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Original Article

Phytochemical screening and antibacterial activity of curry (*Murraya Koenigi)* leaf extracts against enteric pathogens

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ABSTRACT

The present study was carried out for determination of antibacterial activity and phytochemical screening of Curry (*Murraya Koenigi*) leaves solvent extracts. Four solvents were used for extraction. These are Ethanol, Methanol, Diethyl Ether and Acetone. The solvent extraction was done by using Soxlet apparatus. Test microorganisms were screened to confirm their viability and identities using standard microbiological methods. The different solvent extracts of Curry leaves was tested for antimicrobial activity using the standard agar well diffusion method against nine enteric pathogens, these are *E.coli, Salmonella typhi, Sal.para.A, Sal. para. B, Shigella sonnei, Shigella dysentarie, Enterobactor spp., Citrobactor spp. and Klebsiella spp.* The Ethanol extract of Curry leaves showed highest antimicrobial activity against *Shigella sonnei.* The Methanol extract of Curry leaves showed highest antimicrobial activity against *E.coli*. The Diethyl ether extract of Curry leaves showed highest antimicrobial activity against *Sal. para. A* while Acetone extract showed highest antimicrobial activity against *Sal. typhi*. The antimicrobial activity of standard antibiotics Ampicillin and Tetracycline were studied in comparison with Curry leaves solvent extracts. The MIC values were determined by both agar and broth dilution method. The functional chemical group was determined by Fourier Transform Infrared Spectroscopy (FTIR). The phytochemical analysis of Curry leaves solvent extracts showed presence of Alkaloid, Flavonoid, Phytosterol, Saponin, Glycosides, Phenolic compounds, Terpenoid etc.

Keywords: Curry (*Murraya koenigi*), Antimicrobial activity, Phytochemical screening, Enteric pathogens, MIC

INTRODUCTION

The people of India have a very long-standing tradition in the use of natural medicines and the local practices are still quite common in the treatment of diseases [1, 2]. The assessment of plants used in the conventional medicines is anticipated to make available new antimicrobial agents [3, 4].The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. Medicinal plants are considerably useful and economically essential. They contain active constituent which are used in the treatment of many human diseases. Modern research has played a way for the discovery of another plant of potential value to help in a wider range of ailments.

Over the last decade, there has been a renewed interest in plant and the pharmaceutical industry considers plants as a viable option for the discovery of new leads [5]. In fact, it is also estimated that natural products are implicated in the development of 44% of all new drugs, generally as leads for the preparation of semi-synthetic derivatives [6]. Despite the existence of conventional antimicrobial agents, resistant or multi-resistant strains of pathogenic microorganisms are continuously appearing imposing the need for a thorough search for and development of new drugs [7].

In an effort to discover new lead compounds, many research groups screen plant extracts to detect secondary metabolites with relevant biological activities. The plant kingdom has served as an inexhaustible source of useful drugs, foods, additives, flavoring agents, lubricants, coloring agents and gums from time immemorial [8]. Various medicinal plants are rapidly recognized as a source of new chemical compounds, which may be important due to their potential uses in medicine, or their other biological properties.

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Medicinal plants are naturally gifted with invaluable bioactive compounds which form the backbone of traditional medicines. Approximately 80% of the 4,000 million inhabitants of the earth rely on herbal medicines for their primary health care [9].

There has been an increasing interest worldwide on therapeutic values of natural products from plants due to disenchantment with modern synthetic drugs [10, 11]. Moreover; the clinical efficacy of many existing antibiotics is also being threatened by the emergence of multidrug resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Therefore, there has been tremendous upsurge in the demand for the drugs from natural sources.

Curry (*Murraya koenigi*), a member of the family Rutaceae, is a deciduous to semi-evergreen aromatic tree found throughout India. Traditionally, it is used as an analgesic, febrifuge, stomachic, carminative and for the treatment of dysentery and skin eruptions [12, 13]. Curry leaf is commonly used as spice due to aromatic nature of leaves. Carbazole alkaloids, the major constituents of the plants are known to possess cytotoxic, antioxidative, antimutagenic and anti-inflammatory activities [14, 15]. The leaves are rich in mono-terpenoids and ses-quiterpenoids which exhibited antifungal activities [16].Minor furano-coumarins are also reported from seeds [17].In the present investigation, an attempt has been made to investigate antimicrobial screening and phytochemical analysis of Curry leaves solvent extracts.

MATERIALS AND METHODS

Collection of Plant Material: Healthy disease free, indigenously grown mature leaves of Curry was purchased from local market of Solapur (M.S.). The identification of plant material was confirmed by a Botanist in the Dept. of Botany, Walchand College of Arts and Science, Solapur (M.S.).

Test Pathogens: Nine strains of enteric pathogenic bacterial cultures were used in this study. These are *E.coli, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B ,Shigella dysenteriae, Shigella sonnei, Enterobactor spp., Citrobactor spp.* and *Klebsiella spp.* The pure pathogenic bacterial strains were collected from Dept. of Microbiology, V.M. Govt. Medical College, Solapur (M.S.). The collected pure pathogenic bacterial strains were isolated from hospitalized patients at Govt. Civil Hospital, Solapur (M.S.) The cultures were maintained on nutrient agar slants at 4^0C and subcultured for 24hr. before use.

Preparation of Solvent Extracts: Thoroughly washed mature leaves were shade dried and then powdered with the help of electric blender. Twenty five grams of the powder was filled in the thimble and extracted successively with Ethanol, Methanol, Diethyl ether and Acetone using a Soxlet extractor for 48hr.All the extracts were concentrated using rotary flash evaporator and preserved at 5^0C in airtight bottle until further use. All the extracts were subjected to antibacterial activity assay and phytochemical analysis.

Antibacterial Activity Assay: Antimicrobial activity of the Curry leaves solvent extracts was determined by agar well diffusion method on Muller- Hinton agar medium [18]. Cups are made on Muller- Hinton agar plates using cork borer and inoculum containing 10^6 CFU/ml of pathogenic bacteria were spread on the solid plate with the help of sterile glass rod. Then 100ul of solvent extract was placed in the cups made in inoculated plates. All the plates were incubated for 24hr. at 37^0C. and after incubation period zone of inhibition was measured in mm. Antimicrobial activity of Standard antibiotics Ampicillin and Tetracycline were observed in comparison with Curry leaves solvent extracts.

Determination of Minimum Inhibitory Concentration (MIC): MIC was determined by both agar and broth dilution methods [19]. For broth dilution tests, 0.1ml of standardized suspension of bacteria (10^6 CFU/ml) was added to each tube containing different concentrations of solvent extracts (05-50ul/ml) and incubated for 24hr at 37^0C. In agar plating method dilutions having 05-50ul of solvent extracts was placed in the cups on the inoculated plates and incubated as mentioned above.

Phytochemical Analysis

Qualitative Phytochemical Analysis: The Curry leaves solvent extracts was tested for the presence of bioactive compounds by using standard method [20].

Fourier Transform Infrared Spectroscopy (FTIR): FTIR was used to identify the characteristic functional group in the crude Curry leaves powder. A small quantity (5mg) of the powder was dispersed in dry potassium bromide (Kbr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min. to form a Kbr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance

accessory. The IR spectrum was obtained using Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 4000 to 400cm^{-1} for 16times to increase the signal to noise ratio.

RESULTS AND DISCUSSION

In the present study significant antibacterial activity is observed by all solvent extracts of Curry leaves. The antimicrobial activity of Curry leaves solvent extracts are represented in table 1. The result revealed that the Ethanol extract of Curry leaves shows highest antibacterial activity against *Shigella sonnei*. The Methanol extract of Curry leaves shows highest antibacterial activity against *E.coli*. The Diethyl ether extract of Curry leaves shows highest antibacterial activity against *Salmonella paratyphi A.* while Acetone extract of Curry leaves shows highest antibacterial activity against *Salmonella typhi*. The antimicrobial activity of standard antibiotic Ampicillin were found to be maximum against *Citrobactor spp.* while standard antibiotic Tetracycline showed highest antibacterial activity against *Sal. Paratyphi A.*

Minimum inhibitory concentration (MIC) of the different Curry leaves solvent extracts varied against different test pathogens. The MIC of Curry leaves solvent extracts required for test pathogens are represented in table 2. Lowest MIC of 5ul was observed against *Shigella sonnei* by Ethanol extract of Curry leaves while highest MIC of 35ul was observed against *Salmonella paratyphi A.* Lowest MIC of 10ul was observed against *E.coli* by Methanol extract of Curry leaves while highest MIC of 40ul was observed against *Salmonella Paratyphi B.* Lowest MIC of 10ul was observed against *Salmonella paratyphi A* by Diethyl ether extract of Curry leaves while highest MIC of 40ul was observed against *E.coli.* Lowest MIC of 10ul was observed against *Salmonella typhi* by Acetone extract of Curry leaves while highest MIC of 50ul was observed against *Shigella dysenteriae*

The aim of FTIR analysis is to determine the existence of functional group that exists on isolate. The IR spectrum of the crude powder of Curry leaves in the form of Kbr pallet is shown in fig 1.
The absorption at 3375cm^{-1} is due to N-H stretching vibration. The bond at 1627cm^{-1} is due to c=c stretching. The absorption at 1404 cm^{-1} is due to active alpha-methylene group. The absorption at 1068 cm^{-1} is due to olefinic C-H out of plane bending modes. The results of phytochemical analysis of various solvent extracts of Curry leaves are represented in table 3. The phytochemical analysis showed presence of Alkaloid, Flavonoid, Phytosterol, Saponin, Glycosides, Phenolic compounds, Terpenoid etc.

Medicinal plants are valuable and readily available resources for primary health care and complementary health care system, undoubtedly medicinal plants containing substances of medicinal value that have yet to be discovered, though large number of various plants are constantly being screened for their antimicrobial effect, these plants may prove to be a rich source of compounds with possible antimicrobial activity. Several studies have described the antioxidant properties of medicinal plant extracts which are rich in phenolic compounds. Natural antioxidants mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc [21]. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall.

CONCLUSION

The present study suggested that, the various solvent extracts of Curry leaves have a great potential as antimicrobial agents against selected enteric pathogens and they can be used as an alternative medicine in the treatment of enteric disorders. The antimicrobial activity and MIC assays showed promising evidence for the antimicrobial activity of Curry leaves solvent extracts against selected enteric pathogens. Phytochemcal analysis showed presence of antimicrobial substances in the studied extracts. The results revealed the presence of medicinally important constituents in these solvent extracts. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Therefore, the Curry leaves solvent extracts could be seen as a good source for useful drugs.

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Fig1. **Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Crude Curry Leaves.**

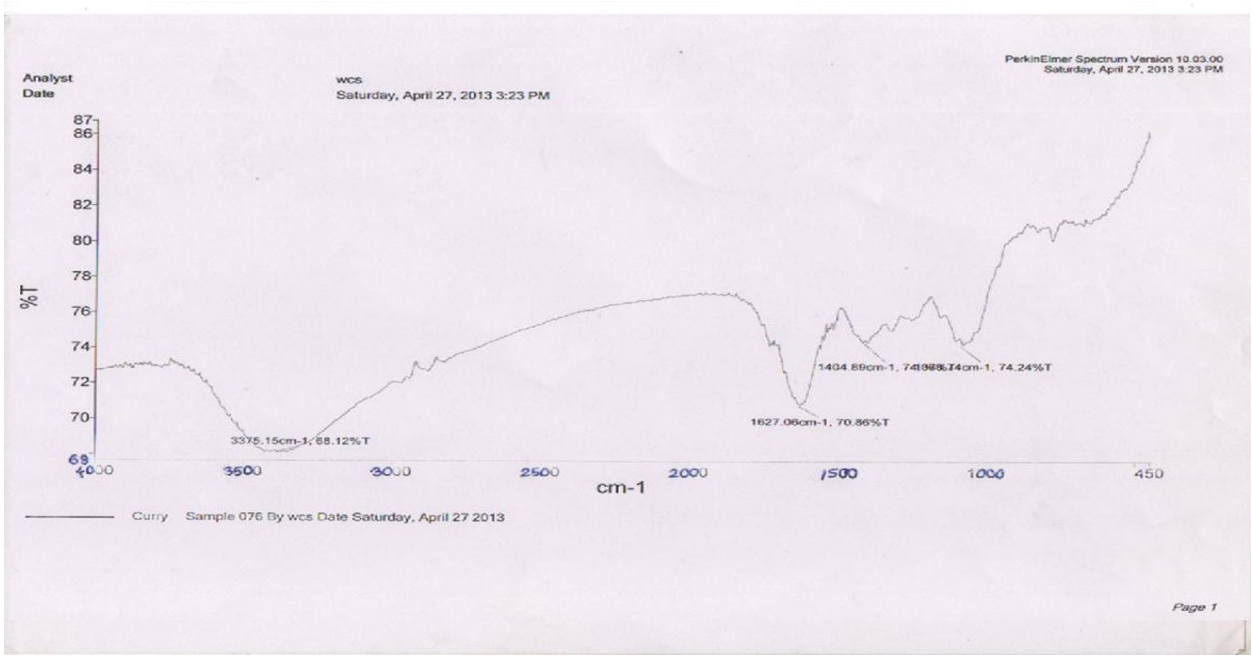

Table 1.Antibacterial Activity of Solvent Extracts of Curry Leaves.

M/O	Zone of Inhibition (mm)					
	Ethanol	Methanol	D.E.	Acetone	Ampi.	Tetra.
E.coli	6mm	5mm	3mm	3mm	13mm	14mm
Sal.typhi	6mm	4mm	3mm	5mm	14mm	11mm
Sal.para A	4mm	4mm	6mm	2mm	10mm	15mm
Sal.para B	4mm	2mm	4mm	3mm	11mm	12mm
Shigella sonnei	7mm	4mm	5mm	3mm	6mm	12mm
Shigella dysenteriae	6mm	4mm	3mm	2mm	12mm	11mm
Enterobactor spp.	5mm	3mm	4mm	3mm	12mm	14mm
Citrobactor spp.	5mm	4mm	3mm	2mm	15mm	13mm
Klebsiella spp.	4mm	3mm	4mm	3mm	9mm	11mm

(Ampi- Ampicillin, Tetra- Tetracycline)

Table 2. MIC of Solvent Extracts of Curry Leaves.

M/O	Minimum Inhibitory Concentration (ul/ml)			
	Ethanol	Methanol	D.E.	Acetone
E.coli	10	10	40	35
Sal.typhi	15	20	35	10
Sal.paratyphi A	35	15	10	50
Sal. paratyphi B	30	40	20	30
Shigella sonnei	05	15	15	40
Shigella dysenteriae	10	15	30	50
Enterobactor spp.	20	30	30	30
Citrobactor spp.	15	20	40	45
Klebsiella spp.	30	25	35	35

(D.E. - Diethyl ether)

Table 3.Phytochemical Analysis of Curry Leaves Solvent Extracts.

Test	Ethanol	Methanol	D.E.	Acetone
Alkaloid	+	+	+	+
Flavonoids	+	+	+	+
Tanin	-	-	-	-
Saponins	+	+	-	+
Glycosides	+	+	-	-
Carbohydrate	+	-	-	+
Phytosterol	+	+	+	+
Phenolic Compounds	+	+	+	+
Terpenoid	+	+	+	+

(+=Present, -=Absent)

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