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Evaluation of Antimicrobial Efficacy of *Murraya Koenigii* Plant Leaf Extract Against *E. coli* Isolated from Different Sources

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Abstract

The current study was carried out to evaluate the anti-microbial efficacy of the leaf extract *Murraya koenigii* plant against *E. coli* isolated from clinical and various other sources like water, soil, skin, floor, juice, basin, incubator and mobile phone. The anti-microbial test showed that the ethanolic extract was more potent than aqueous extract of *Murraya koenigii* leaves against *E. coli*. The sensitivity of the bacterial isolates from each sample having lowest potential against the extract was examined against four different antibiotics. It was observed that all isolate showed complete resistance to Amoxicillin and were sensitive to Ciprofloxacin, Chloramphenicol and Gentamycin. Qualitative analysis of active compounds in extract of *Murraya koenigii* leaves was done using different chemical reagents. The result showed the presence of phenols, flavonoids, terpenoids, tannins and quinones. The Total Antioxidant Capacity was calculated to be 40.42%. UV-VIS spectrophotometric analysis of extract of *Murraya koenigii* leaves in different solvents like distilled water, ethanol and methanol was done. The analysis exhibited peaks at wavelength 420 and 500 nm for methanol and 500nm for distilled water and ethanol.

Keywords: *Murraya koenigii*, *E. coli*, Antimicrobial Activity

1. Introduction

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. (Tugume P. *et. al.* 2019). One of which is *Murraya koenigii* plant. The Curry Tree (*Murraya koenigii*) is tropical to the subtropical small tree in the family *Rutaceae*, which is native to India. It is reported to have anti-oxidant, anti-diabetic, anti-carcinogenic, anti-dysenteric, stimulant, hypoglycemic and antimicrobial activities (Ningappa *et. al.*2008). The extract of curry leaves shows very significant antibacterial activity against many broad ranges of bacteria mainly *E. coli.*

Escherichia coli is Gram-negative, facultative anaerobic and rod-shaped bacterium of the genus *Escherichia*. Pathogenic *E. coli* contamination of the environment may occur through manure and other animal wastes, wastewaters from slaughterhouses and effluent from wastewater treatment plants *(Balière et. al.* 2015). Most pathogenic *E. coli* are transmitted by fecal-oral route from food materials, water, animals, and environment. Depending on the pathotype and the system, *E. coli* infection may cause a range of

syndromes including watery, mucoid, or bloody diarrhea; abdominal cramps; urinary tract infection syndromes; and meningitis. Also, syndromes have been reported as food poisoning outbreak, travel-related illness, or animal or contaminated environment contact-related diseases.

In the past few years, antibiotics helped saving a significant number of lives and reduced the illness of several million people across the world (Jones *et. al.* 2007). However, the emergence and rapid spread of extended-spectrum cephalosporin and carbapenem resistance in *Enterobacteriaceae* is becoming a global health challenge. In addition, antibiotic-resistant *E. coli* are also increasing and becoming a major threat for global human health. The increasing resistance to cephalosporin, especially the parallel rise in the frequency of multidrug-resistant *E. coli*, constitutes an increasing concern for the treatment of *E. coli* disease (Wu D *et al.*2021).

To find alternative medicine medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs, or use to complement or neutralize drag's negative effects (synergic medicine), also medical plants can prevent the appearance of some diseases, this will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment.

Phytochemically curry leaves are rich source of organic compounds with different chemical composition such as alkaloids, flavonoids carbohydrates, sterol and volatile oil (Shaikh *et. al.* 2020). The presences of important phytochemicals make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. Antioxidants are the substance that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance (Praveen *et. al.* 2007). Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Khalaf *et. al.* 2008).

This study aims to detect the antimicrobial activity of the leaves of plant *Murraya koenigii* via assessing its phytochemical constituents and antioxidant activity against enteropathogenic *E. coli.*

2. Materials and Methods

Collection of samples

For this study 30 samples from various sources were taken for the isolation of *E. coli.* Clinical samples such as blood and urine were collected from Mapari Pathology Laboratory, Akola. Soil samples were collected from Rhizospheric area of Rose, Hibiscus and Mango plants in a cleaned polythene bag. Sewage water samples from local drains of Akola city, domestic water samples from residential area and various juice samples from local vendors of Akola City were collected. Other samples like skin, floor, basin and mobile were collected using sterile cotton swabs. The swabs were rotated and rubbed against sampled surface several times and then kept in sterile test tubes until inoculation.

Isolation and Identification

Each of the collected samples without much delay was inoculated onto EMB (Eosin Methylene Blue) agar plates and incubated at 37°C for 24 hours. The colonies which produced metallic green sheen with dark centered colonies were selected and were sub-cultured to assess the uniformity of colony appearance. Further identification of the bacteria was done based on colony characteristics observed on the EMB Agar plate, morphological characteristics by Gram's staining, sugar fermentation test, and a series of biochemical tests.

Extract Preparation

Murraya koenigii leaves were collected locally from Akola city of Maharashtra, India. The curry leaves were washed thoroughly and dried under shadow at room temperature for 5 days or until constant weight. Then the dried leaves were ground to fine powder in a mixer grinder.

1) Aqueous Extract:

The curry leaf powder of about 20gm in 100ml of double distilled water and kept at room temperature for 2 days. The extract was filtered through Whatman No. 1 filter paper followed by muslin cloth.

2) Ethanolic Extract:

The powdered curry leaf was mixed with 70% ethanol for 24 to 48 hours. It was then filtered using Whatman No. 1 filter paper. The extract was concentrated further in a water bath at 40°C by heating and evaporating the solvent.

Antimicrobial Activity

Antibacterial activity using plant extract was evaluated by Agar Well Diffusion Method on Nutrient Agar. Each isolate of *E. coli* was uniformly swabbed onto the individual plates using sterile cotton swabs. Wells were made by using sterile borer. A micropipette was used to pour the extract solution into each well on all plates. After incubation at 30o C for 24 hours the zone of inhibition was measured in millimeters.

Antibiotic Susceptibility Test

Antibiotic susceptibility test was carried out using agar disc diffusion method on Nutrient Agar. Each strain of *E. coli* was uniformly swabbed onto the individual plates using sterile cotton swabs. After the inoculation, different antibiotic discs were placed on the medium using sterile forceps. The antibiotics used in the study include Ciprofloxacin (5µg/disc), Chloramphenicol (30µg/disc), Amoxicillin (30µg/disc), and Gentamicin (10µg/disc). The plates were incubated at 30o C for 24 hours. After incubation, clear zone of inhibition was measured and results were noted.

Spectrophotometric analysis of *Murraya koenigii* leaf extract

The extract was centrifuged at 3000rpm for 10min and filtered through Whatman No.1 filter paper. The sample is diluted to 1:10 with the different solvent. The extract was scanned at wave length ranging from 400 to 680 nm using UV-VIS Spectrophotometer and the characteristic peaks were detected.

Phytochemical Screening

Qualitative phytochemical analysis of the curry leaf extracts was carried out to detect the presence of phytochemicals by following the procedures stated below:

1. Test for phenol

2ml of *Murraya koenigii* extract was taken in a test tube and few drops of 1% ferric chloride were added in it. Presence of phenol was confirmed by the appearance of green/blue/ bluish green/ brown/ brownish red color (Sophiyamole L *et. al.* 2017).

2. Test for flavonoids

2 ml of Murraya koenigii leaf extract solution was taken in a test tube and 3 ml of diluted ammonia was added to the solution and then 1 ml concentrated sulphuric acid was added into the solution. The tube was observed for appearance of yellow color indicating the presence of flavonoids.

3. Test for terpenoids

3ml Murraya koenigii leaf extract dissolved in 1ml of chloroform in a test tube and 1ml of concentrated sulphuric acid was added in the test tube. An intense red- brown indicates the presence of terpenoids (Sophiyamole L *et. al.* 2017).

4. Test for tannins

About 0.5g of dried powder of Murraya koenigii plant sample was boiled in 4ml of water in a test tube and then filtered. Few drops of 0.1% Ferric chloride were added to observe brownish green or blue-black coloration indicating the presence of tannin (Sophiyamole L *et. al.* 2017).

5. Test for Quinone

A few drops of concentrated hydrochloric acid were added to 1ml of extract. A yellowish-brown color was observed for the presence of quinone.

Antioxidant Activity

Total antioxidant activity of curry leaf extract was determined spectrophotometrically by phospho-molybdenum assay. Phosphomolybdenum reagent solution was prepared by mixing 0.46g sodium phosphate, 78.4mg ammonium molybdate and 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 95oC for 90mins. Absorbance was read at 695nm against reagent blank in a UV-VIS Spectrophotometer. The antioxidant capacity was estimate using the following formula: % Inhibition = $\frac{\text{Abs.of control}-\text{Abs.of sample}}{\text{Abs.of control}} \times 100$

3. Results And Discussion

During the current research work total 30 samples were analyzed including clinical samples such as blood and urine samples where as other samples were collected from different sources such as tap water, sewage water, soil, skin, pineapple juice, sugarcane juice, basin, floor, mobile, incubator and shoes. From total 30 samples, 23 samples were isolated as Gram negative coccobacilli. On the basis of cultural, morphological and biochemical characteristics, the isolates obtained were tentatively confirmed as *Escherichia coli*. In the study, M. Kavitha (2017) reported the antimicrobial efficiency of *Murraya koenigii* against bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Similar reports were given by Pagu *et.al.* (2020). They isolated *Escherichia coli* from urine sample of UTI patients for observing the antimicrobial effect of aqueous extract of *Murraya koenigii* leaves.

In the current investigation, Aqueous and Ethanolic extract of leaves of *Murraya koenigii* plant were prepared to detect its antimicrobial activity against *E. coli*. The ethanolic extract obtained from *M. koenigii* were less viscous in nature and brownish in color. The Antimicrobial Activity of aqueous and ethanolic extract of *Murraya koenigii* leaves was evaluated by Agar Well Diffusion Method on Nutrient Agar. It was observed that ethanolic extract of leaves of *Murraya koenigii* was more effective than the aqueous extract (Table 1; Figure 1)

Ramamurthy and Prasanth (2018) reported similar findings of *Murraya koenigii* plant extracts demonstrating greater antibacterial activity against *Escherichia coli.* Sethi *et. al.* (2012) found the highest antibacterial activity against *Escherichia* (29mm) with methanolic extract of Leaf and Bark of *Murraya.* According to the study done by Arun Thanavel *et.al.* (2017) *Murraya koenigii*'s wintertime acetone extract had more actively than other extract against *E. coli* (16mm), *K.pneumoniae* (17mm), *S.typhi* (16mm) and *P.mirabilis* (15mm).

The isolates from each sample having highest potential as antimicrobial was selected for the antibiotic susceptibility testing. It was noticed that all the isolates showed complete resistance to Amoxicillin and were sensitive to Ciprofloxacin, Chloramphenicol, and Gentamycin. The M2 (mobile) isolate was found to be the most susceptible to the antibiotics used forming a zone of clearance of 34 mm for Ciprofloxacin, 28 mm for Chloramphenicol and 26 mm for Gentamycin. The least sensitive isolate for Ciprofloxacin was

S1 (soil) forming 21 mm zone of inhibition. The least zone of 10 mm was recorded for Chloramphenicol against the isolates obtained from Bl (blood) and T2 (tap water). For Gentamycin 17 mm zone was obtained against *E. coli* obtained from incubator (Table 2; Figure 3).

According to the research done by Arun Thangavel *et.al.* (2017) it was revealed that Ciprofloxacin had a high level of inhibitory effect against *E. coli* found in urine samples from UTI patients. Also, Olerumi *et.al.* (2011) in his study observed that the Ciprofloxacin and Oflaxin could inhibit the growth of gram-negative bacteria. The results of Nehia Hussein *et. al.* (2017) showed that *E. coli* resistance to Erythromycin, Ciprofloxacin, Cefotaxime, Tobramycin, Doxycycline, and Clindamycin are 100%, 97%, 94%, 100%, 89% and 93% respectively, while both chloramphenicol and gentamicin were inhibited 22.33 mm and 18.66 mm respectively.

The qualitative UV-VIS spectrum profile of extract of *Murraya koenigii* in different solvents like distilled water, ethanol and methanol were studied at different wavelength ranging from 400 to 680 nm due to sharpness of peaks and proper baseline. The profile showed the peaks at 420 and 500 nm with absorption of 0.43 and 0.38 respectively for methanol; 0.41 and 0.37 respectively for ethanol, while for distilled water the profile showed peak at 500 nm with absorption of 0.17 (Figure 5). The result is in accordance with Pagu *et.al.* (2020) who scanned at wave length ranging from 200 to 1100 nm exhibited four peaks 751.55, 954.65, 1,008.20 and 1,053.00. Similar study was done by K. Kalaichelvi *et.al.* using *Micrococca mercuralis* petroleum ether extract at selected wavelength from 200 nm to 700 nm due to sharpness of peak and proper baseline. The profile showed the peaks at 214, 446 and 472 nm with the absorption of 0.599, 0.655, and 0.550 respectively.

The preliminary phytochemical screening of *Murraya koenigii* plant was carried out in current research to check the presence of phytochemicals in the plant. It was seen that all the phytochemical constituents such as phenols, flavonoids, terpenoids, tannins and quinones were present in the *Murraya koenigii* leaf extract (Table 3). Similar results for the presence of phytochemicals like flavonoids, phenols, tannins, terpenoids and various other like alkaloids, carbohydrates amino acids and proteins in the Murraya koenigii. L aqueous extracts was reported by Pagu *et.al.* (2020). Also, Rashmi and Naveen (2016) reported the presence of phytochemical constituents viz. alkaloid, carbohydrate, phenols, tannins and terpenoids in the *Murraya koenigii* L aqueous extract.

Total antioxidant capacity was determined by phosphomolybdenum assay. The total antioxidant capacity of aqueous extract of *Murraya koenigii* was determined to be 40.42%. Another study done by Rajesh *et.al.* (2017) revealed 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%. Pagu *et. al.* (2020) also the evaluated the antioxidant activity via phosphomolybdenum assay; the total antioxidant capacity was found to be 27.29 AAE/100 g of crude aqueous leaf extract.

Table 1: Antimicrobial Activity of *Murraya koenigii* against *E. coli* isolated from different sources

Isolates	**Aqueous Extract**	**Ethanolic Extract**
	Zone of Inhibition (in mm)	
Blood (Bl)	14.0	16.0
Urine 1 (U1)	16.0	18.0
Urine 2 (U2)	10.0	12.0
Urine 3 (U3)	16.6	20.4

Tap water 1 (TW1)	14.0	14.0
Tap water 2 (TW2)	17.2	18.6
Sewage Water 1 (SW1)	18.4	19.0
Sewage Water 2 (SW2)	19.0	20.0
Sewage Water 3 (SW3)	17.0	20.5
Soil 1 (S1)	16.5	17.0
Soil 2 (S2)	12.0	14.7
Hand1 (H1)	13.0	15.0
Hand 2 (H2)	12.0	14.0
Sugarcane Juice (SJ)	18.3	18.5
Basin 1 (B1)	16.2	18.0
Basin 2 (B2)	19.0	19.4
Floor 1 (F3)	18.0	20.0
Floor 2 (F2)	14.7	14.0
Floor 3 (F3)	12.8	14.2
Mobile 1 (M2)	18.2	18.3
Mobile 2 (M2)	18.4	21.0
Incubator (I)	15.3	21.8

Figure 1: Antimicrobial Activity of leaf extract of *Murraya koenigii* plant against *E. coli* isolated from different sources

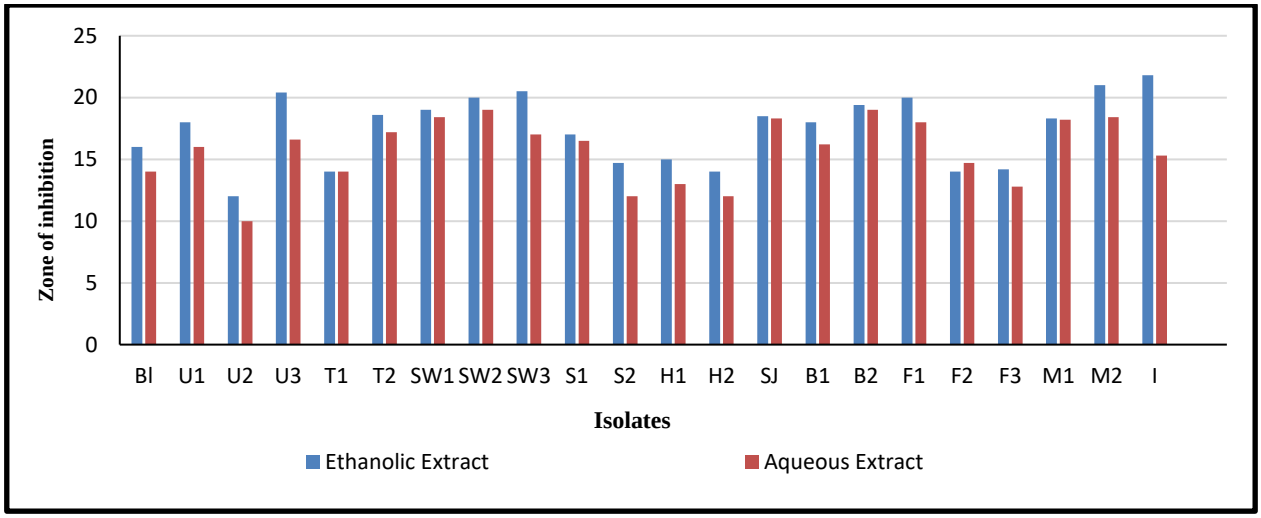

Figure 2: Inhibitory effect of Aqueous and Ethanolic extract of *Murraya koenigii leaves* on *E. coli* isolates

Table 2: Antibiotic Susceptibility Test against *E. coli* isolates

Isolates	Ciprofloxacin	Chloramphenicol	Amoxicillin	Gentamycin
	Zone of Inhibition (in mm)			
Bl	24	10	-	18
U3	22	12	-	21
T_2	24	10	-	19
SW_2	22	25	-	18
S_1	21	22	-	20
H_1	30	25	-	22
SJ	23	28	-	21
B_2	33	22	-	18
F_1	22	27	-	19
M_2	34	28	-	26
I	24	11	-	17

Figure 3: Antibiotic Susceptibility Test against *E. coli* isolates

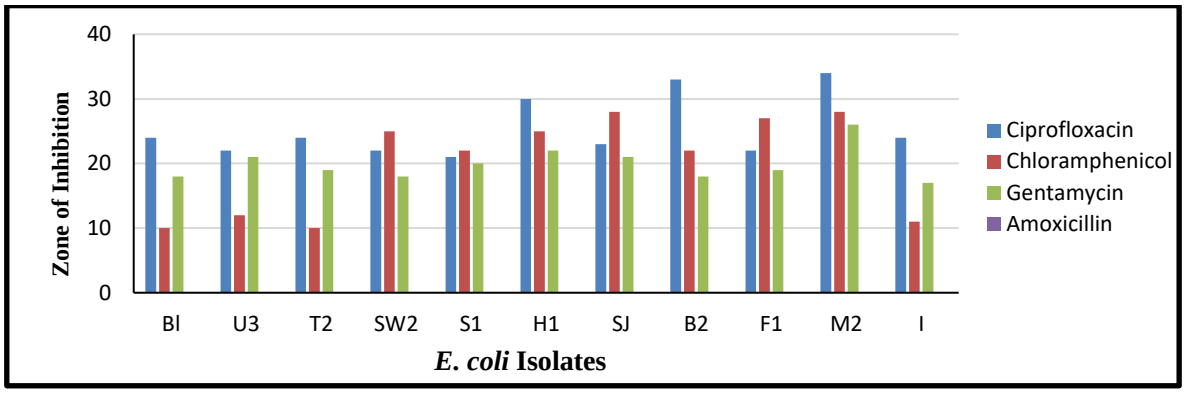

Figure 4: Sensitivity of *E. coli* isolates to Antibiotics

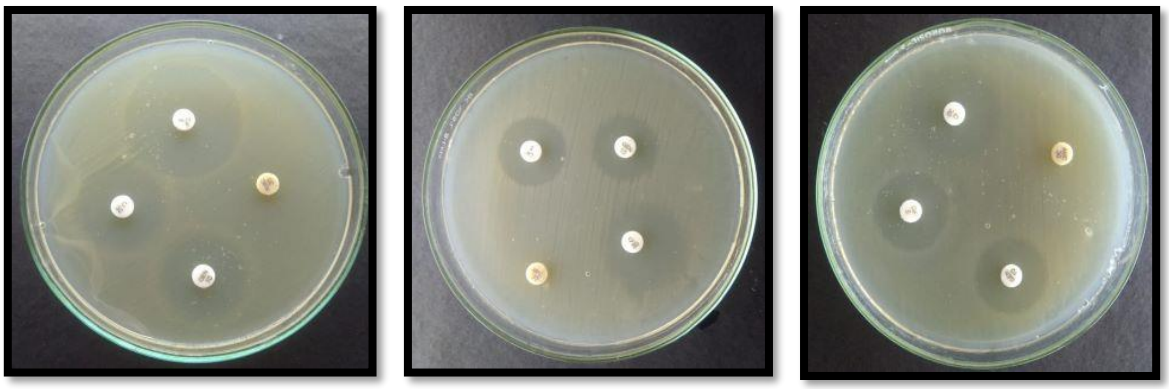

Figure 5: Spectrophotometric Analysis of Murraya koenigii plant leaves extract in different solvent

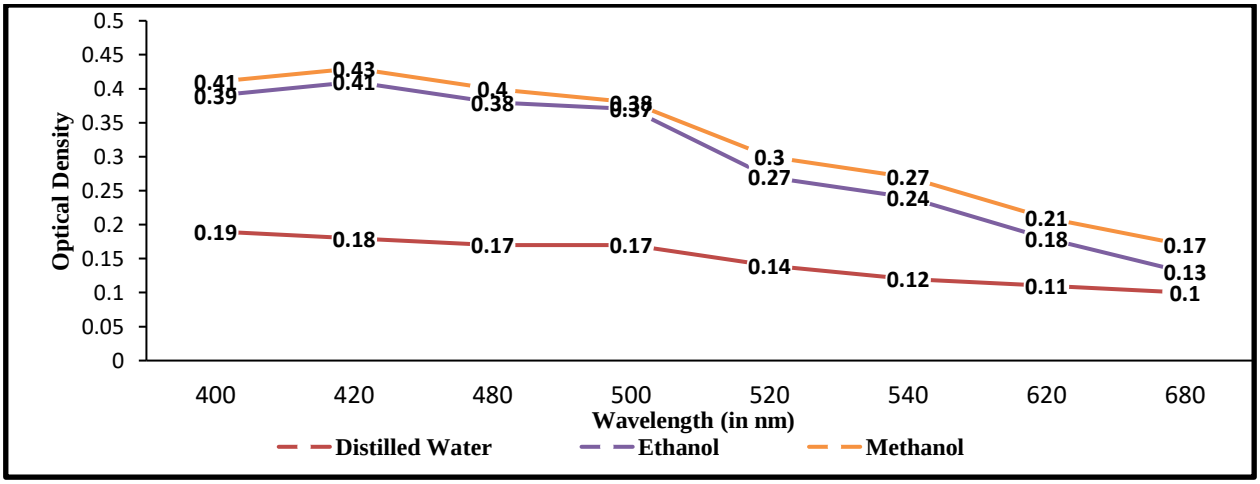

Table 3: Preliminary phytochemical analysis *Murraya koenigii* plant leaves extract

Phytochemicals	Color Change	Leaf Extract
Phenols	Brown	+
Flavonoids	Yellow	+
Terpenoids	Red-brown	+
Tannins	Brownish green	+
Quinones	Yellowish brown	+

4. Conclusion

The current research is related to the antibacterial activity of *Murraya koenigii* against *E. coli* obtained from clinical and various other sources. *Murraya koenigii* is a source of several bioactive compounds including phenols, flavonoids, terpenoids, tannins and quinones, which could be responsible for the inhibition of enteropathogenic *E. coli*. Thus, *Murraya koenigii* plant can be used as an effective antimicrobial agent in new drugs against *E. coli* and also could be used as raw material in water purification. However, further study needs to include bioavailability, efficiency enhancement in clinical investigation and in water purification.

5. References

1. Baliere, C., Rinc_e, A., Blanco, J., Dahbi, G., Harel, J., Vogeleer, P., Giard, J.-C., Mariani-Kurkdjian, P. et al. (2015) Prevalence and characterization of Shiga toxin-producing and enteropathogenic *Escherichia coli* in shellfish harvesting areas and their watersheds. Front Microbiol 6,1356.
2. Jones RN, Sader HS, Fritsche TR, Pottumarthy S. Comparisons of parenteral broad-spectrum cephalosporins tested against bacterial isolates from pediatric patients: report from the SENTRY antimicrobial surveillance program (1998-2004). Diagn Microbiol Infect Dis. (2007) 57:109–16. doi: 10.1016/j.diagmicrobio.2006.06.011
3. Kalaichelvi K, Dhivya SM. Screening of phytoconstituents, UV-VIS Spectrum and FTIR analysis of *Micrococca mercurialis* (L.) Benth. International Journal of Herbal Medicine, 2017; 5(6): 40-44.
4. Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z and Farah H: Antioxidant activity of some common plants. Turk J Biol 2008; 32: 51-5.
5. M. Kavitha Antibacterial and antioxidant activity of leaf organic extracts of local cultivars of Murraya koenigii (L.) Spreng from Tamil Nādu Bioscience Biotechnology Research Communications 10(2): 359-364 (2017).
6. M. Veera Pagu, K. R. Jeya and S. Vennila, Evaluation of phytochemicals, antioxidant potential and invitro antibacterial efficiency of *Murraya koenigii* leaves against uropathogenic *E.coli*, 2020, ISO 9001:2015 Certified Journal, Volume 10, Issue 1, 1185-1195.
7. Nehia Hussein, Noor A. Hanon. The antimicrobial Activity of some medical plants extracts used against some types of bacteria that causes urinary tract infection, Al-Mustansiriyah Journal of Science, 2017; DOI: http://doi.org/10.23851/mjs.v28i3.177
8. Ningappa MB, Dinesha R, Srinivas L: Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. Food Chemistry 2008; 106, 720–728.
9. Praveen K and Awang B: Antioxidant activity, total phenolic and flavonoid content of *morinda citrifolia* fruit extracts from various extraction processes. JESTEC 2007; 2: 70-80.
10. Rajesh ST, Sharmistha B, Shuchi K. Assessment of antioxidant activity of leaves of *Murraya koenigii* extracts and its comparative efficacy analysis in different solvents. J. Pharm. Sci. Res, 2017; 9: 288–291.
11. Ramamurthy V, Prasanth AS. Phytochemical screening and antibacterial activity of Murraya koenigii. World Journal of Pharmaceutical Research, 2018; 7(5): 1830-1837.
12. Rashmi JB, Naveen G. Phytochemical analysis and antibacterial activity of different leaf extracts of Murraya koenigii. International Journal of Biochemistry and Biomolecules, 2016; 2(2): 1-5.

13. Shaikh H, Shaikh S, Priya R. A review on Indian traditional herb *Murraya koenigii* its nutritive & medicinal properties in human health management. World Journal of Pharmaceutical Research, 2020; 9(6): 850-863.
14. Sonia Sethi, Shrikant, Gupta B Lal. Antimicrobial Activity of Medicinal Plants on Urinary Tract Pathogens International Journal of Pharmacy and Pharmaceutical Sciences Vol 4, Issue 2, 2012.
15. Sophiyamole L, Dr Reshma J k, Sakhi Prabhachandh R, Nimmi Babychan. Phytochemical analysis of *Murraya koenigii* in urban and coastal area, 2017 Journal of Emerging Technologies and Innovative Research (JETIR) www.jetir.org.
16. Tugume P, Nyakoojo C. Ethno-pharmacological survey of herbal remedies used in the treatment of paediatric diseases in Buhunga parish, Rukungiri District, Uganda. BMC Complementary and Alternative Medicine, 2019; 19(1): 353.
17. Wu D, Ding Y, Yao K, Gao W and Wang Y (2021) Antimicrobial Resistance Analysis of Clinical Escherichia coli Isolates in Neonatal Ward. Front. Pediatr. 9: 670470.doi: 10.3389/fped.2021.670470