JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF *MURRAYA KOENIGII* (CURRY LEAVES)

[1]Sonia Parashar*, [1]Shivani, [2]Kajal, [2]Jatin, [2]Kailash
[1]Assistant Professor, Shri Baba Mastnath Institute of Pharmaceutical Sciences & Research, Asthal Bohar, Rohtak, Haryana, India
[2]Bachelor of Pharmacy student, Shri Baba Mastnath Institute of Pharmaceutical Sciences & Research, Asthal Bohar, Rohtak, Haryana, India

Abstract:
Traditional healers have utilized plants to treat a variety of human illnesses, and India is renowned for its abundant biodiversity. Today, the same plants are being utilized for human benefit. The study of traditional plant applications has gained popularity worldwide since the turn of the century since plant-based natural products are the most valuable source for creating novel medications to treat a wide range of illnesses. In the Ayurvedic medical system, curry leaves, or *Murraya koenigii*, are a widespread and significant herb of Indian origin. They are rich in carbazole alkaloids, which have strong pharmacological and biological effects. Therefore, the current study was conducted to examine the antibacterial activity, antioxidant capacity, and phytochemical screening of the hydroalcoholic extract of *M. koenigii* leaves against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethnobotanical information on the plant's traditional uses served as the basis for the selection. The antibacterial and antioxidant properties of the leaf hydroalcoholic extract were investigated. The antibacterial potential against the chosen bacterial strains was assessed using the zone of inhibition and the agar well diffusion experiment. Using the DPPH radical assay, the extract's capacity to scavenge free radicals was also evaluated. The results showed the presence of alkaloids, flavonoids, carbohydrates, tannins, phenols, aminoacids and proteins in phytochemical analysis. With a zone of inhibition of 27 mm at a dosage of 100 mg/mL, the hydroalcoholic extract demonstrated outstanding antibacterial activity against both of the chosen bacteria. With a % DPPH scavenging value of 47.03%, the extract also demonstrated strong antioxidant activity. Because *M. koenigii* leaves have strong antibacterial and antioxidative qualities, they may be used for therapeutic purposes.

Keywords: *Murraya koenigii*, Antioxidant, Antimicrobial, Agar well diffusion.

I. INTRODUCTION

Medicinal plants have served as fundamental sources of therapeutic compounds throughout human history, providing the foundation for approximately 60% of all pharmaceuticals currently in clinical use (Cragg & Newman, 2013). The growing concern over antibiotic resistance and oxidative stress-related diseases has intensified research into natural compounds that possess both antimicrobial and antioxidant properties. Among the vast array of medicinal plants, *Murraya koenigii* (L.) Spreng, commonly known as curry leaves or sweet neem, has emerged as a promising candidate for pharmaceutical and nutraceutical applications due to its remarkable bioactive potential.

Murraya koenigii belongs to the family Rutaceae and is indigenous to the Indian subcontinent, where it has been traditionally utilized for centuries in Ayurvedic medicine and culinary practices (Arulselvan et al., 2016). The plant is characterized by its pinnately compound leaves, small white flowers, and dark purple berries, and can grow up to 4-6 meters in height. Beyond its widespread use as a flavoring agent in South Asian cuisine, curry leaves have been recognized in traditional medicine systems for treating various ailments including diabetes, diarrhea, dysentery, and skin infections (Ningappa et al., 2008).

1.1 Phytochemical Composition and Bioactive Compounds of *M. koenigii*

The therapeutic potential of *M. koenigii* is attributed to its rich phytochemical profile, which includes alkaloids, flavonoids, phenolic compounds, essential oils, and glycosides. The primary alkaloids identified in curry leaves include carbazole alkaloids such as mahanimbine, murrayanol, mahanine, koenoline, koenigicine, and murrayazolidine (Adebajo et al., 2006). These carbazole alkaloids are considered the most significant bioactive constituents responsible for the plant's pharmacological activities.

The essential oil composition of *M. koenigii* leaves has been extensively studied, revealing the presence of numerous volatile compounds including β-pinene, sabinene, α-pinene, β-caryophyllene, and α-terpinene as major constituents (Chowdhury et al., 2008). The phenolic compounds present in curry leaves, including gallic acid, catechin, rutin, and quercetin, contribute significantly to the plant's antioxidant capacity (Tachibana et al., 2003). The concentration and composition of these bioactive compounds can vary depending on factors such as geographical location, harvesting time, extraction methods, and storage conditions.

1.2 Antioxidant Properties and Mechanisms of *M. koenigii*

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, plays a crucial role in the pathogenesis of numerous diseases including cardiovascular disorders, neurodegenerative diseases, cancer, and diabetes (Halliwell, 2012). The antioxidant properties of *M. koenigii* have been demonstrated through various in vitro and in vivo studies, showing its ability to scavenge free radicals, chelate metal ions, and enhance endogenous antioxidant enzyme activities.

Studies have reported that methanolic and aqueous extracts of curry leaves exhibit significant antioxidant activity, with DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activities ranging from 70-85% at optimal concentrations (Rao et al., 2007). The antioxidant mechanisms of *M. koenigii* involve multiple pathways, including direct radical scavenging, inhibition of lipid peroxidation, and upregulation of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Kesari et al., 2012).

The phenolic compounds present in curry leaves, particularly flavonoids and hydroxycinnamic acid derivatives, are primarily responsible for the antioxidant activity. These compounds can donate hydrogen atoms or electrons to neutralize free radicals, thereby preventing oxidative damage to cellular components including DNA, proteins, and lipids (Xie et al., 2015). Additionally, the carbazole alkaloids in *M. koenigii* have been shown to exhibit antioxidant properties through their ability to chelate transition metals and prevent Fenton reaction-mediated oxidative stress.

1.3 Antibacterial Properties and Mechanisms of Action of *M. koenigii*

The emergence of antibiotic-resistant bacterial strains has created an urgent need for alternative antimicrobial agents derived from natural sources. *Murraya koenigii* has demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacteria, making it a potential candidate for developing new antimicrobial therapeutics (Ghasemzadeh et al., 2016).

Research has shown that various extracts of curry leaves exhibit antibacterial activity against clinically important pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Salmonella typhimurium* (Kumar et al., 2013). The minimum inhibitory concentrations (MIC) of curry leaf extracts against these pathogens typically range from 25-200 µg/mL, depending on the extraction method and bacterial strain tested.

The antibacterial mechanisms of *M. koenigii* are multifaceted and involve disruption of bacterial cell membrane integrity, inhibition of essential enzymes, interference with DNA replication and protein synthesis, and generation of reactive oxygen species within bacterial cells (Petersson et al., 2010). The carbazole alkaloids, particularly mahanimbine and murrayanol, have been identified as the primary compounds responsible for antibacterial activity. These alkaloids can penetrate bacterial cell walls and membranes, leading to leakage of cellular contents and ultimately bacterial cell death.

The essential oils from curry leaves also contribute to antibacterial activity through their lipophilic nature, which allows them to interact with bacterial cell membranes and disrupt membrane fluidity and permeability (Pandey et al., 2014). Furthermore, the phenolic compounds present in *M. koenigii* can form complexes with bacterial proteins and enzymes, leading to their inactivation and subsequent bacterial growth inhibition.

1.4 Clinical Relevance and Therapeutic Applications of *M. koenigii*

The dual antioxidant and antibacterial properties of *M. koenigii* make it particularly valuable for treating conditions where oxidative stress and bacterial infections coexist. Wound healing represents one such application, where bacterial contamination and oxidative damage can significantly impair the healing process. Studies have demonstrated that topical application of curry leaf extracts can accelerate wound healing by providing antimicrobial protection while simultaneously reducing oxidative stress and promoting tissue regeneration (Gupta et al., 2014).

The potential applications of *M. koenigii* extend to the food industry, where natural preservatives with both antioxidant and antimicrobial properties are highly sought after. The incorporation of curry leaf extracts in food products can help prevent lipid oxidation, extend shelf life, and provide protection against foodborne pathogens (Sharma et al., 2010). This dual functionality makes curry leaves an attractive alternative to synthetic preservatives, which are increasingly being scrutinized for their potential health risks.

This research aims to provide a comprehensive evaluation of the antioxidant and antibacterial potential of *Murraya koenigii*, contributing to the growing body of evidence supporting its therapeutic applications. The investigation will focus on phytochemical screening of the leaf extract responsible for these properties and assessing their potential for developing novel therapeutic interventions.

II. MATERIAL AND METHOD

2.1 Plant Material

The whole plant of *Murraya koenigii* was collected from local nursery of Rohtak. The collected plant was identified and authenticated from valid sources.

Figure 1: *Murraya koenigii* leaflets

2.2 Preparation of plant extract

The collected leaves were thoroughly washed; shade dried and powdered using a mechanical grinder. The powder kept in air tight bottle for further study. Hydroalcoholic extract was prepared using Methanol and water in 7:3 ratio. Extract was prepared by adding 60 g of powdered material in 800 ml of solvent. The contents were filtered out. Filtrate was allowed to evaporate on a hot water bath until the desired concentration of the extract was obtained.

2.3 Preliminary Phytochemical Investigation: Qualitative phytochemical analysis of the aqueous curry leaf extracts was carried out to detect the presence of phytochemicals such as alkaloid, flavonoids, terpenoids, sterols, tannins, glycosides etc. as per standard procedures.

2.4 Powder microscopy of *M. koenigii L* leaves

The powdered sample of *M. koenigii L* was subjected to powder microscopic studies using a compound microscope with 60 X eyepiece.

2.5 Antioxidant activity of *Murraya koenigii* extract

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is based on the ability of antioxidants to scavenge the stable free radical DPPH•, resulting in a color change from deep violet to yellow. This change can be quantitatively measured by a decrease in absorbance at 517 nm using a spectrophotometer. For antioxidant activity, DPPH is dissolved in methanol to obtain a 0.1 mM solution (e.g., 3.9 mg DPPH in 100 mL methanol) and the solution is protected from light and used freshly for the assay. Different concentrations (20-100 µg/ml) of *M. koenigii L* extract and standard (ascorbic acid) were prepared. Mix 3.9 mL of DPPH solution with 100 µL of test sample solution. For control, mix 3.9 mL of DPPH solution with 100µL of methanol (without sample). For blank, use methanol only. Incubate the reaction mixtures in the dark for 30 minutes at 37 °C temperature. Measure the absorbance at 517 nm against the blank using a UV-Vis spectrophotometer. The control contains all the reagents except samples and standards. The decrease in absorbance was recorded, and the percentage of free radical scavenging activity was calculated using below-mentioned formula. The absorbance of the resulting solutions against the corresponding blank solutions was measured using a UV-Vis spectrophotometer (UV-3600 Plus, Schimadzu, Japan).

$$\text{DPPH activity (\%)} = (A_{control} - A_{test})/A_{control} \times 100$$

Where

$A_{control}$ = Absorbance of control (DPPH + methanol)

A_{test} = Absorbance of DPPH + sample

2.6 Antimicrobial activity of *Murraya koenigii* extract

The antimicrobial activity of the extract was determined by agar well diffusion method against selected bacterial strains. Overnight grown culture of pure bacterial isolates (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) inoculated in Luria Bertani broth (secondary culture), incubated at 37°C under shaking until turbidity matches to 0.5 MacFarland was used for the study. The culture was swabbed back and forth on the Muller Hinton Agar plate and the plate was left to dry for 10 min. Then, a hole with a diameter of 8 mm was punched aseptically with a sterile cork-borer and 80 µL of the desired concentrations of the extract viz. 100 mg/mL, 50 mg/mL, 25 mg/mL and standard drug ampicillin (50 ug/mL) was introduced into the wells. After that, agar plates were incubated under suitable conditions, that is 37°C for 20 h. After incubation the plates were observed for the zone of inhibition.

III. RESULTS & DISCUSSION

3.1 Extractive value

Hydroalcoholic extract of *M. koenigii* L was prepared by adding 60 g powdered leaves in methanol and water (7:3) by maceration process. The extractive value came out to be 13.32 & w/w.

Figure 2: Extract of *M. koenigii L* leaves

3.2 Preliminary phytochemical screening

A variety of phytochemicals, including flavonoids, alkaloids, cardiac glycosides, carbohydrates, proteins and amino acids, phenols, saponin, terpenoids, and tannins, were screened in an aqueous extract of *M. koenigii* L leaves. Table 1 displays the findings of the qualitative phytochemical analysis of the aqueous extracts of *M. koenigii* L. Rashmi and Naveen observed similar findings about the presence of phytochemicals such as carbohydrates, alkaloids, phenols, terpenoids, and tannins in the aqueous extracts of *M. koenigii* L L (Rashmi & Naveen, 2016). The phytochemicals in the aqueous leaf extract of *M. koenigii* L were examined by Farooq et al (Farooq et al, 2019), who reported the presence of flavonoids, carbohydrates, phenolic compounds, saponin, lipids, and fixed oils. Various phytochemicals were reported to be present in *M. koenigii* L by a number of writers (Prabakaran et al, 2013; Pujan et al, 2019).

Table 1: Phytochemical analysis of *Murraya koenigii* L leaves aqueous extract

Phytochemicals	Hydroalcoholic leaf extract
Alkaloids	+
Carbohydrates	+
Cardiac glycosides	-
Flavonoids	+
Phenol	+
Amino acids & Proteins	+
Saponins	-
Tannins	+
Terpenoids	-

3.3 Powder microscopy of *M. koenigii* L leaves

The microscopic investigations of powdered M. koenigii leaves showed different structures like parenchyma cells, fragments of epidermis, trichomes, calcium oxalate crystals, starch grains, oil glands and xylem/phloem. The powder microscopy findings of *M. koenigii* leaves are shown in Figure 4.

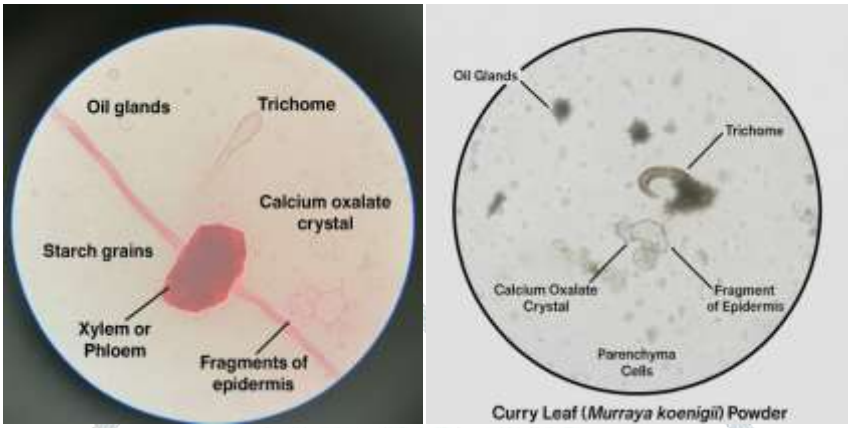

Figure 3: Powder microscopy of *M. koenigii* L leaves

3.4 Antioxidant activity of *Murraya koenigii* L extract

The antioxidant activity of *M. koenigii* L extract was evaluated using DPPH free radical scavenging assay. Ascorbic acid was used as standard in the assay. The DPPH scavenging activity of *M. koenigii* L extract was found to be 47.03 % at a concentration of 100 µg/mL. While ascorbic acid showed higher scavenging activity of 91.58 % at the same concentration.

Table 2: % DPPH scavenging effect of *M. koenigii* L leaf extract

Concentration (µg/ml)	% DPPH scavenging
20	35.84
40	38.95
60	41.26
80	44.45
100	47.03

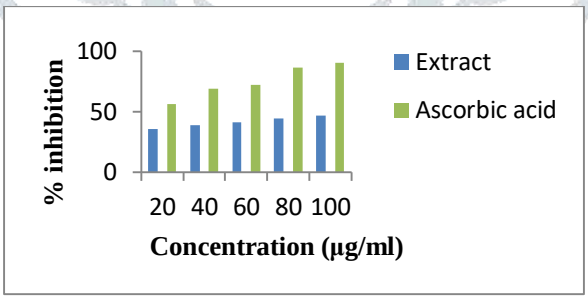

Figure 4: DPPH scavenging activity showing % inhibition of DPPH with increasing concentration of *M. koenigii* L leaf extract & standard

3.5 Antimicrobial activity of *Murraya koenigii* L extract

The antimicrobial activity of the *M. koenigii* L extract was evaluated against *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria by agar well diffusion method. From the results it was observed that zone of inhibition of *M. koenigii* L extract came out to be 27 mm at at a concentration of 100 mg/ml and that of the standard drug Ampicillin was found to be 18 mm at a concentration of 50 µg/mL. Extracts from *M. koenigii* L have shown antibacterial properties against a broad range of microorganisms (Panghal et al, 2011; Abuga et al, 2020). Several carbazole alkaloids found in the extracts of *M. koenigii* L are responsible for this characteristic (Jain et al, 2017; Verma, 2018).

Table 3: Zone of inhibition of *M. koenigii* L leaf extract

Concentration of extract (mg/ml)	Zone of inhibition (mm)	
	P. aeruginosa	***S. aureus***
100	27	27
50	18	18
25	-	-
Control	-	-
Ampicillin (50 µg/mL)	19	18

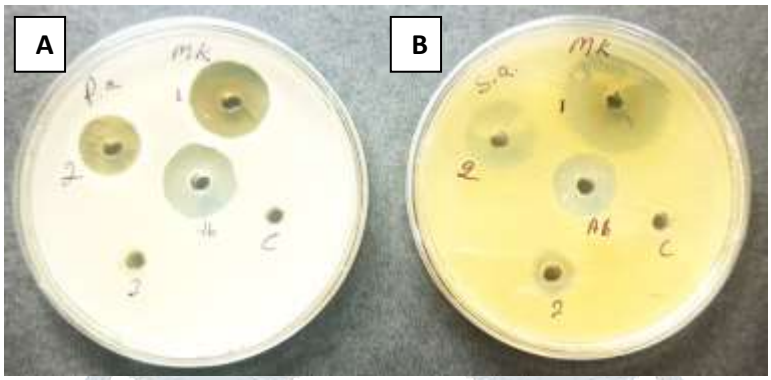

Figure 5: Antimicrobial activity of *M. koenigii* L leaf extract showing zone of inhibition : A) *P. aeruginosa*: 1-100mg/ml extract; 2-50 mg/ml extract; Ab-Ampicillin; C-25 mg/ml extract; D-Control B) S. aureus: 1-100mg/ml extract; 2-50 mg/ml extract; Ab-Ampicillin; C-25 mg/ml extract; D-Control

IV. CONCLUSION

The current study demonstrated good antimicrobial and antioxidant efficacy of *M. koenigii* L. Polyphenols, alkaloids, flavonoids, and terpenoids are among the bioactive substances found in *M. koenigii* L leaf extract that may be in charge of inhibiting infections. *M. koeniigi* L can thus be used as an efficient antibacterial agent in novel medications to treat various bacterial infections brought on by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. However, more research is required to understand the molecular mechanisms underlying the roles of different elements in antimicrobial inhibition and radical scavenging, as well as experimental studies on bioavailability and clinical investigation efficiency enhancement.

V. ACKNOWLEDGMENT

The authors would like to thank Department of Pharmacy, Baba Mastnath University, for providing access to all the facilities needed to carry out the research work.

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