An In Vitro Investigation of Antimicrobial Efficacy Of *Murraya Koenigii* Leaves (Curry Leaf Plant) And Its Bioactive Component Quercetin Against Selected Pathogenic Microorganisms

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Abstract
Murraya Koenigii, (family: Rutaceae) commonly known as curry leaf plant, is a green leafy plant found all over India. They have been long used for their medicinal properties. Owing to the presence of various bioactive compounds such as phytochemicals they can act on various microorganisms (including bacteria) inhibiting their growth or killing them as a whole. This study focuses on the demonstration of their antibacterial properties. For this, leaf samples from two different locations were taken to produce a comparative result. Four test bacteria were taken viz *Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae and Staphylococcus aureus.* Methanolic extracts were prepared for the leaf samples and standard agar well diffusion method was chosen for the study. Furthermore, quercetin, a bioactive compound found in the leaves of *M. koenigii,* was tested to demonstrate its individual antibacterial activity. The present study showed the effective antibacterial activity of both leaf samples along with its bioactive component quercetin, and quercetin showed effective results inhibition against different bacterial strains. Therefore, Quercetin can be used as a potent therapeutic in pharmaceutical industry.

Keywords: *Murraya koenigii*, Rutaceae, antibacterial, bio active compound, quercitin, phytochemicals

1. INTRODUCTION

Since ancient times human beings have utilised different kinds of plants for various purposes. From being used as food, for sheltering, or for treatment of various diseases, plants play an important role in our everyday life. Our survivability and livelihood depend on the commercial utilisation of plants by a large margin. Either as medicine or as ingredients of medicine plants have been seen all over the globe. The medicinal practices in India such as Ayurveda. Siddha and Unami till date utilise different plants for disease treatment (Jayasree, et al, 2014). India as a country is known for its vast collection of medicinal plants. These plants produce bioactive compounds, called phytochemicals which attribute to their medicinal properties. These compounds help the plant fight against disease causing pathogens. Therefore the isolation and utilisation of these phytochemicals can be useful in preventing or treating various kinds of diseases. Some examples of phytochemicals produced are alkaloids, flavonoids, glycosides, tannins, etc. The WHO estimates that around 80% of the world's population depend on traditional medicine for their living, which makes around 2 billion people to wholly rely on medicinal plants for their livelihood. (Ifeoma Lois, et al,

2018; Tan et al., 2022; Balakrishnan et al., 2020). Chemical antibiotics have been popularly used as medicinal drugs but their effectiveness have a major downside as there are chances that the pathogenic microorganisms can develop resistance against the antibiotic. In this case phytochemicals produced by plants facilitating as antimicrobial agents can come in handy.

Murraya Koenigii (family - Rutaceae) are a plant native to India and south Asian countries. They are commonly found as an evergreen or deciduous tree all over India. Ranging from their roots, barks to the leaves, these plants have valuable properties. Their usage as a commercial plant is evident not only in India but other parts of the world as well. They are characterised by the distinct aroma of their leaves making them a common ingredient in Indian food recipes. The leaves further have antimicrobial and antioxidant properties imbibed in them. Additionally they are rich in a wide range of phytochemicals as well. The leaves therefore help combat against a wide range of diseases including diabetes, ulcer, diarrhoea, cancer, dysentery to name a few. Their quality is such that even after drying they retain much of their valuable properties and their flavour (Balakrishnan et al., 2020; Aniqa et al., 2021; Abeysinghe, 2021). As mentioned earlier, secondary metabolites produced by plants which are bioactive phytochemicals are known to possess a wide range of pharmacological activities. These compounds show anti-diabetic, anti inflammatory, anti-cancer, antimalarial activities, etc just to name a few. Notably *M. koenigii* possess phytochemicals like quercetin, flavonoids, glycosides, anthraquinones, tannins, etc. Quercetin is a notable phytochemical present not only in *M. Koenigii* but also other plants like apples, berries, vegetables and some seeds, nuts, flowers, etc. As a phytochemical it exhibits numerous therapeutic activities among which it can act as an antimicrobial agent. It can help inhibit the growth of various bacteria and fungi. This study aims to evaluate the antibacterial efficacies of the leaf samples that we had collected from two different locations. Results of the antibacterial efficacy of quercetin was used as additional comparative data.

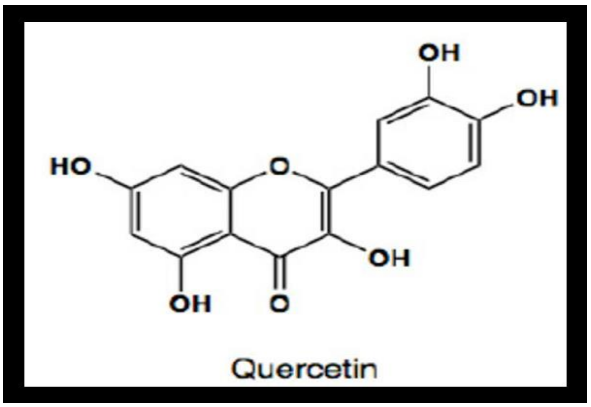

Fig 1:- Chemical structure of Quercetin

1.2 TAXONOMY

1)Kingdom;- *Plantae*
2)Sub-kingdom;- *Tracheobionta*
3)Super division;- *Spermatophyta*
4)Division;- *Magnoliophyta*
5)Class;- *Magnoliospida*
6)Sub-class;- *Rosidae*
7)Order;- *Sapinda*
8)Family;- *Rutaceae*
9)Genus;- *Murraya*
10)Species;- *koenigii*

1.3 DISTRIBUTION

Murraya Koenigii is found commonly as a bush or a tree (evergreen and deciduous) all over India. Ranging from the Himalayan region to the southern part of India.

Fig 2 :- Commonly occurring M. koenigii bush

1.4 Traditional uses of Murraya Koenigii;-
Different parts of the plant are said to have different properties. As a result they are used to treat different ailments. The barks and the roots are used to cure bites of poisonous snakes and other animals. (Nishan et al., 2014-15). The leaves are highly valuable and are used in baths in their freshly plucked and dry state. Raw green leaves are eaten directly as a remedial measure for diarrhea and dysentery. These leaves can be then steamed and used to treat fever, indigestion, constipation etc. the leaves can also be used as a source for calcium, and vitamins like vitamin A, vitamin C, B etc. Crushed leaves are used as ointments to relieve skin burns, boils etc Apart from this the leaves also help keep the hair black and healthy (Handral et al., 2012). Diseases in which M. Koenigii showed potential effect: Diabetes, ulcer, dysentery, diarrhoea, fungal diseases, bacterial diseases, inflammation, mental ailments like depression, hair fall, blood disorders, fever, etc.

3. MATERIALS

- Nutrient agar (HiMedia)
- Mueller Hinton agar (HiMedia)
- Methanol (SD Fine Chemicals Ltd)
- Soxhlet Apparatus (Zexter)
- Nutrient broth (HiMedia)
- Micropipette and pipette box
- Test tubes, petri plates, beakers and other glasswares
- Fehling's solution (HiMedia)

4. METHODS

4.1 Plant sample collection

Leaves of *M. koenigii* were located from two different locations for the study. One sample was collected from Darjeeling, West Bengal while the other from Dehradun, Uttarakhand.

- Leaf sample designated as 'MU' was collected from near the campus of Uttaranchal University, Uttarakhand. (The sample weighed at around 20g)
- Leaf samples designated as 'MD' were collected from the forest area of Singbulli Tea estate, Darjeeling, West Bengal. (The sample weighed at around 20g)

The leaves after collection were washed thoroughly with distilled water and then left to shade dry for about a week. After drying they were crushed using a blender to powder form for preparation of the plant extract.

Fig 3:- Dried MD leaf sample

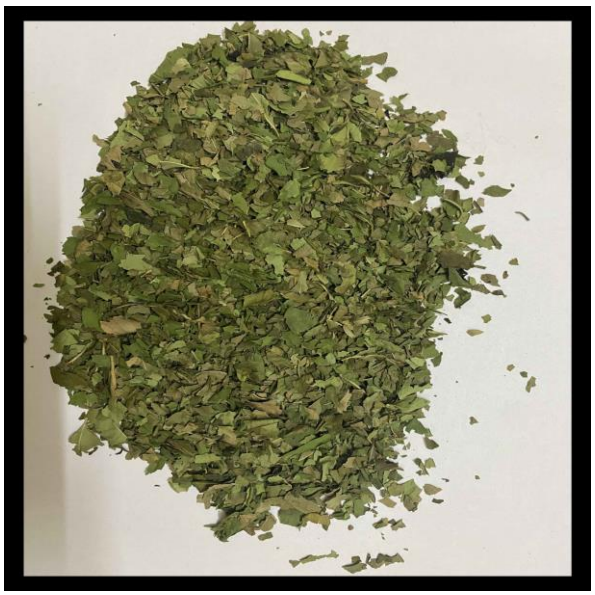

Fig 4:- Dried MU leaf sample

4.2 EXTRACT PREPARATION

Soxhlet extraction method was followed for the leaf extract preparation. The powdered leaves of both the samples were extracted using methanol as solvent in the Soxhlet apparatus for a period of 12-14 hrs. After the extracts were collected from the apparatus they were filtered using Whatman filter paper. The filtrate was then left to dry in the oven for 1-2 hrs at around 35 C. After a semi solid mass was formed the extracts were stored in eppendorf tubes and refrigerated at 5°C until further use.

Fig 5 :- Soxhlet apparatus.

4.3 ANTIBACTERIAL ACTIVITY ANALYSIS

4.3.1. Test bacteria

Four bacterial strains were used for the study viz *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis*. The four strains were collected from Uttaranchal University, School of Applied and Life Sciences laboratory. The pure cultures of the strains were subcultured in nutrient broth tests tubes 24 hours prior to experimentation.

4.3.2. Evaluation of antibacterial activity of leaf samples (MU & MD) by agar well diffusion assay

Agar well diffusion method was adopted for the evaluation of the antibacterial efficacy of the leaf extracts. Nutrient agar media was the media used. All the experimentations were done under a sterile laminar air flow. The experimentation procedure preceded with the sterilisation of the required lab equipment in an autoclave. For this study 8 petri plates (4 each for one leaf extract sample) along with the prepared media and other requirements including test tubes, beakers were autoclaved. The leaf extracts were divided into four concentrations, 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml (Plant extract). After autoclaving was done, media was poured onto the petri plates and left to solidify. After 2-3 hours of solidification, 15 µl of test bacteria from the test tubes were pipetted onto the media plates and spread with a sterile glass spreader, four strains were spread on four separate plates. After that 6 wells were made on each of the plates. 4 wells for the 4 concentrations of the extracts and 2 wells for two control solutions. The two control solutions used were methanol (100 µl) and antibiotic solution (100 µl). The antibiotic used for the study was amoxicillin (10mg/ml). The plates were then incubated for 24 hours at 37°C. Zone of inhibition was recorded after incubation.

4.3.3. Evaluation of antibacterial activity of Quercetin compound

To observe the antibacterial activity of quercetin, quercetin compound was purchased from the market. Physically the compound obtained was yellowish in powdered form. Media used was Mueller Hilton agar. A stock solution was prepared (10mg/ml), by dissolving 10mg of quercetin powder in 1ml of solvent. From this stock solution four working solutions were prepared. 10, 20, 30 and 40 mg/ml of the stock solution was taken and in four different eppendorf tubes. Similar to leaf sample procedure, 6 wells were made on the media plates where the four concentrations (100 µl each) and two control solvents were added. Amoxicillin antibiotic (positive control) and methanol (negative control) were used as control agents both added in 100 µl concentration as well. Plates were incubated at 37°C for 24 hours and the zone of inhibition was recorded.

5. RESULTS

The zones of inhibition formed were all measured in mm. In terms of the zone of inhibitions formed, antibacterial efficacies of The MU sample, MD sample and Quercetin were recorded in table 1, 2 and 3 respectively. Zone of inhibition was recorded for all the 3 samples while clear zones were recorded for the plates with *P. aeruginosa* strain. Larger zones were observed for MD sample as compared to MU sample. Quercetin on the other hand demonstrated larger zones compared to the leaf extracts. Control agent methanol showed no ZOI while antibiotic samples showed clear large ZOI. Appearance of these ZOI shows the antibacterial activity of the leaves of *M. koenigii* regardless of location while solidifying the status of quercetin as an antibacterial agent.

Table 1:- Zone of inhibition (in mm) of MD Sample

Bacterial strain	20 mg/ml (ZOI in mm)	40 mg/ml (ZOI in mm)	60 mg/ml (ZOI in mm)	80 mg/ml (ZOI in mm)	Amoxicillin solution (ZOI in mm)
P. aeruginosa	**5**	**7**	**12**	**14**	**25**
B. subtilis	**12**	**17**	**19**	**20**	**29**
K. pneumoniae	**12**	**16**	**19**	**22**	**28**
S. aureus	**10**	**13**	**17**	**18**	**20**

Table 2:- Zone of inhibition (in mm) of MU sample

Bacterial strain	20mg/ml (ZOI in mm)	40 mg/ml (ZOI in mm)	60 mg/ml (ZOI in mm)	80 mg/ml (ZOI in mm)	Amoxicillin solution (ZOI in mm)
P. aeruginosa	**4**	**5**	**6**	**14**	**20**
B. subtilis	**1**	**6**	**6**	**7**	**20**
K. pneumoniae	**1**	**2**	**4**	**5**	**18**
S. aureus	**2**	**3**	**5**	**7**	**16**

Table 3:- Zone of inhibition (in mm) of Quercetin sample

Bacterial strain	10mg/ml (ZOI in mm)	20mg/ml (ZOI in mm)	30mg/ml (ZOI in mm)	40mg/ml (ZOI in mm)	Amoxicillin solution (ZOI in mm)
P. aeruginosa	**7**	**18**	**20**	**22**	**25**
B. subtilis	**10**	**14**	**16**	**24**	**23**
K. pneumoniae	**10**	**17**	**18**	**20**	**22**
S. aureus	**15**	**17**	**20**	**22**	**20**

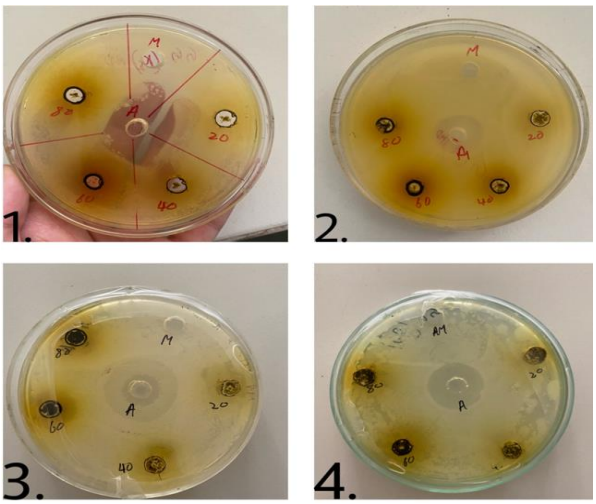

Figure :- **ZOI of MD sample**
1: ZOI on *K. pneumoniae* plate
2: ZOI on *S. aureus* plate
3: ZOI on *B. subtilis* plate
4: ZOI on *P. aeruginosa* plate

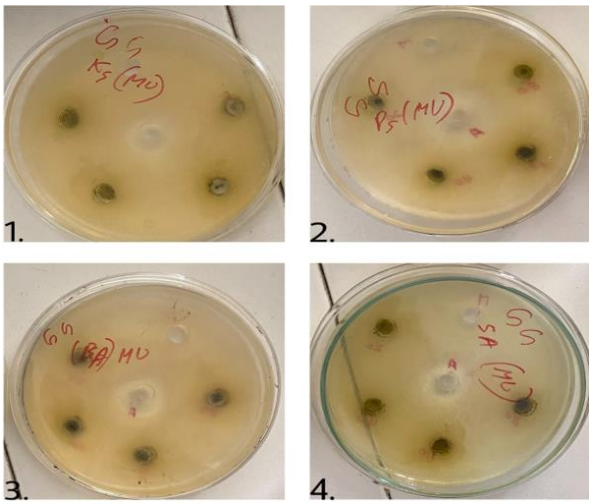

Figure :- **ZOI of MU sample**
1: ZOI on *K. pneumoniae* plate
2: ZOI on *P. aeruginosa* plate
3: ZOI on *B. subtilis* plate
4: ZOI on *S. aureus* plate

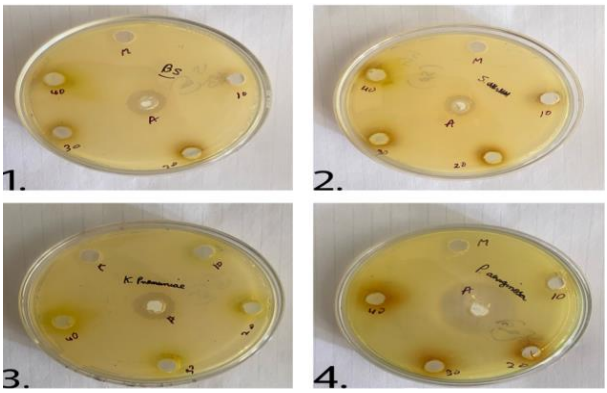

Figure :- **ZOI of Quercetin sample**
1: ZOI on *B. subtilis* plate
2: ZOI on *S. aureus* plate
3: ZOI on *K. pneumoniae* plate
4: ZOI on *P. aeruginosa* plate

6. DISCUSSION

The results of our study demonstrate how geography and the climatic factor of different locations can play a part in demonstrating the antibacterial mechanism of the leaf extracts. Physically the leaf samples collected from West Bengal were less green and smaller in size compared to the ones collected in Uttarakhand. Furthermore the differences in the size of the zone of inhibition formed stand as evidence to support our claims. The *P. aeruginosa* strain showed the highest susceptibility to the extract and Quercetin sample as well. It is known to cause various infections in the body related to the urinary tract, bones, lungs, etc. Control solution methanol did not display formation of zones in any of the samples while the antibiotic sample showed the formation of zones in a mean range of 20-25 (mm) for all the samples used. Compared to the other studies related to antibacterial efficacy related with *M. Koenigii* the zones formed in this study were smaller and less clear owing to the fact that less concentrations of the leaf extract were used since our main motive was to produce a comparative result than a single demonstrative one. This study also made a motive to compare the antibacterial efficacy of a plant sample with a bioactive compound found in the plant itself, where the data generated helped in the success. In a nutshell, because of the presence of the various phytochemicals in the leaves of *Murraya koenigii*, their viability as an antibacterial agent is pronounced.

7. CONCLUSION

M. koenigii exhibited good antibacterial potential in the assays conducted. The methanolic extracts of *M. koenigii* showed antibacterial activity against *S. aureus, E. coli, B. subtilis* and *P. aeruginosa*. The highest inhibitory activity was observed in the Darjeeling sample of *M. koenigii* (MD) as compared to Uttarakhand sample of *M. koenigii* (MU) towards bacterial strains. Quercetin, one of the bioactive compounds of *M. koenigii* also tested for their antibacterial activity and showed significant results against bacterial strains. The present study concludes that a bioactive component found in *M. koenigii* leaves also possess antibacterial activity, and this compound can be used solely for various pharmacological aspects. Therefore, *M. koenigii* leaves can be explored as a promising source of antioxidants compounds which can be further used as a potent target in the pharmaceutical industry.

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