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EVALUATION OF PHYTOCHEMICALS, ANTIOXIDANT POTENTIAL AND INVITRO ANTIBACTERIAL EFFICACY OF MURRAYA KOENIGII LEAVES AGAINST UROPATHOGENIC E.COLI

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ABSTRACT

Urinary tract infection (UTI) is the second most common infectious disease prevailing in developed and developing countries. Although a number of antimicrobial agents are available for the treatment and management of urinary tract infection, some microbes develop resistance to many antibiotics. *Murraya koenigii* (Curry leaves) a commonly used medicinally important herb of Indian origin in the Ayurvedic system of medicine are rich sources of carbazole alkaloids, which produce potent biological activities and pharmacological effects. Hence the present study were undertaken to investigate phytochemical screening, antioxidant activity and antimicrobial acitivity of aqueous extracts of *Murraya koenigii* leaves against the uropathogenic *E.coli* isolate from the urine samples of urinary tract infected patients. The results showed the presence of alkaloid, carbohydrate, flavonoids, phenols, phylobatannins, tannins, aminoacids and proteins in phytochemical analysis, exhibited four peaks 751.55, 954.65, 1,008.20 and 1,053.00 in UV-Vis analysis and characteristic peak in FTIR determination revealed the presence of alcohols, phenols, alkene, alkane, aromatics, amines etc. The minimum inhibitory concentration (MIC) of crude curry leaf aqueous extract was found to be10mg/ml against uropathogenic *E.coli*, MBC 20mg/ml, MBC/MIC value 2 and total antioxidant activity (TAC) 27.29 AAE/100 g. Thereupon *Murraya koenigii* can be exploited as a potent therapeutic agent against uropathogenic *E.coli*.

KEYWORDS: Urinary tract Infection (UTI), *Murraya koenigii*, Curry leaves, Uropathogenic *E.coli*, MIC, MBC.

INTRODUCTION

Ever since man has started his life, he suffers with never ending struggle. Some time he is busy with collections of food and other time from diseases inflicted on them, or their domestic animals. In their surrounding, the plants and other natural substances are available to cure the diseases. Traditional medicine is still recognized as the preferred primary health care system in many communities, with over 60% of the world's population and about 80% in developing countries depending directly on medicinal plants for their medical purposes.[1] This is due to a number of reasons including affordability, accessibility and low cost.[2] Urinary tract infections are very common infections and lead to significant amount of morbidity and mortality.[3] Urinary tract infection (UTI) was mostly caused by Gram negative (GN) bacteria, predominately by *Escherichia coli* is followed in prevalence by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Citrobacter freundii*, *Proteus vulgaris* and *Klebsiella oxytoca*. and by Gram positive, (GP) *Staphylococcus aureus*, *Enterococcus faecalis*.[4,5] The bacterial resistance of microorganisms that are isolated from human urinary infections is well recognized, resulting in a reduction of therapeutic efficacy, making such treatments ineffective and expensive, prolonging the course of the disease, increasing the incidence of complications and increasing the mortality rate.[6] Thus, the lack of new therapeutic agents to replace those that have become ineffective has necessitated the search to discover new alter-natives to treat UTIs more effectively.

Phytochemically curry leaves are rich source of organic compounds with different chemical composition such as alkaloids, flavonoids carbohydrates, sterol and volatile oil.[7] Traditionally, the plant is used as a stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhea, dysentery.[8] The leaves are reported to have great medicinal value such as antibacterial, anti-inflammatory, antifeedant etc.[9-11] Thus in the present research the antimicrobial properties of the leaf extracts of *Murraya koenigii* against *E.coli* that commonly causes urinary tract infection and antioxidant potential was evaluated.

MATERIALS AND METHODS

Plant material

M.koenigii leaves were collected locally from various locations of Perambalur, Tamil Nadu, India. The curry leaves were authenticated at Hans Roever KVK, Valikandapuram, Perambalur, Tamilnadu- 621115 and documented properly. The fresh curry laves were washed thoroughly and dried under shadow at room temperature for 3 days or until constant weight. Then the dried leaves were ground to fine powder in a mixer grinder (figure 1A-1C).

Extract preparation

The powdered curry leaf of about 40gm were immersed in 200 ml of double distilled water and kept at room temperature for two days (figure 1D). The extracts were filtered through Whatman No. 1 filter paper followed by muslin cloth and concentrated to dryness.[12] The stock was used for phytochemical screening, antioxidant and antibacterial activity.

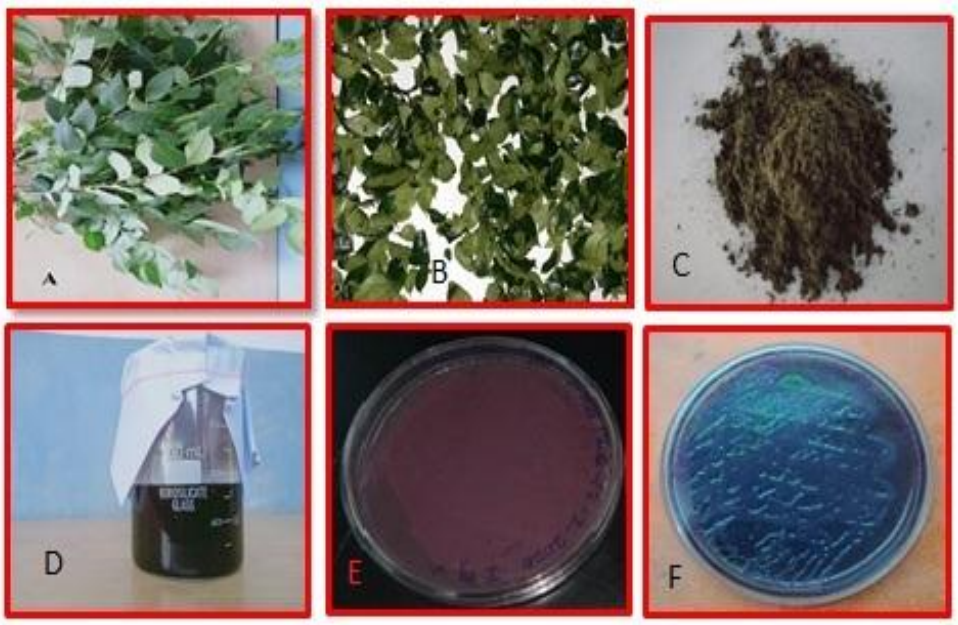


Figure 1: A. Fresh Curry leaves, B. Curry leaves dried under shadow, C. Powered Curry leaves, D. Aqueous Extract of Curry leaves, E. Characteristic pink color colonies in MacConkey agar plate - *E.coli,* F. Mettalic sheen in EMB (Eosin Methylene Blue) agar plate- *E.coli.*

Phytochemical screening

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.[13]

Spectroscopic analysis of extract

The extract was centrifuged at 3000rpm for 10min and filtered through Whatmann No.1 filter paper. The sample is diluted to 1:10 with the same solvent. The extract was scanned at wave length ranging from 200 to 1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.[14] The peak values of the UV-Vis were recorded.

Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm 1 with a resolution of 4cm^{1}.[15]

Antimicrobial activity of curry leaf extract

Test organism

Fourteen urine samples collected aseptically in a sterile container from UTI patients at Government Head quarter Hospital, Perambalur, Tamilnadu were used for isolation of uropathogenic *E.coli* immediately after transport to the laboratory. By following standard microbiological procedures *E.coli* was isolated, identified (figure 1E&1F) and maintained as pure culture in the Microbial culture Lab, P.G. and Research Department of Microbiology, Thanthai Hans Roever College (Autonomous) Perambalur, Tamilnadu.

Preparation of bacterial suspension

A pure single colony from the stock culture was inoculated onto Muller Hinton broth was incubated at 37^{0}C and bacterial suspension was adjusted to 0.5 McFarland standard.[16]

MIC aassay

Mininum inhibitory concentration was demonstrated by rezasurin turbidometric microbroth dilution method. Microwell plate containing 50µl of different concentration of curry leaf aqueous extract 160,80,40,20,10,5,2.5,1.25, 0.625mg/ml. was prepared. To each well 30ul of MHB, 10ul of bacterial suspension and 10ul of resazurin was added and incubated at 37^{0} C for 18- 24hrs after covering the plate with lid. The assay was performed in triplicate along with a positive control antibiotic, negative control and sterility control. The MIC value was determined to be the lowest concentration of the plant extract that did not induce the colour change.[17]

MBC was determined by inoculating 100ul of culture from the micro well exhibiting no growth /no colour change to sterile plate count agar and incubated at 37^0 C for 18- 24hrs. The lowest concentration of curry leaf where no bacterial growth was detected in the agar plate was considered as MBC.[18]

Total antioxidant activity

Total antioxidant activity of curry leaf extract was determined spectrophotometrically by phosphomolybdenum assay.[19] Phosphomolybdenum reagent solution was prepared by mixing 0.46g sodium phosphate, 78.4mg ammonium molybdate 3.367ml sulphuric acid for 100ml in water. The reaction mixture containing1 ml of curry leaf extract (1mg/ml) and 3ml of phosphomolybdenum reagent was incubated at 95^0C for 90mins. Absorbance was read at 695nm against reagent blank in a UV-VIS Spectrophotometer (Systronics). The total antioxidant capacity was expressed as ascorbic acid equivalent (AAE).[20]

RESULTS AN DISCUSSION

Aqueous extract of *Murraya koenigii*. L leaves were subjected to screening of different phytochemicals alkaloid, carbohydrate, cardiac glycosides, flavonoids, phenols, phylobatannins, aminoacids and protein, saponin, tannins and terpenoids. The results of qualitative phytochemical analysis of *Murraya koenigii*. L aqueous extracts are presented in table 1. Similar results of the presence of phytochemicals alkaloid, carbohydrate, phenols, tannins and terpenoids in the *Murraya koenigii*. L aqueous extracts was reported by Rashmi and Naveen.[21] Farooq et al.[22] analyzed the phytochemicals in the *Murraya koenigii*. L aqueous leaf extract and promulgate the presence of carbohydrate, flavonoids saponin, phenolic compounds and fixed oils and fats. Several authors reported the presence of different phytochemicals in the *Murraya koenigii*. L.[23-25]

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

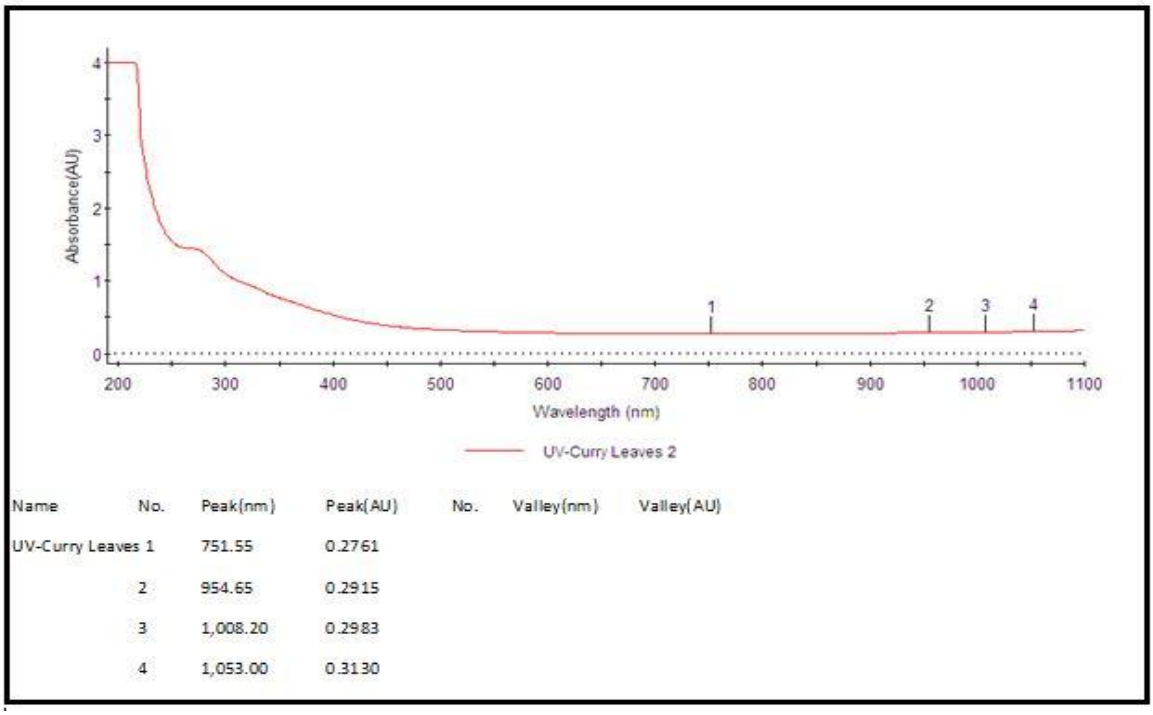
Phytochemicals	Aqueous leaf extract
Alkaloids	+
Carbohydrates	+
Cardiac glycosides	-
Flavonoids	+
Phenol	+
Phylobatannins	+
Amino acids & Proteins	+
Saponin	-
Steroids	-
Tannins	+
Terpenoids	-

+ indicates the presence of phytochemicals - indicates the absence of phytochemicals

Spectroscopic analysis

The crude *Murraya koenigii*. L leaf extract was filtered through Whatmann No.1 filter paper after centrifugation and scanned at wave length ranging from 200 to 1100 nm exhibited four peaks 751.55, 954.65, 1,008.20 and 1,053.00. The results of peak values and absorbance are as in figure 2.

FTIR analysis was performed to determine the chemical bonds in a molecule by producing infrared absorption spectra of pigments.[26] Measurements were carried out at infra-red spectra between 400-4,000nm. The FTIR analysis of the aqueous extract of *Murraya koenigii*.L revealed the characteristic peaks (figure 3) corresponding functional groups O-H, C-H, C=C, C-C, C-O evidenced the presence of alcohols, phenols, alkene, alkane, aromatics, amines etc. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity.[27] Flavonoid and its derivatives exert a varied range of anti-inflammatory, antibacterial, anticancer, antiviral and anti-allergic activities.[28]



Name	No.	Peak(nm)	Peak(AU)	No.	Valley(nm)	Valley(AU)
UV-Curry Leaves	1	751.55	0.2761			
	2	954.65	0.2915			
	3	1,008.20	0.2983			
	4	1,053.00	0.3130			

Figure 2: UV-vis spectrum analysis of curry leaves aqueous extract.

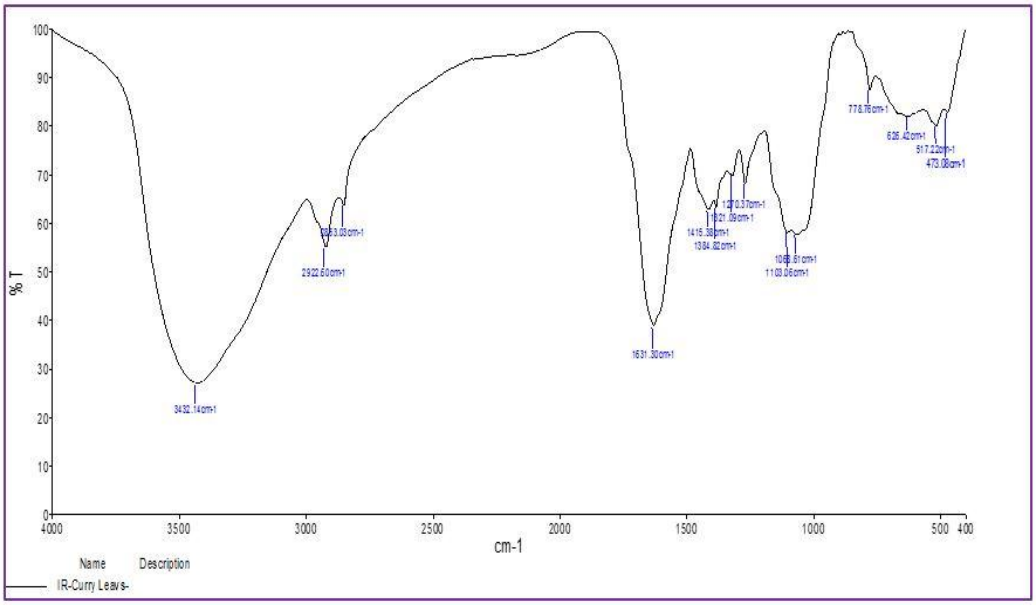


Figure 3: FTIR Analysis of curry leaves aqueous extract.

Antibacterial activity

Invitro antibacterial activity of the *Murraya koenigii*. L leaf extract was evaluated by resazurin microtiter-plate assay. The minimum inhibitory concentration of crude curry leaf extract was 10mg/ml, MBC 20mg/ml and the MBC/MIC value was 2 against uropathogenic *E.coli* (table 2). Our results are in accordance with previous results of Nithya and Aminu.[29] that the leaves of *Murraya Koenigii* L. exhibited significant antibacterial activity against urinary tract infection causative pathogens *E. coli* and *K. pneumoniae*. Similar results of Murraya koenigii plant extracts exhibiting higher antibacterial activity against Escherichia coli also reported by Ramamurthy and Prasanth.[30]

M. koenigii extracts have demonstrated antibacterial effects on a wide variety of microorganisms.[31,32] This property is attributed to several carbazole alkaloids present in the *M. koenigii* extracts.[33-,35]

Table 2: Antibcterial activity of *Murraya koenigii*. L leaves aqueous extract against *E.coli.*

MIC	MBC	MBC / MIC ratio
10mg/ml	20mg/ml	2

Total antioxidant capacity

Total antioxidant capacity was determined by phosphomolybdenum assay. The TAC of *M.koenigii* was determined to be 27.29 AAE/100 g of crude aqueous leaf extract. Rajesh et

al.[36] evaluated that the total antioxidant activity of acetone extract of *M. koenigii* old leaves was found to be 151.58%, and for young leaves in petroleum ether, the value was 72.23%. The antioxidant potential of the crude extracts of *M. koenigii* were probably due to the presence of flavonoids and phenolic derivatives.[37,38]

CONCLUSION

The current research revealed the antibacterial activity of *M.koenigii* against uropathogenic E.coli and significant antioxidant efficacy. *M. koenigii* is a source of several bioactive compounds, including alkaloids (carbazole), polyphenol, terpenoids, and flavonoids which could be responsible for the inhibition of pathogens causing Urinary Tract Infections. Thus, *Murraya koeniig*i can be used as an effective antimicrobial agents in new drugs against uropathogenic E.coli, a common causative agents of urinary tract infections. However further study need to include molecular mechanism behind the role of various constituents on radical scavenging and antimicrobial inhibition, experimental studies on bioavailability and efficiency enhancement in clinical investigations.

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