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Phytochemical screening and antibacterial efficacy of curry leaves in water purification and fertilizer use

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The phytochemical components and antibacterial effects of the leaf extract of *Murraya koenigii* and the water purification capacity was investigated in the present study. Treated and untreated water samples were studied for physio-chemical characteristics including hardness, TDS, nitrate, BOD and COD and bacteriological analysis using MPN method. Water and ethanol were used as solvents for curry leaf extract preparation which was further utilized for antibacterial and qualitative phytochemical assays. The *in vitro* antibacterial activity assay was performed by the agar well diffusion method. The results showed that both the aqueous and ethanolic leaf extracts had significant antibacterial activity in terms of zone of inhibition against *Escherichia coli*. The ethanolic extract showed a maximum reduction in MPN count of 56.25%. The phytochemical analysis of leaf extract showed the presence of bioactive agents like tannins, flavonoids, terpenoids, phenolic compounds and absence of glycosides, saponins, anthraquinones.

The aim of this study is to investigate the antibacterial properties, phytochemical composition, and water purification potential of curry leaf powder. Phytochemicals found in plants are bioactive compounds that have been shown to offer numerous health benefits, such as reducing the risk of cancer and strokes, as well as exhibiting antimicrobial effects. This work is unique in that it explores both the medicinal and environmental applications of curry leaves, specifically in the context of water purification. By examining the antibacterial and water treatment abilities of curry leaf powder, this study seeks to provide new insights into the versatile uses of curry leaves, with implications for both health and sustainability.

Keywords: agar well diffusion, antibacterial activity, chemical oxygen demand, leaves extract, most probable number, phytochemical components, water purification

INTRODUCTION

Different plant parts are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides which have been found to have antimicrobial properties. The studies about the effect of leaf extracts against different types of bacteria and for sustainable water purification are still one of the most important fields of research [1-2]. The extracts thus obtained may be used as medicinal agents normally expected to contain phytochemicals. Curry leaves are a part of small deciduous aromatic shrub scientifically named *Murraya koenigii* belonging to the family *Rutaceae* commonly considered as natural medicinal plant. Curry leaves have a blood pressure lowering effect, antibacterial activity, antifungal activity, antiprotozoal activity, laxative effect, anti-diarrhoeal activity, wound healing action, anti-cancer activity, antidiabetic activity, anti- inflammatory action, antioxidant, cholesterol lowering effect, antiulcer activity and anti-tumor activity [3-4]. Curry leaf extract showed abroad spectrum of very significant antibacterial activities by producing a clear zone of inhibition against *Staphylococcus, Streptococcus* and *Proteus* [5]. The present study was carried out to evaluate the antibacterial and phytochemical activity, as well as water purification efficacy of curry leaf powder. Phytochemicals are naturally bioactive components found in plants which may reduce cancer, strokes, hinder the aging process and have antimicrobial properties [6-7].

MATERIAL AND METHODS

Collection of plant material and extract preparation

Mature leaves of *Murraya koenigii*, commonly known as curry leaves, were harvested from a botanical garden. After collection, the leaves were sorted and cleaned with tap water, followed by rinsing with distilled water. Subsequently, they were dried in an oven at the optimal temperature and then ground into a powder using a mixer and grinder. This curry leaf powder was utilized to prepare various extracts. For the extraction process, 10 g of the dried leaf powder was placed in a 250 ml conical flask, and 100 ml of water and ethanol were added separately.

The flasks were tightly sealed with polyethylene sheets and vigorously shaken on a shaker for 48 h.

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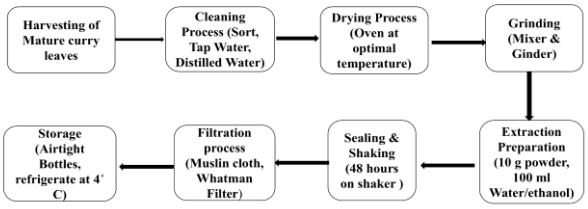
After extraction, both the water and the ethanol extracts were initially filtered through muslin cloth and then through Whatman No.1 filter paper. The resulting filtrates were stored in airtight sample bottles in a refrigerator at 4ºC until further use [4-5].

LITERATURE

Flow Diagram for Curry Leaf Extraction Process



Some studies on curry leaf extract (*Murraya koenigii* L.) highlights the presence of several bioactive compounds including alkaloids, flavonoids, saponins, polyphenols, and tannins. The alkaloid content was found to be 23.73%, as indicated by a positive test that resulted in a white precipitate. Flavonoids were present at a lower concentration of 1.24%, with a pink color formation confirming their presence. Saponins were detected at 8.74%, with foam formation within approximately one min serving as the positive indicator. The polyphenol content was 4.4%, identified by a greenish-black color during testing, while tannins were present at 5.2%, confirmed by a greenish-orange color. UV-Vis spectrophotometry was used to measure the absorbance at various wavelengths, with the levels of flavonoids, polyphenols, and tannins correlating with higher absorbance values at increasing concentrations. These findings suggest that curry leaf extract is rich in bioactive compounds, making it a potential ingredient for both animal feed and medicinal applications [8]. The methanolic extract of *T. cordifolia* stem was most effective against *Staphylococcus aureus*, with *Escherichia coli* being the least sensitive. Amoxicillin was the most potent antibiotic, followed by others like ampicillin and chloramphenicol. A synergistic effect was noted between amoxicillin and *Murraya koenigii* leaf extract, showing maximum inhibition against *Staphylococcus aureus*. Increasing the extract concentration reduced microbial growth. *Murraya* leaves contain bioactive compounds such as steroids, saponins, flavonoids, and alkaloids, which contribute to its antimicrobial properties [9]. Some researchers evaluated the drying behavior and kinetics of curry leaf and pulp, comparing different drying methods. It finds that CHD (conductive hydro drying), particularly at 40°C, is the most efficient for drying time, proximate composition, and antioxidant activity, with the best color retention. CHD at 80°C and freeze drying (FD) excel in preserving phytochemicals and achieving low water activity, essential for longer shelf life**.** FD and CHD at 80°C are also most effective for moisture removal and improving flow properties. The research highlights that while TD (tray drying) is energy-efficient for pulp, CHD offers a better balance of time-saving and quality preservation, making it ideal for maintaining the nutritional and medicinal properties of curry leaves. Overall, CHD is superior for optimizing the drying process to enhance both health and culinary benefits of curry leaf [10]. The health benefits and applications of *Murraya koenigii* (curry leaves), emphasizing their nutritional composition and functional properties. The study reveals that *M. koenigii* leaf powder is a valuable source of fiber, low in fat, and rich in phenolic compounds, making it an effective natural antioxidant. The difference in phenolic and flavonoid content offers insights for future research into their specific health effects. Using advanced techniques like HPLC, SEM, FTIR, and GC-MS, the study provides a comprehensive analysis of its composition and potential uses. These findings lay a strong foundation for developing functional foods, supplements, or therapeutic agents based on the nutritional and bioactive properties of curry leaves, with promising implications for global health and well-being [11].

Culture media and inoculum preparation

Pure culture isolates of *E. coli* were cultivated in nutrient agar broth by inoculating a loopful of culture into sterile nutrient broth, followed by incubation at 37°C for 48 h. From these broth cultures, a loopful was extracted and spread onto sterile nutrient agar plates using a sterile cotton swab to produce a dense, uniform lawn culture. The nutrient agar and broth were prepared individually in distilled water and sterilized by autoclaving at 120°C for 15 min under a pressure of 15 lb. Bacterial cultures of *E. coli* were separately introduced into the nutrient broth and subjected to shaking conditions for 24 h.

Comparison between the present work and published works

Plant leaf	Phyto-chemical components	Antibacterial target	Antibacterial effect	Ref.
Murraya koenigii (Curry leaves)	Alkaloids, Glycosides, Tannins, Saponins, Flavonoids, Steroids	*E. coli*, Coliforms (Water sample)	Significant reduction in coliform count (56.25%) in treated water samples	Present work
Murraya koenigii (Curry leaves)	Alkaloids, Flavonoids, Glycosides, Saponins, Tannins	*Staphylococcus aureus*, *Streptococcus*, *Proteus*	Broad-spectrum antibacterial activity, clear inhibition zones	[5]
Murraya koenigii (Curry leaves)	Alkaloids, Glycosides, Tannins, Flavonoids, Steroids	*E. coli*, *Klebsiella pneumoniae*	Antibacterial activity against *E. coli* and *Klebsiella pneumoniae*	[4]
Murraya koenigii (Curry leaves)	Alkaloids, Flavonoids, Tannins	*E. coli*, *Salmonella*	Significant antibacterial effects	[1]
Murraya koenigii (Curry leaves)	Alkaloids, Flavonoids, Glycosides, Tannins	*E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*	Antibacterial activity against multiple strains	[3]

Collection and analysis of water sample

A sample of pond water was gathered and preserved in pre-cleaned plastic containers. It was then subjected to both physio-chemical and bacteriological analysis using standard protocols, both prior to and following treatment with curry leaf extract. Various parameters including total hardness, nitrate levels, fluoride content, total dissolved solids (TDS), and chemical oxygen demand (COD) were measured in the water samples.

Treatment of effluent

A sample of pond water was treated with 10 ml of alcoholic curry leaf extract and left to incubate for a period of 48 h. Following incubation, the sample was filtered and subjected to analysis. Total dissolved solids (TDS) were determined using the oven drying method, hardness was assessed through EBT-EDTA titration, nitrate and fluoride levels were measured using an Aqua water testing kit, and chemical oxygen demand (COD) was quantified *via* the ferroin-FAS titrimetric method [12].

Most probable number (MPN) test

It is a method used to estimate the viable microorganisms in a sample by means of replicate lactose broth, both single strength and double strength tubes prepared in ten-fold dilution. It is a most commonly used applied test for quality testing of water to ensure whether the water is safe or not in terms of bacteria present in it. A group of bacteria commonly referred to as faecal coliforms act as indicator of faecal contamination of water.

Phytochemical screening of the extract

A known volume of alcoholic curry leaf extract was subjected to the phytochemical tests [13] for alkaloids, glycosides, tannins, flavonoids, saponins, terpenoids and phenolic compounds.

Test for alkaloids: Filtrates were treated with Wagner's reagent (iodine in potassium iodide) Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for glycosides: The extracts were hydrolyzed with HCl solutions and neutralized with Na_2SO_4 solutions. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of glycoside.

Test for tannins ($FeCl_3$ solution test): 10% alcoholic ferric chloride solution was added to 2 ml extract. The formation of green color indicated the positive result.

Test for flavonoids: About 0.2 g of the extracts were shaken with 5 ml of distilled water and then a few drops of 10% lead acetate solution was added. A yellow or dirty white precipitate shows the presence of flavonoids.

Test for saponins: 5 ml of hot distilled water was mixed with 1 ml of extract solution and shaken vigorously for two min. The test tube was left for a while and then was checked for the presence or absence of frothing formation as an indication of presence of saponins.

Test for terpenoids: To 200 mg of the dry crude extract, 2 ml of chloroform and 3 ml of concentrated sulfuric acid were added. Formation of a reddish-brown color is an indication of the presence of terpenoids.

Test for phenolic compounds: 1 ml of the methanol extract was treated with 10% ethanolic ferric chloride. Change in color from blue-green to dark blue is an indication of the presence of phenolic compounds.

Antibacterial activity assay by agar well diffusion

The antibacterial activity of the curry leaf extract was assessed against pre-prepared 20 ml of sterilized Müller-Hinton agar plates. These plates were inoculated with 100 µl of fresh *E. coli* culture, which was evenly spread across the surface using a sterile swap spreader to ensure uniform microbial growth. Following inoculation, heat-sterilized cork borers with a diameter of 10 mm were employed to create wells in the agar medium. Subsequently, 50 µL of the curry leaf extract was loaded into the wells and tested against the inoculated organism. Diluted curry leaf extract was poured into the wells. The plates were then placed in an incubator at 37°C for 24 h and subsequently examined for clear zones of inhibition, which were measured. Sterile water was used as a control in this experiment.

Similarly, for another set of experiments, bacterial inoculum was uniformly spread onto sterile Petri plates and allowed to solidify. Following this, four wells were created in each plate using a cork borer with a diameter of 10 mm. Different concentrations (100%, 10%, and 5%) of ethanol extracts from selected plant sources were added to three respective wells, while one well was filled with 70% ethyl alcohol. The Petri plates were then incubated for 18-24 h and observed for bacterial growth. The zone of inhibition surrounding bacterial growth was measured in mm.

RESULTS AND DISCUSSION

Aqueous test of water sample

Table 1. Water quality parameters after treatment with ethanolic extract

S. No	Parameter (mg/l)	Before treatment	After treatment	% Removal
1.	TDS	578	380	34
2.	Total hardness	300	90	70
3.	Fluorides	2.5	0.5	90
4.	Nitrate	200	50	75
5.	COD	124	100	19

Treatment with the curry leaf extract resulted in a significant reduction in the hardness (300 to 90 mg/l), fluorides (2.5 to 0.5 mg/l), nitrate (200 to 50 mg/l) of the water sample at a concentration of 100 mg/l, which was in accordance with previous literature (Table 1). Treatment with curry leaf extracts presented moderate reductions in the TDS (578 to 380 mg/L) and COD (124 to 100 mg/l) of the pond water samples [14-15]. These findings strongly validate the high coagulation potential of the curry leaf extract in purification of water [16].

Chemical oxygen demand (COD) analysis

Chemical oxygen demand (COD) is a measure of the capacity of water to consume oxygen during the decomposition of organic matter and the oxidation of inorganic chemicals. The higher the value of COD, the lower is the dissolved oxygen in water. An aliquot of the sample was digested for 2 h at 150°C in the presence of dichromate and sulfuric acid. The resulting solution was titrated to a colored endpoint with ferroin indicator.

Equations for COD:

$$\text{COD} = \frac{(a-b)\times N\times 8\times 1000}{Volume\ of\ Sample\ (mL)}$$

Result of water sample without treatment:

$$\text{COD} = \frac{(8.1\text{-}5)\times 0.1\times 1000\times 8}{20} = 124 \text{ mg/l}$$

The total chemical oxygen demand in untreated water sample was 124 mg/l.

Result of water sample after treatment

$$\text{COD} = \frac{(8.1\text{-}5.6)\times 0.1\times 1000\times 8}{20} = 100 \text{ mg/l}$$

The chemical oxygen demand in the water sample after treatment with curry leaves extract was 100 mg/l.

Phytochemical analysis of curry leaf ethanolic extract

A small portion of ethanolic extract was subjected to the phytochemical testing for alkaloids, glycosides, tannins, flavonoids, saponins, terpenoids, phenolic compounds and anthraquinones (Table 2).

Table 2. Phytochemical analysis test results

S. no	Phytochemical test	Results
1.	Alkaloids	-
2.	Glycosides	-
3.	Tannins	+
4.	Flavonoids	+
5.	Saponins	-
6.	Terpenoids	+
7.	Phenolic compound	+
8.	Anthraquinones	-

[(+) means present, (-) means absent]

Presence of different phytochemicals in leaves of *M. koenigii* Linn., is shown in Table 2. The results are in confirmation to [4, 6].

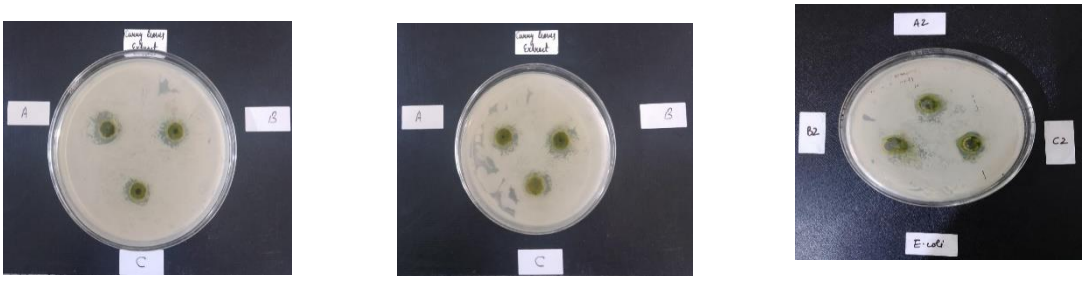
Antimicrobial activity (mm) of M. koenigii leaves extracts against test organisms

Antimicrobial test for the ethanolic and aqueous leaves extract was carried out against *E. coli* on the already prepared 20 ml of sterilized Muller-Hinton agar plates which were inoculated and incubated at 37^0C for 24 h. A heat-sterilized 10 mm cork borer was then used to make wells in the already inoculated medium and the plant sample to be tested against each test organism [17]. The sterile petri plates were filled with sterilized nutritional agar medium (20 ml) and allowed to harden. The *E. coli* broth culture was swabbed on an agar plate using a sterile bud. The wells (5 mm in diameter) were drilled into the agar with a sterile borer. Curry leaves extract was aseptically placed into each well and incubated at 37°C for 24 h. A measurement of the inhibitory zone was made (Table 3). Antibacterial activity was measured with ruler using the diameter of the inhibition zone which was measured in millimetres [18]. It was shown that the bigger the zone, the higher is the antibacterial activity but the lack of zone of inhibition does not necessarily mean absence of activity. A zone is generally shown by antimicrobial agents which kill the microbes present on as well as around the treated area [13,19]. From Table 3 it is observed that ethanolic extract is more effective for varying concentrations against *E. coli* culture as compared to the aqueous extract.

Table 3. Antimicrobial activity results for both extracts

Bacteria	Concentration	Aqueous extract	Ethanolic extract
Escherichia coli (*E. coli*)	100 ppm	5 mm	15 mm
	50 ppm	9 mm	12 mm
	25 ppm	5 mm	12 mm

Aqueous extract result



100 ppm 50 ppm 25 ppm

Fig. 1. Zones of inhibition by aqueous curry leaf extract for 100 ppm, 50 ppm and 25 ppm.

Ethanolic extract result

100 ppm 50 ppm 25 ppm

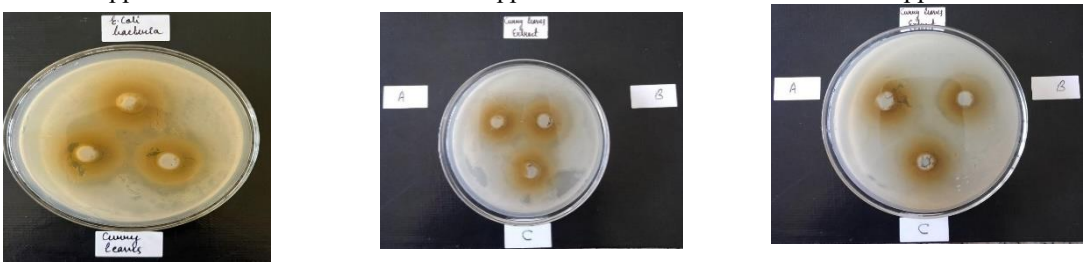


Fig. 2. Zones of inhibition by ethanolic leaf extract for 100 ppm, 50 ppm, 25 ppm.

Total coliform using the most probable number (MPN) method

The most probable number (MPN) method is a statistical method used to estimate the viable numbers of coliform bacteria in a water sample by inoculating lactose broth in 10-fold dilutions and is based on the principle of extinction dilution [20]. The presumptive test involves inoculating multiple tubes of liquid growth medium with different volumes of the sample being tested. Dispense the double strength medium in 6 tubes (10 ml in each tube) and single strength medium in 3 tubes (10 ml in each tube) and add Durham tube in an inverted position. The test tubes were then incubated at 37^0C for 24-48 h. The test involves observing the media color change and/or formation of gas bubbles in the tubes from the confirmed test.

The number of tubes showing gas production were counted and the figure was compared to a table developed by American Public Health Association (Table 4). The number was the MPN of coliform per 100 ml of the water sample [17, 21].

Table 4. Most probable number (MPN) results

S.no	Pond water with ethanolic extract	Untreated water sample	Treated water sample	MPN count of untreated water sample	MPN count of treated water sample	% Reduction in MPN count
1.	10ml	3	2	16	7	56.25
2.	1ml	2	1			
3.	0.1ml	1	1			

MAJOR CHALLENGES

The study on *Murraya koenigii* (curry leaves) extracts shows its potential for antibacterial activity and water purification. However, several challenges need to be addressed. These include variations in phytochemical composition due to factors like location and extraction methods, which can affect consistency. Additionally, the extraction process may be inefficient, leading to high costs and low yields. The exact mechanisms behind the antimicrobial and water-purifying effects are not fully understood, requiring further investigation. Scaling up production for commercial use presents logistical and financial difficulties, and the safety of the extract needs thorough evaluation. Regulatory approval is another challenge, along with the need to assess its effectiveness against a broader range of pathogens. Overcoming these challenges will be essential for the widespread use of curry leaf extract in different applications.

CONCLUSIONS

From the present research it follows that the curry leaves extract was successful in increasing the purification of water by reduction in various parameters. There was a significant reduction (56.25%) of coliform count in treated water sample with curry leaves extract [22] as seen from Table 4. The phytochemical analysis revealed the presence of bioactive compounds which are responsible for the *in vitro* antimicrobial action of the ethanolic extract of *Murraya koenigii* on *E. coli* strain. The results showed that extracts obtained from the leaves of the plant *Murraya koenigii* are rich sources of potent phytochemicals and have inhibitory effects on the tested microbes. From previous studies and the current work, it is clear that the plant is a rich source of alkaloids, glycosides, tannins, saponins, flavonoids and steroids. These bioactive complex phytochemicals can be used for the development of potent drugs, medicines or antimicrobial agents that can be used for various purposes for human welfare upon further extensive and systematic studies.

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