Bioscene

Bioscene
Volume- 21 Number- 04
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

Phytochemical Analysis and Antimicrobial Activity of Plant Extract Murrayakoneigii

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Abstract:- Murrayakoenigii also known as a curry-leaf tree is usedas a medicinal plant in India. In the present study, different were extracted and tested against Staphylococcus aureus by agar well diffusion method. Alkaloid, carbohydrate, and tannins were present in all extracted samples, however cardiac glycosides and phlorotannins were extracted only in methanolic and ethanolic extracts of plant leaves sample.In all solvent extractsfrom leaves of M. koenigii and minimum activity was recorded at 25 ppm of plant extract concentration. Maximum antibiotic activity was recorded with aqueous extract of leaves against S. aureus. In the case of P. aeruginosa, all extracts were failed to show antibacterial activity.

Keywords: Murrayakoneigii, phytochemicals, Bacillus cereus, Pseudomonas aeuriginosa and terpenoids.

1. 0 Introduction

Various medicinal systems like Ayurveda, Amchi, etc., and local health traditions are practiced in India, which utilize a large number of plants for the treatment of various human and animal diseases. Those plants used for medicinal purposesare known as medicinal plants. The medicinal value of a plant depends on numerous active compounds such as carbohydrates, proteins, enzymes, oils, terpenoids, phenolic compounds, etc., improving help improve the life and treatment of disease. Many plants have antimicrobial principles such as tannins, essential oils, and other aromatic compounds. These compounds protect the plant from microbial infection and Deterioration [1]. The presence of important phytochemicals makes the plant useful against different diseases and has a strong ability toprovideuseful drugs forhuman use. Phytochemicals are regarded as secondary metabolites produced invery smallamountsas the plant haslittle need for them. They are produced naturally in whole parts of the plant body; like bark, leaves, stem, root, flower, etc. [2]. The quantity and quality of phytochemicals present in plant parts may differ from one part to another [3]. One of the medicinally important herbs is M. Koenigii, has placed in thefamily Rutaceae and is normally known as the curry leaftree. The plant is a local of India, Sri Lanka, and other SouthAsian nations. It is discovered wherever in the Indian subcontinent [4]. The plant is exceptionally esteemed for its leaves a vital fixing in Indian cooking to advance edacity and processing. The leaves, roots,and bark are tonic, for stomach pain. Leaves are utilized inside as a part of

loose bowels, likewise checking to regurgitate[5]. The leaves and roots are biting, harsh, cooling, hostile to helminthic, mollifies the warmth of the body, thirst, irritation, and tingling (see Image 01). It is additionally helpful in leukoderma and blood issues. The new scientific research aimed atphytochemical investigation, biological evaluation on experimental models, toxicity studies, analysis of the molecular mechanism of actions of isolated phytoprinciples, and their clinical trials [10]. Plants are the source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay [11]. For instance, plantsrich in tannins have antibacterial potential due to their character that allows them to react with protein to form stable water-soluble compounds thereby killing the bacteria by directly damaging its cell membrane [12]. The first step towards this goal is a phytochemical investigation of plant material, followed by the in vitro antibacterial activity assay [8]. By keeping this on mind, the present investigation was conducted to study the phytochemical constituents, followed by the antibacterial activity indifferent solvent extracts of M. koenigii Linn. against three pathogenic bacteria namely, B. cereus, P. aeuriginosa and Staphylococcus aureus.

Image 01 curry leaf tree

2.0 Materials and Methods

2.1 Collection of Plant Materials

Leaves of M. koenigii L were collected from the campus of S.G.R.R. (PG) College, Dehradun during March-April, 2022 and authenticated by the Department of Botany. Samples were packed in sterilized polypropylene bags and brought to the Department of Botany, S.V.R.M. College, Nagaram, Guntur District, and Andhra Pradesh, INDIA for further work.

2.2 Collection of Test Organisms

The test organism'sB. cereus, P. aeuriginosa and S. aureus used in the present study were clinical isolates procured from Shri MahantIndiresh Hospital, S.V.R.M.College, Nagaram, Guntur District, and Andhra Pradesh, INDIA. All the test organisms were maintained in a refrigerator at 4°C in nutrient agar slants until required. Extraction Procedure Freshly collected leaves samples of M. koenigii were washed with fresh water 2–3 times dried under the shade at room temperature and then blended to powder using an electric blender. Powdered leaves passed through a 2 mm sieve and stored in a sterile air-tight container for further use. Extraction of leaves sample was done in three different solvents viz. Distilled water, 100% methanol and 100% ethanol. For this purpose, 10 g of dry powdered leaves was placed in a conical flask of 250 ml capacity and 100 ml of different solvents viz. Water, Methanol, and Ethanol were added separately. Flasks were tightly sealed with polythene sheet and allowed to be shaken vigorously on shaker for 48 h. Extracts were filtered using muslin cloth first then followed by Whatman No.1 filter paper. The filtrates were then stored in an airtight sample bottle in a refrigerator at 4°C until required.

2.3 Phytochemical Analysis of Plant Extracts

The presence of phytochemicals is determined based on standard qualitative test procedures and these procedures are as follows:

2.4 Test for Alkaloid (Wagner's test):

3-5 drops of Wagner's reagent were added to 5 ml extract the formation of red/brown precipitate indicated a positive result.

2.5 Test for Carbohydrates (Molisch's test):

A few drops of Molisch's reagent were added to 2 ml extract and 2 ml conc. H_2SO_4 was also added. Allowed to stand for 2-3 min. The formation of red/dull viol of the two layers indicated a positive result.

2.6 Test for Cardiac glycosides (Keller kiliani test):

2 ml of glacial acetic acid was added to 5 ml extract then few drops of ferric chloride were also added with 1 ml conc. H_2SO_4. The formation of brown/violet/green ring indicated the positive result.

2.7 Test for Flavonoids (Alkaline reagent test):

Few drops of 20% NaOH solution were added to 2 ml extract which showed yellow color within a second and became colorless on addition of dilute HCL which indicated the positive result.

2.8 Test for Phenols (Ferric Chloride test):

Aqueous 5% ferric chloride was added to 2 mL extract. The formation of deep blue/black color indicated the positive result.

2.9 Test for Phylobatannins (Precipitate test):

1 ml of HCL was added to the 2 ml extract which was boiled and marked the volume of 1 mL. The formation of red precipitate indicated the positive result.

2.10 Test for Amino acids and Proteins(Ninhydrin test):

2-5 drops of ninhydrin solution were added to 2 ml extract and boiled in a water bath for 1-2 minutes. The formation of purple color indicated apositive result. Test for Tannins (FeCl3 solution test): 10% alcoholic ferric chloride solution was added to 2 ml extract. The formation of blue/green color indicated apositive result.

2.11 Test for Terpenoids (Salkowski test):

In 1 ml of chloroform, 2 ml extract was added, and a few drops of conc. H2SO4 wasalso added. The formation of reddish-brown precipitate indicated thepositive result.

2.12 Test for Quinons:

Few drops of conc.HClwasadded to 2 ml extract. The formation of yellow precipitate indicated apositive result.

2.13 Antibacterial Activity Using Agar Well Diffusion Method

M. koenigii leaf extracts were screened for in-vitro antibacterial activity against B. cereus,P. aeuriginosa and S. aureus in comparison with standard antibiotic streptomycin (25, 50, and 100 µg/ml) by Agar Well Diffusion Method. A lawnculture was prepared using test organismson Nutrient Agar. Inoculated plates were kept aside for 5 min to dry up the culture and then using a well cutter, three wells were made in each plate at the required distance. Using sterilized micropipettes 20µl of different extracts were added in the well. For each bacterial strain, controls were maintained where pure solvents without extracts were used. All plates were incubated at 37°C for overnight. The activity of the leaf extract was determined by measuring the diameter of the zone of inhibition.

3.0 Results

Phytochemical Test Crude extracts (Aqueous, Methanol and Ethanol) of M. koenigii Linn were subjected to different phytochemical screening for alkaloid, carbohydrate, tannins, terepnoids, cardiac glycosides, flavonoids, phenols, phylobatannins, quinons, amino acids and protein. Presence of different phytochemicals in leaves of M. koenigiiLinn., are showed in Table 1. Out of ten tested phytochemicals, alkaloid, carbohydrate, tannins, terepnoids were present in all extracted samples, however cardiac glycosides and phylobatannins were extracted only in methanolic and ethanolic extracts of plant leaves sample. Test for presence of phenolic compounds showed positive results in aqueous and ethanolic extracts, while quinons only extracted in aqueous medium. Flavonoids and Amino acids and protein were showed negative results in all crude extracts.

Table1. Phytochemical analysis of leaves of m. koenigii extracted in different solvents

Phytochemicals	Aqueous	Methanol	Ethanol
Alkaloids	++	+	+
Carbohydrate	+++	+++	+++
Cardiac glycosides	-	+++	+++
Flavonoids	-	-	-
Phenols	++	-	++
Phylobatannins	-	+	+
Amino acids and protein	-	-	-
Tannins	+	+	+
Terepnoids	+	++	+
Quinons	++	-	-

+ Presence of compound; + + + good results; – absence of compound.

3.1 Antibacterial Activity

In all solvent extracts from leaves of M. koenigii and minimum activity was recorded at 25 % plant extract concentration. Maximum antibiotic activity was recorded with aqueous extract of leaves against S. aureus (14.5±0.6 mm) at 100% plant extract, which was at par with methanolic extract against B. cereus (14.5±0.6 mm) at same concentration. All extracts of leaves samples showed antibacterial activity against B. cereus, however methanolic extract showed better results at all tested concentrations and ethanolic and aqueous extracts showed equal potential to inhibit bacterial growth. Aqueous extract showed antibacterial activity against S. aureus, however methanolic and ethanolic extracts showed activity only in crude (100%) extract while diluted extract (25–50%) unable to showed positive results. In case of P. aeruginosa, all extracts were failed to show antibacterial activity (Table 2).

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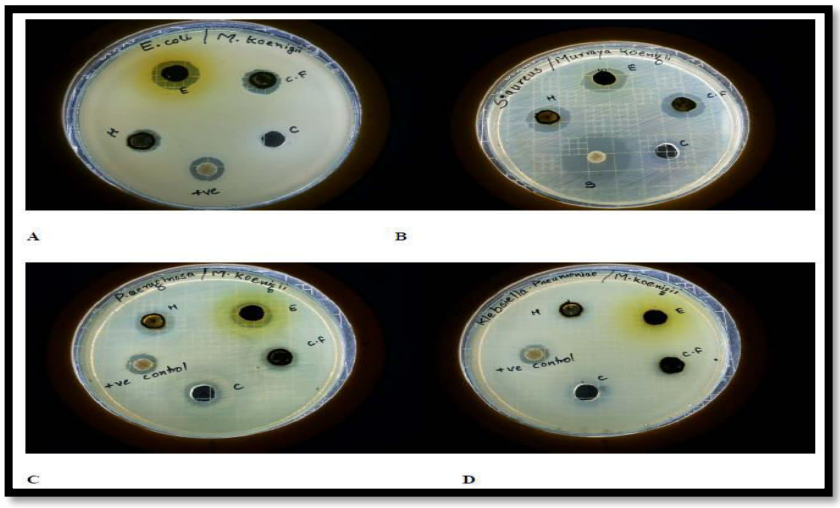

Figure 1 : MHA Plates exhibiting zone of inhibition of M.koenigii organic extracts against

(A) Escherichia coli, (B) Staphylococcus aureus , (C) Pseudomonas aeruginosa ,

Table 2. Antibacterial activity (in mm) of M. koenigii leaves extracts against test organisms.

Bacteria	Concentration	Control	Extracts		
			Aqueous	Methanol	Ethanol
B. cereus	100 rpm	23.0±1.5	11.0±0.5	14.5±0.6	11.5±0.5
	50 rpm	21.0±1.1	10.0±0.5	11.0±0.52	10.5±1.5
	25 rpm	20.0±1.0	5.7±0.2	13.5±0.6	10.5±0.5
P. aeruginosa	100 rpm	20.0±0.9	-	-	-
	50 rpm	18.0±0.7	-	-	-
	25 rpm	14.5±0.5	-	-	-
S. aureus	100 rpm	21.5±1.2	14.5±0.6	11.5±0.5	10.5±0.5
	50 rpm	19.0±0.9	12.0±0.5	-	-
	25 rpm	15.0±0.6	10.5±0.6	-	-

*Values are expressed as mean of three replicates SD (Standard deviation).

4.0 Discussion

Alkaloids isolated from plant are commonly reported to have antimicrobial properties. The antibacterial activity of the leaf extract of M. koenigii as recorded in present study may therefore be attributed to the presence of above phytochemicals i.e. Alkaloids, Carbohydrates, Cardiac glycosides, Phenol, Phylobatannins, Tannins, Terpinoids, in Ethanol extracts and Alkaloids,

Carbohydrate, Cardiac glycosides, Phylobatannins, Tannins, Terpinoids, in Methanol extracts and Alkaloids, Carbohydrate, Phenols, Terpinoids, Tannins, Quinons in Aqueous extracts. In present study aqueous extracts have the higher solubility for more phytoconstituents, and hence consequently showed highest antibacterial activity. It indicates that leaves of M. koenigii may possess compounds with antimicrobial properties which are effective against bacteria B. cereus, P. aeuriginosa, S. aureus. Handral et al. [10] reported antibacterial activity of M. koenigii leaves extract against P. aeruriginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia and Salmonella typhi. In the global food industry more priority is given to natural preservatives as there is increase occurrence of resistance in pathogenic strains against chemical food preservative. The screening of plant extracts and plant product for antimicrobial activity has shown that high plants represent a potential source of novel antibiotic prototype.

5.0 Conclusion

It is concluded that the plant extract of M. koenigii possess antimicrobial activity against tested organisms. The zone of inhibition varied suggests the varying degree of efficacy and differentphytochemicals constituents of herb on the target organism. The antimicrobial activity of the plants may be due to presence of various active principles in their leaf. Future studies are needed to isolates and characterize the bioactive principles to develop new antimicrobial drugs.

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