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Phytochemical analysis and antibacterial screening of curry leaves (*Murraya koenigii*)

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Abstract

Murraya koenigii belongs to the Rutaceae family and is a pharmacologically potent plant that is highly valued for its specific aroma and functional compounds. Carbon tetrachloride, chloroform, ethyl acetate, and methyl alcohol extracts of *M. koenigii* leaf powder were extracted using the Soxhlet extraction method. The amount of methanol extract was found to be the highest, while that of carbon tetrachloride was the least. It was subjected to qualitative analysis of bioactive compounds as well as their antibacterial properties. The phytoconstituents analysis revealed the existence of terpenoids, glycosides, and quinone in the carbon tetrachloride extract. Similarly, chloroform extract revealed the presence of glycosides, ethyl acetate extract constitutes polyphenols, terpenoids, glycosides, tannins, flavonoids, and quinone, whereas methanol extract contains all tested phytochemicals, alkaloids, polyphenols, glycosides, tannins, flavonoids, and saponins except quinone. The different extracts obtained from *Murraya koenigii* were subjected to antibacterial screening tests in microbes, both in Gram-positive and Gram-negative bacterial strains. The result showed that carbon tetrachloride extract and the chloroform extract *S. aureus* ATCC 25923 with 13- and 12-mm diameter zones of inhibition, respectively, and the chloroform extract exhibited notable activity against *E. coli* ATCC 25922 (Gram-negative bacteria) with a zone of inhibition of 16 mm, but was resistant towards carbon tetrachloride extract, with no zone of inhibition.

Keywords: Antibacterial screening, *Murraya koenigii*, Phytoconstituents, Soxhlet extraction

Introduction

Murraya koenigii, commonly known as the curry leaf plant, is an aromatic evergreen shrub that belongs to the family **Rutaceae**. It is naturally distributed across the subtropical to lower temperate regions of Nepal, where it thrives in a broad range of climatic conditions (Pradhan et al., 2024). The leaves of *Murraya koenigii* hold dual significance serving both as a natural medicinal resource and as a highly valued flavoring ingredient. In culinary practices, they are valued for their distinctive aroma

and taste, which arise primarily from the presence of volatile essential oils. Beyond their flavoring capacity, the leaves are traditionally recognized for their role in promoting digestion and overall gastrointestinal health (Ramamurthy & Prasanth, 2018).

Phytochemical investigations reveal that *Murraya koenigii* is a rich repository of biofunctional organic molecules such as alkaloids, carbohydrates, flavonoids, sterols, tannins, and phenolic compounds. These secondary metabolites have been successfully extracted using different polar solvents, including petroleum ether, ethyl acetate, chloroform, ethanol, and distilled water (Gaidhane et al., 2020). Since a high number of the population in developing countries relies heavily on herbal medicines for first-level medical care, this plant continues to hold a prominent position in traditional medical systems (Pokhrel & Neupane, 2021).

Ethnomedicinally, fresh green leaves are often consumed raw to relieve digestive disorders such as dysentery, diarrhea, and vomiting (Acharya & Acharya, 2009). The leaves and roots are also employed for their anthelmintic, analgesic, and inflammation-reducing properties (Balakrishnan et al., 2020) and have been traditionally used in the management of piles, skin irritation, itching, leukoderma, and several blood-related disorders. Modern pharmacological studies further confirm the broad therapeutic potential of *Murraya koenigii*, reporting antioxidant, antimicrobial, hypoglycemic, cholesterol-lowering and ulcer inhibiting activities (Shaikh et al., 2020).

Owing to its abundance of natural antioxidants, extracts of *Murraya koenigii* have found diverse applications—not only as therapeutic agents in the treatment and prevention of diseases but also as natural preservatives in food systems and as functional ingredients in the pharmaceutical and nutraceutical industries **(**Rana & Yamini, 2022). Thus, *Murraya koenigii* stands as a multipurpose plant of both cultural and biomedical importance, bridging traditional knowledge with modern scientific validation. Therefore, this research aims to perform phytochemical screening of different extracts and evaluate the antibacterial activity of *Murraya koenigii* leaf extract.

Materials and Methods

Murraya koenigii leaves, natively known as "Curry patta," were obtained from Dhulikhel, Kavrepalanchok, Nepal. The leaves of the plant were cleaned properly under the tap water to remove other contaminants like dust, soil, insects, eggs, larvae, etc., and air dried in shade (for about two weeks). The leaves were dried and grinded to fine powder by using an electric blender. Then

it was collected and stored in a clean plastic bag. It was used for the extraction process in different solvents like carbon tetrachloride (CCl_4), chloroform ($CHCl_3$), ethyl acetate (EtOAc), and methanol (MeOH) (laboratory grade, of Thermo Fischer Scientific India Pvt. Ltd., Mumbai).

Methods

Extraction of different extracts of *Murraya koenigii* leaves

The dried powder of leaves (30 g) was placed into the thimble and extracted in carbon tetrachloride (CCl_4, 200 mL), chloroform ($CHCl_3$, 200 mL), ethyl acetate (EtOAc, 300 mL), and methyl alcohol (MeOH, 300 mL) by the Soxhlet extraction method, respectively. The filtrate was concentrated under a water bath maintaining 60 °C to get a solid or semi-solid extract and stored in the freezer for further investigation.

Phytoconstituent analysis of *Murraya koenigii* leaves extract

Methods employed for phytoconstituent analysis are mainly based on the standard protocol given by Ciulei 1982. All plant materials were completely extracted by Soxhlet extractor in order of increasing polarity (carbon tetrachloride, chloroform, ethyl acetate, and methyl alcohol) and qualitatively analyzed for phytochemical constituents. The phytochemical screening was carried out to investigate biologically active, non-nutritive compounds that help with the flavor, color, and other characteristics of the plants, like alkaloids, tannins, glycosides, saponins, and flavorins, among others.

Biological screenings of different extracts of *Murraya koenigii*

The chemical constituents present in the plant are responsible for its biological activity. The biological assay means the study of the effect of crude plant extract on the organisms. This present work includes the assay of different leaf extracts of *Murraya koenigii* and their antibacterial activities.

Antibacterial assay

The antibacterial property of plant extract is helpful in assessing the bioactive compounds in the plant. Antibacterial activities of methanol, ethyl acetate, chloroform, and carbon tetrachloride were carried out at the Nepalese Farming Institute, Maitidevi, Kathmandu. The antimicrobial assay of plant extracts was performed in Mueller-Hinton Agar (MHA) by agar well diffusion method.

Preparation of media

3.8 g of agar nutrient was taken in a conical flask, and distilled water was added to it and was made up to the final volume of 100 mL (38 g/mL). Then, it was boiled with continuous shaking and was autoclaved at 121 °C for 15 min. After that, the sterilized

media was cooled to 50 ℃ and transferred into a petri dish of 90 mm diameter in the ratio 30 mL/plate and labelled appropriately. The poured medium was allowed to solidify. Finally, the nutrient agar plates were prepared.

Preparation of inocula

The inoculum was made by transferring 3-4 colonies from the nutrient agar to sterile normal saline. The turbidity of inoculums was made equivalent to 0.5 McFarland standard (approximately 10^8 CFU/mL). The lawn culture of the test inoculums was prepared by swabbing MHA (Mueller-Hinton agar) containing 2% NaCl with a sterile cotton swab dipped into the inoculums.

Preparation of plant extracts

Plant extract of 0.2 g/mL of carbon tetrachloride extract, 0.06 g chloroform extract, 20 mg/mL ethyl acetate extract, and 20 mg/mL methyl alcohol extract were taken, respectively. Similarly, 30 mg/mL, 20 mg/mL, and 20 mg/mL of respective extracts, i.e., carbon tetrachloride, ethyl acetate, and methanol, were dissolved in DMSO and stored at 4 ℃.

Microorganisms included in the study

Active cultures of two standard strains of bacteria were provided by the Nepalese Farming Institute, Maitidevi, Kathmandu, Nepal. The following organisms were included in the study; Gram positive bacteria (American Type Culture Collection), *Staphylococcus aureus* ATCC 25923, and one Gram-negative bacterium, *Escherichia coli* ATCC 25922. Microorganisms were cultured in the Nutrient Broth (NB) and were kept viable by sub-culturing them in Nutrient Agar (NA). Purity of organisms was maintained by sub-culturing using the streak plate technique.

Agar well diffusion method

Sterile Mueller-Hinton agar (MHA) plates were made having a uniform thickness of 4 mm. Respective inocula of organisms of selected bacterial strains were carpet cultured using a sterile cotton swab uniformly. It was allowed to diffuse for 15 minutes at room temperature in a laminar hood, 7 wells of 6 mm. Each well was filled with different extracts and a negative solvent control (DMSO), respectively.

Further study was done in chloroform extract in which 2 well was made. Then 45 µL of chloroform solvent and chloroform leaves extract were placed in the well, respectively. It was then allowed to diffuse for about 60 minutes at room temperature and incubated for 12-18 hours at 37 ℃. After incubation, plates were observed for the formation of a clear zone around the well, which corresponds to the anti-bacterial activity of the tested compounds. Zone of inhibition (ZoI) was recorded (Parekh et al., 2005).

Results and Discussion

Extractive values of plants in different solvents.

The physical state and percentage yields of different extracts obtained are shown in **Table 1**. The total amount of crude extract obtained from the Soxhlet extraction method was 14.80 g. The percentage (%) yield of methanol extract was found highest while that of carbon tetrachloride extract was the least. This is due to variations in the polarity of extracted solvents, which could affect a large variation in the degree of bioactive compounds in the extract.

Table 1: *Percentage (%) yield of different extracts obtained from Murraya koenigii*

SN	Solvent used in Soxhlet extraction	Amount of Plant material (g)	Physical state	% yield
1	Carbon tetrachloride	30.00	Solid	14.38
2	Chloroform	30.00	Oily liquid	21.90
3	Ethyl acetate	30.00	Solid	29.19
4	Methanol	30.00	Viscous gel	34.52

Phytoconstituent analysis

The outcomes of phytoconstituent analysis of *Murraya koenigii* leaves in different extracts are presented in **Table 2**. Phytoconstituent screening of four different extracts of *Murraya koenigii* showed the presence of different phytochemical constituents. There is a vast difference in the phytoconstituents present in plants; hence, no specific solvent is efficient in extracting all the compounds.

Table 2: *Phytochemical Screening of Murraya koenigii extracts*

SN	Phytochemicals	Solvent extracts			
		Carbon tetra chloride	Chloroform	Ethyl acetate	Methanol
1	**Alkaloids:**				
	Mayer's test	-	-	-	+
	Wagner's test	-	-	-	+
	Dragendroff's test	-	-	-	+
2	Flavonoid test	-	-	-	+
	Lead acetate test	-	-	+	+
3	Polyphenol	-	-	+	+
4	Terpenoids	+	-	+	+
5	Glycosides test	+	-	+	+
6	Saponin test	-	+	-	+
7	Tannins	-	-	+	+
8	Quinone	+	-	+	-

NOTE: (+) indicates present and (-) indicates absent

The phytoconstituents of *Murraya koenigii* are more exposed by the more polar solvent, i.e. methanol) than less polar solvent i.e., ethyl acetate, chloroform, and carbon tetrachloride, respectively. The yield of the methanol extract of *Murraya koenigii* is higher than that of ethyl acetate, carbon tetrachloride, and chloroform. Methanol extract contains higher polar compounds, which produce a higher yield of phenolic compound concentration than non-polar ones (Do et al., 2014). This is supported by the fact that methanol extracts show the highest positive tests of alkaloids, flavonoids, terpenoids, polyphenols, saponins, glycosides, etc.

Ethyl acetate, which is moderately polar, also revealed the presence of almost similar contents. The variation in yield can be justified by the greater activity of methyl alcohol to dissolve the bioactive compounds and higher heating stability, which assists the retention of thermolabile volatile compounds. Chloroform extract and carbon tetrachloride, which were nonpolar in nature, were positive to only a few of the phytochemicals, like saponins and terpenoids, glycosides, quinones, etc.

Similar phytochemical components, such as alkaloids, flavonoids, phenolics, terpenoids, saponins, etc., were found in the methanol extract by Ali et al. (2021). Generally, water-soluble tannins show antibacterial properties and are toxic in nature because they can readily form metal complexes (Akiyama et al., 2001). However, saponin was present in the case of the chloroform extract in this study. Environmental variables may be the cause of this variation in component extraction. The findings indicate that chloroform is a distinct solvent that is essential for both the quantitative and qualitative examination of the numerous phytochemicals that are extracted from *Murraya koenigii* (Ali et al., 2021).

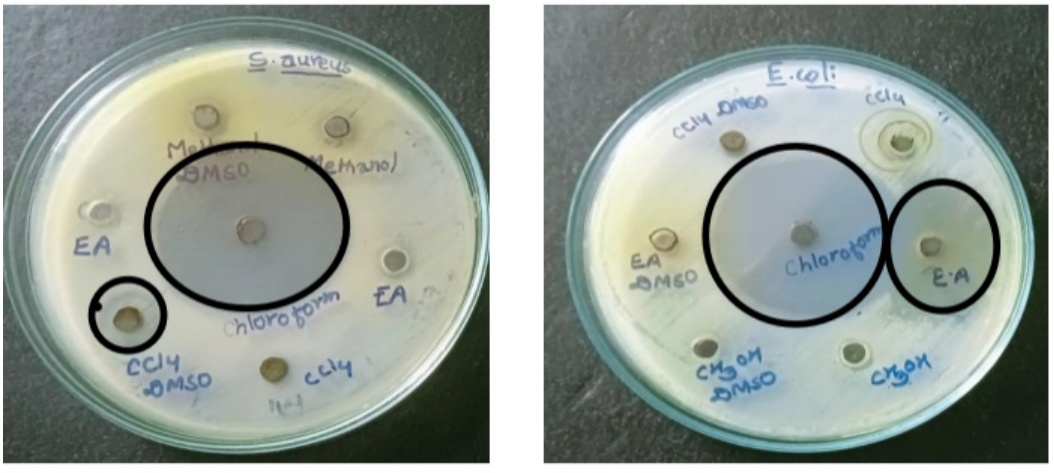
Antibacterial activity of *Murraya koenigii* leaf extract

The biological assay of *Murraya koenigii* is based on an antibacterial test. Its leaves have antibacterial efficacy against Gram-negative and Gram-positive bacterial pathogens. The result of the antibacterial efficacy of methanol, ethyl acetate, chloroform, and carbon tetrachloride extracts of *Murraya koenigii* has been displayed in **Table 3**.

Table 3: Antibacterial activities of extracts of *Murraya koenigii*

SN	Sample of the plant extract	Inhibition Zone (mm)	
		Staphylococcus aureus **ATCC 25923**	***Escherichia coli*** **ATCC 2592224**
1	Methanol	-	-
2	Ethyl acetate	-	-
3	Chloroform	12	16
4	Carbon tetrachloride	13	-

Antibacterial potential of the extract is a property of a compound present in the extract that kills bacteria or inhibits their growth. This property of plant extract is shown because of the presence of secondary metabolites. The antibacterial activity of chloroform and carbon tetrachloride extract of *Murraya koenigii* was investigated in terms of an inhibition zone. The result showed the carbon tetrachloride *Murraya koenigii* leaf extract displayed inhibitory effects against *Staphylococcus aureus* with a 13 mm zone of inhibition, while *E. coli* was resistant against the extract as it hadn't shown any zone of inhibition. Chloroform itself exerts antibacterial activity. The antibacterial activity of chloroform on *S. aureus* and *E. coli* was found to be 27 mm and 31 mm, respectively. Similarly, the antibacterial activity of the chloroform extract of *M. koenigii* was effective against *S. aureus* and *E. coli*, with the zone of inhibition being 12 mm and 16 mm, respectively. Similarly, Abeysinghe et al. (2021) mentioned that the extract of ethanol exhibited an 11.9 mm zone of inhibition against *S. aureus*. The result showed that the Gram-positive *S. aureus* bacteria were susceptible to carbon tetrachloride and chloroform extract while Gram-negative bacteria *E. coli* were only susceptible to chloroform extract but sensitive to carbon tetrachloride extract of *M. koenigii*. *S. aureus* bacteria cause meningitis, pulmonary infections, and several urinary tract infections (Otto, 2010). Consequently, chloroform extracts may be useful in curing pulmonary infections, skin inflammation, and other urinary tract disorders. On the other hand, *E. coli* is a causative agent of diarrhea, pneumonia, and urinary tract infections. Extracts also exhibit therapeutic potential against diarrhea, pneumonia, and inflammatory disorders of the urinary tract.



(i) (ii)

Figure 1: *Antibacterial activity shown by plant extract on different bacteria, (i) S. aureus and (ii) E.coli*

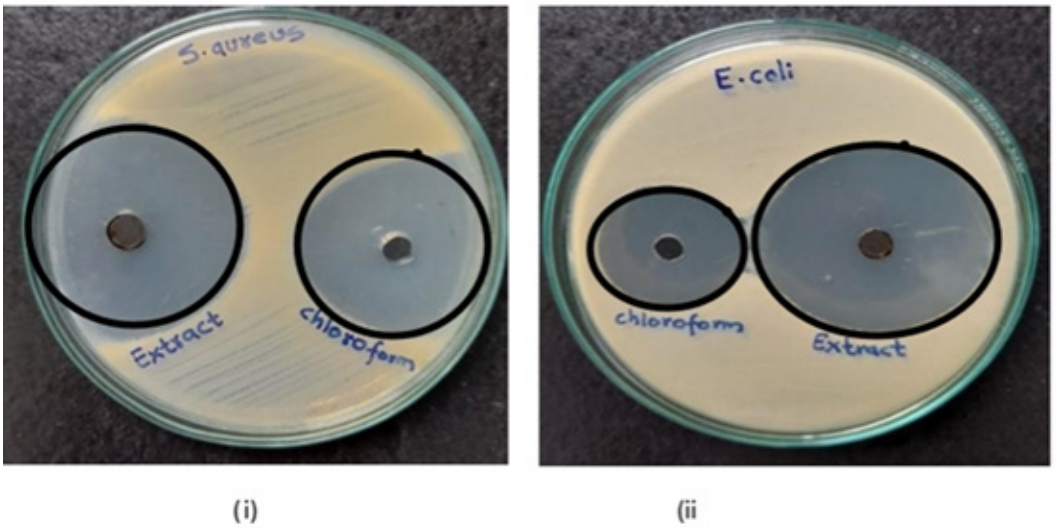


Figure 2: *Antibacterial activity shown by chloroform and its plant extract on (i) S. aureus and (ii) E. coli bacteria (i) S. aureus* and (ii) *E. coli*

In the current work, the anti-bacterial activity of methanol and ethyl acetate extracts was found to be negative. This may be due to the environmental condition as well as due to contamination in the laboratory procedures. There may be production of such compounds which destroy these compounds. The compound may be decomposed during the working process. In the Soxhlet extraction process, heat may decompose such compounds.

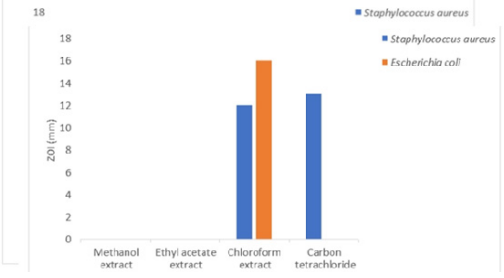


Figure 3: *Bar diagram showing antibacterial properties by plant extract on different*

Conclusion

The leaves of *Murraya koenigii* that were dried and powdered were subjected to successive extraction with carbon tetrachloride, chloroform, ethyl acetate, and methyl alcohol *via*. Soxhlet extraction method. The methanol extract yield was found to be the highest, while that of the carbon tetrachloride extract was the least.

The phytoconstituent analysis of different *Murraya koenigii* leaf extracts revealed the presence of major phytoconstituents such as alkaloids, flavonoids, polyphenols, glycosides, saponins, terpenoids, etc. Bioassay of carbon tetrachloride showed effective to Gram-positive bacteria (*Staphylococcus aureus*) but was resistant towards Gram-negative bacteria

(*Escherichia coli*). Bioassay of chloroform extract showed effectiveness towards both Gram-positive and Gram-negative bacteria. *Murraya koenigii* extracts have demonstrated antibacterial effect, which showed it could be effectively used for the prevention of bacterial infections, a natural remedy in everyday meals. Also, these herbal medicines are cheaper and more convenient than other systems of medicines. Hence, this plant may assist as a beneficial resource in the food processing sector and clinical medicine.

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Author's Contribution

Conceptualization: SP, Investigation: MKC, SP; Methodology: MKC, SP; Data curation: SP; Data analysis: SP; Writing - original draft: MKC, SP; Writing - review and editing: SP.

Conflict of Interest

The authors declare that there is no conflict of interest.

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