % to 99.3 % with IC_{50} = 150 ± 4 µg/mL. The crude

methanol extract generally showed the range of the IC_{50} values as100-300 µg/mL while the active fractions have shown the IC_{50} value of 15-25 µg/mL. Our results showed strong antioxidant activity of the methanol extract of the plant which showed that the extract contains compounds that efficiently scavenge both radical cations and neutral radicals. The relatively high phenolic contents may be correlated to the observed radical scavenging activity due to presence of phenolic hydroxyls and flavonoid moieties which donate hydrogen/ electrons to neutralize the DPPH and ABTS radicals and hence supporting the antioxidant activity of the plant.

Metal-Chelating Activity

The methanol extract of the plant showed dose-dependent Fe^{2+} chelation activity from 29.93 % (100 µL) to 72.11 % (500 µL) with the IC_{50} = 340 ± 6 µg/mL. A recent study indicated that the plant extracts have shown as high as > 60–70 % ferrous chelation at higher concentrations. Our results are well align with the reported values and that the activity may be attributed to the presence of the phenolic compounds such as caffeic and ferulic acids and flavonoids. The metal chelation help prevent Fenton chemistry (Fe^{2+}-catalyzed •OH production) and hence complements radical-scavenging activity by preventing oxidative damage. The outcome of the assay can be affected by pH, $FeSO_4$ concentration and ferrozine stoichiometry, which may explain interested variability.

Antiglycation Activity

In the BSA-glucose model, the methanol extract inhibited the AGE formation in a dose-dependent manner (64.38 % - 84.21 %) with IC_{50} = 270 ± 7 µg/mL compared to aminoguanidine control (92.8–98.07 %) at similar concentrations. Our results are similar tot eh earlier report which showed that the significant antiglycation activity of the plant may be attributed to the presence of phenolic compounds in the plant. This further indicated that while the phenolic rich plants possess the antioxidant activity, they also possess antiglycation activity, as both carbonyl trapping and metal chelation limit oxidative glycation pathways.

Antimicrobial Activity

The aqueous, chloroform, n-hexane and ethyl acetate extracts of the leaves of the plant was subjected to the agar diffusion assay. The ethyl acetate was found most active with the mean relative inhibition 54.38 ± 1.8 %) followed by the chloroform extract (43.33 %), n-hexane extract (24.19 %) and aqueous extract (16.77 %). The ethyl acetate extract showed activity against the bacterial strains, *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. enterica* were inhibition zones of 18, 20, 16 and 15 mm, respectively. The results indicated that the semi-polar fractions; ethyl acetate and methanol extract contain alkaloids and phenolic compounds which possess more antibacterial effects compared to the non-polar extract, hexane or highly polar, aqueous extract. Our results indicated that the ethyl acetate and methanol extracts showed strongest antimicrobial activity. As these extracts are rich in carbazole alkaloids and terpenoids, they cause enzyme inhibition by the membrane disruption and DNA-interference.

Integrative Discussion and Methodological Considerations

The extracts of the plant having relatively high phenolic contents showed shows a significant DPPH/ABTS scavenging activity. Such extracts also showed Fe^{2+} chelation and effective antiglycation activities. The semi-polar fraction extracts showed significant antimicrobial activity. The wide spectrum of biological activities of the extracts of the plant including antioxidant, metal chelation, antiglycation and antimicrobial activities further indicated that the extracts of the plant are a source of biological active phytochemicals.

Implications and Recommendations

Our findings revealed that methanol extracts of the leaves of *Murraya koenigii* contain promising multifunctional chemical constituents having antioxidant, metal-chelating, antiglycation and antimicrobial properties. Based on the results, following recommendation may be presented:

1. Fractionation and bioassay-guided isolation to identify lead compounds- carbazole alkaloids, phenolic compounds and flavonoids;
2. Standardization of the extraction method to ensure reproducibility of results and comparison of the results.
3. Mechanistic studies to understand the mode of action.
4. Preliminary *in vivo* toxicity and efficacy studies to assess nutraceutical or therapeutic potential.
5. Recent advances in nano-encapsulation may also be employed to improve bioavailability of the active fractions

Conclusion

The present investigation demonstrates that the methanol extract of the leaves of *Murraya koenigii* possesses remarkable multifunctional bioactivities that substantiated the earlier findings. The extract yielded significant phenolic (155.5 ± 2.3 µg GAE/ mL) and flavonoid (28.77 ± 1.1 µg QE/ mL) contents. The contents contribute significantly towards antioxidant capacity (DPPH IC_{50} = 180 ± 5 µg/mL and ABTS IC_{50} = 150 ± 4 µg/mL) of the plant extracts. The strong metal-chelating efficiency (72.11 % inhibition; IC_{50} = 340 ± 6 µg/mL) and pronounced antiglycation activity (84.21% inhibition; IC_{50} = 270 ± 7 µg/mL) confirm a synergistic mechanism involving radical scavenging, transition-metal binding, and suppression of advanced glycation end-product formation. The ethyl acetate fraction exhibited the highest antimicrobial efficacy (54.38 ± 1.8 %) against pathogenic bacteria including *E. coli* and *P. aeruginosa*. The presence of carbazole alkaloids, flavonoids, and phenolic acids in the extracts of the plant contributed towards the biological activities. Our findings validates

that the plant is a promising natural source of antioxidants, metal chelators, antiglycation agents, and antimicrobial compounds. Standardization of extraction, bioassay-guided fractionation, and mechanistic studies are recommended to develop nutraceutical or therapeutic applications of the bioactive principles of the plant.

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