Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 1797-1802
Received: 20-05-2017
Accepted: 21-06-2017

Hariom Kumar Singh
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, Uttar Pradesh, India

Amit Alexander Charan
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, Uttar Pradesh, India

Aradhana Irene Charan
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, Uttar Pradesh, India

Sudhanshu Matthew Prasad
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, Uttar Pradesh, India

Correspondence
Amit Alexander Charan
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, Uttar Pradesh, India

Antifungal and antibacterial activity of methanolic, ethanolic and acetonic leaf extracts of curry leaves (*Murraya koenigii*)

Hariom Kumar Singh, Amit Alexander Charan, Aradhana Irene Charan and Sudhanshu Matthew Prasad

Abstract
In this study the antibacterial and antifungal activities of selected plants, *Murraya koenigii* (Curry leaves) were tested against selected fungi (*Aspergillus niger*, *Fusarium oxysporum*, *Penicillium notatum* and *Trichoderma viride*) and selected bacteria (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*) cultures respectively. In evaluating antioxidant property and phytochemical analysis, all two plants were screened for antifungal and antibacterial activities. Antifungal and antibacterial activities were evaluated using well diffusion method. Inhibition of fungal growth and bacterial growth were investigated using PDA and NA well diffusion method. The total flavonoid content in crude methanolic, ethanolic and acetonic extracts and minimum inhibitory, minimum fungicidal and minimum bactericidal concentrations were obtained from *Murraya koenigii* leaves.

Keywords: *Murraya koenigii*, antifungal and antibacterial etc

Introduction
In the ancient literatures such as Charak Samhita and Sushrut Samhita which are known as encyclopedia of ayurvedic medicine herbs are found to have medicinal property. *Murraya koenigii*, commonly known as curry leaf or kari pattain Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 species. *Murraya koenigii* is a highly values plant for its characteristic aroma and medicinal value. It is an important export commodity from India as it fetches good foreign revenue. A number of chemical constituents from every part of the plant have been extracted. The most important chemical constituents responsible for its intense characteristic aroma are P-gurjunene, P-caryophyllene, P-elemene and O-phellandrene (Shah *et al.*, 2008) [9]. *Aspergillus niger* is a filamentous fungus that commonly occurs in the environment and is generally regarded as non-pathogenic (Blumenthal, 2004) [2]. *Fusarium* is a genus of filamentous fungi that contains many agronomically important plant pathogens, mycotoxin producers, and opportunistic human pathogens (Nelson and Hansen, 1997) [6]. *Penicillium* is a genus of Ascomycetous fungi of major importance in the environment, food and drug production (Pitt, 1979) [8]. *Trichoderma* species are cosmopolitan fungi, frequently present in all types of soil, manure and decaying plant tissues (Kubicek and Harman, 1998) [4]. *Bacillus cereus* is a spore-forming bacterium that occurs naturally in many kinds of foods and can cause illness in humans (Berthold-Pluta *et al.*, 2015) [1]. *Escherichia coli* are bacteria that are found in the gut of humans and animals. Most strains of *Escherichia coli* are harmless (Wulf *et al.* 2008) [10]. *Salmonella typhi* causes typhoid fever in humans. Typhoid fever, a systemic febrile illness, is transmitted by the Fecal-oral route, mainly by contaminated food and water in the developing world (Pang *et al.*, 1998) [7]. *Staphylococcus aureus* is a Gram-positive spherical bacterium approximately 1µm in diameter (Heyman, 2004) [3].

Materials and Methods
The experiment was conducted at the laboratory of the Department of Molecular and Cellular Engineering, Jacob Institute of Biotechnology and Bioengineering, SHUATS, Allahabad, Uttar Pradesh situated at 25.4131ºN latitude and 18.8479ºE longitude. The fresh leaves of *Murraya koenigii* were collected from the Department of Horticulture and central field, SHUATS, Allahabad which were surface sterilized simply by washing under tap water and Distilled water and dried in shed for 20 days. After drying, leaves and petals of *Murraya koenigii* were grounded in a grinder mixer to powdered form and stored for further use. The antifungal activity of plant leaves was tested against the selected fungi *viz. Aspergillus niger, Fusarium*

oxysporum, *Penicillium notatum* and *Trichoderma viride* and selected bacteria *viz. Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* cultures respectively. Fungal and bacterial cultures were collected from Microbial Culture Collection Bank, SHUATS. The culture was sub cultured on Nutrient agar slants and stored at 4^0C till use. Plant extracts were prepared using organic solvents *viz.* Ethanol, methanol and acetone. Total flavonoid content (Morena *et al*., 2000) [5] was also determined for *Murraya koenigii.*

Results and Discussion

Plant extracts were prepared from dried powdered samples. Ethanolic, methanolic and acetonic extracts were taken to study the antifungal and antibacterial activity of the leaves of *Murraya koenigii.* Distilled water was taken as control. Well diffusion method was used in this present study in order to get the antifungal properties of the different plant extracts against the test organism.

Results for antifungal activity of *Murraya koenigii* in ethanolic, methanolic and acetonic extracts:

(A.) Antifungal activity of curry leaves (*Murraya koenigii*) against *Aspergillus niger*

Table 1 and Figure 1 clearly indicated that the ZOI for methanolic extract of *M. koenigii* was maximum against *Aspergillus niger* and ethanolic extract showed minimum ZOI against *Aspergillus niger* whereas acetonic extract showed comparatively lowest zone of inhibition against *Aspergillus niger*. DW (control) showed no ZOI.

Table 1: Antifungal activity of curry leaves (*Murraya koenigii*) against *Aspergillus niger*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
M. koenigii	20 ± 1.0	18 ± 1.0	11±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

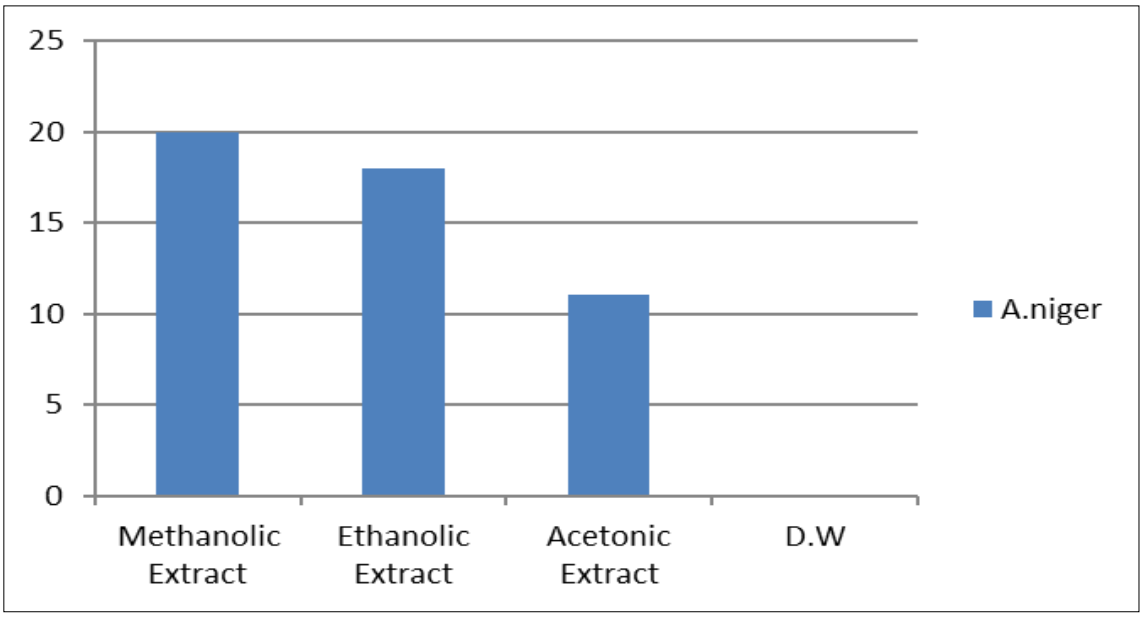

Fig 1: Antifungal activity of curry leaves (*Murraya koenigii*) against *Aspergillus niger*

(B.) Antifungal activity of curry leaves (*Murraya koenigii*) against *Fusarium oxysporum*

Table 2 and Figure 2 clearly indicated that the ZOI for methanolic extract of *Murraya koenigii* was maximum against *Fusarium oxysporum* and ethanolic extract showed minimum ZOI against *Fusarium oxysporum* whereas acetonic extract showed maximum zone of inhibition against *Fusarium oxysporum.* DW (control) showed no ZOI.

Table 2: Antifungal activity of *Murraya koenigii* against *Fusarium oxysporum*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic Extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
M. koenigii	18±1.0	16±1.0	18±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

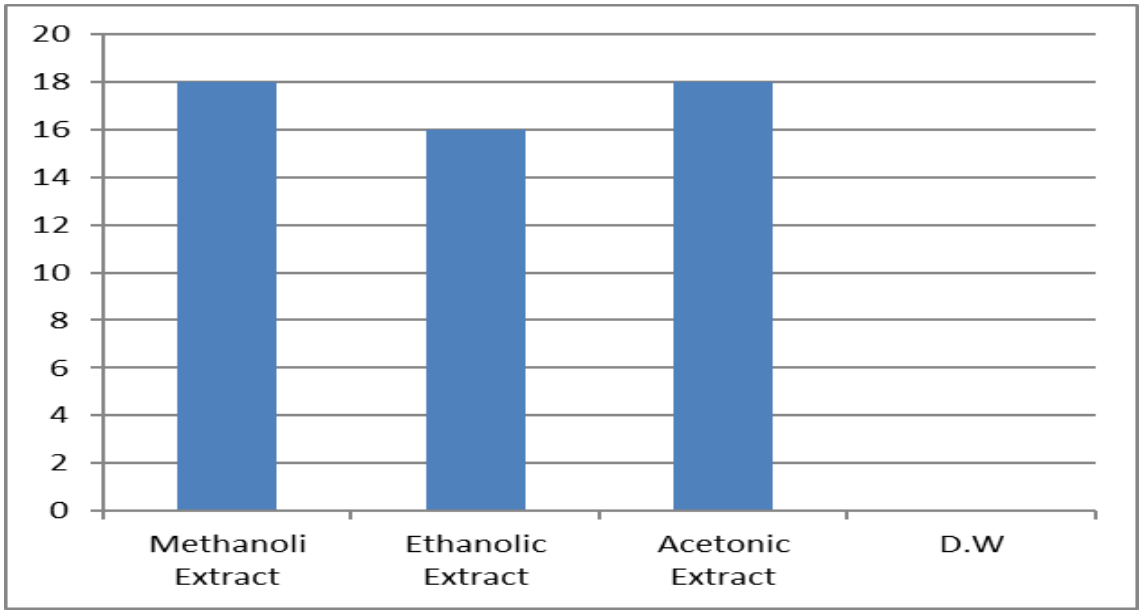

Fig 2: Antifungal activity of *Murraya koenigii* against *Fusarium oxysporum*

(C.) Antifungal activity for curry leaves (*Murraya koenigii*) against *Penicillium notatum*

Table 3 and Figure 3 clearly indicated that the ZOI for methanolic extract of *Murraya koenigii* was minimum against *Penicillium notatum* ethanolic extract showed lowest ZOI against *Penicillium notatum* whereas acetonic extract showed maximum zone of inhibition against *Penicillium notatum*. DW (control) showed no ZOI.

Table 3: Antifungal activity of *Murraya koenigii* against *Penicillium notatum*

Solvent / Plant species	Methanolic Extract (ZOI in mm)	Ethanolic Extract (ZOI in mm)	Acetonic Extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
M. koenigii	20 ± 1.0	15 ± 1.0	22±1.0	00

(Yaxis - ZOI in mm; X axis - Extracts of organic solvent)

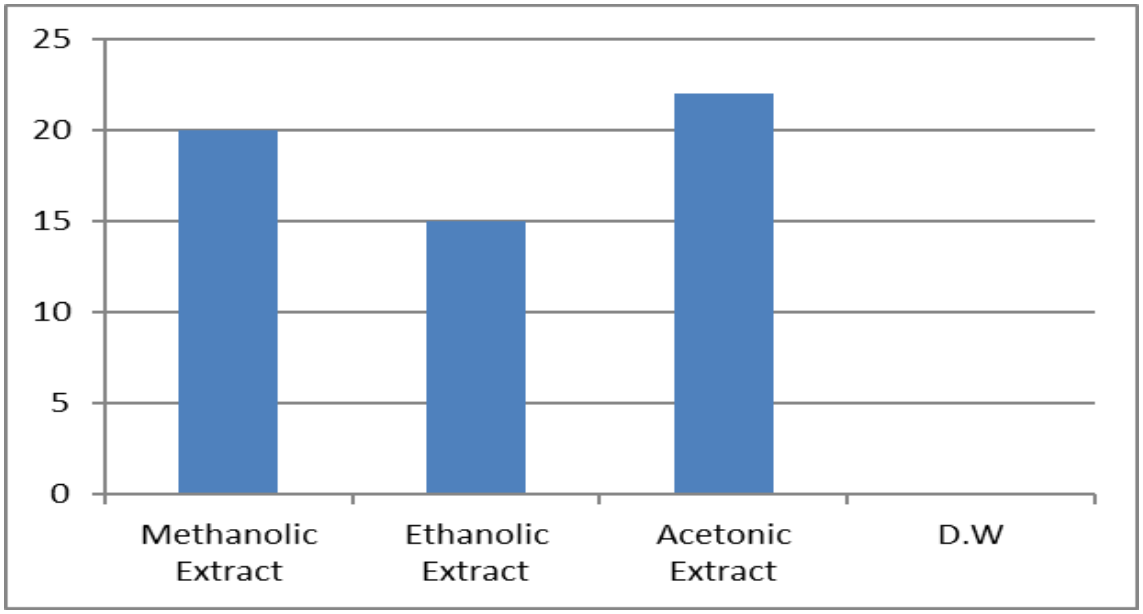

Fig 3: Antifungal activity of *Murraya koenigii* against *Penicillium notatum*

(D.) Antifungal activity of curry leaves (*Murraya koenigii*) against *Trichoderma viride*

Table 4 and Figure 4 clearly indicated that the ZOI for methanolic extract of *Murraya koenigii* was maximum against *Trichoderma viride* and ethanolic extract showed minimum ZOI against *Trichoderma viride* whereas acetonic extract showed comparatively lowest zone of inhibition against *Trichoderma viride*. DW (control) showed no ZOI.

Table 4: Antifungal activity of curry leaves (*Murraya koenigii*) against *Trichoderma viride*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
M. koenigii	22 ± 1.0	20 ± 1.0	18±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

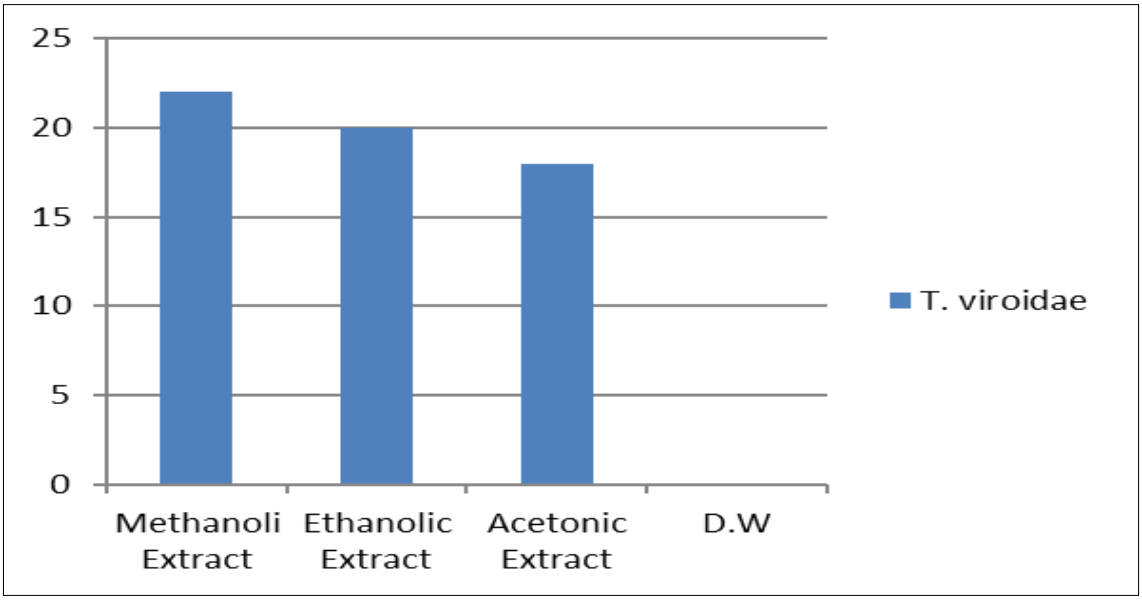

Fig 4: Antifungal activity of curry leaves (*Murraya koenigii*) against *Trichoderma viride*

Results for antibacterial activity of *Murraya koenigii* in ethanolic, methanolic and acetonic extracts

(A.) Antibacterial activity of curry leaves (*Murraya koenigii*) against *Bacillus cereus*

Table 5 and Figure 5 clearly indicated that the ZOI for methanolic extract of curry leaves (*Murraya koenigii*) was maximum against *Bacillus aureus* and ethanolic extract showed minimum ZOI against *Bacillus aureus* whereas acetonic extract showed maximum zone of inhibition against *Bacillus aureus*. DW (control) showed no ZOI.

Table 5: Antibacterial activity of curry leaves (*Murraya koenigii*) against *Bacillus cereus*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
M. koenigii	10 ± 1.0	9 ± 1.0	10±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

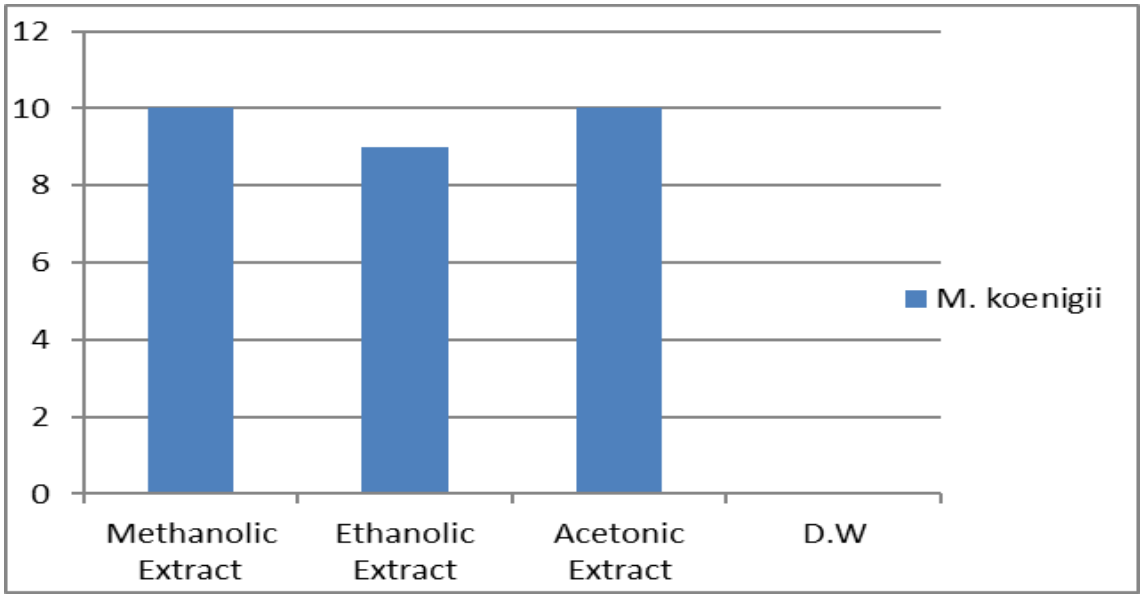

Fig 5: Antifungal activity of curry leaves (*Murraya koenigii*) against *Bacillus cereus*

(B.) Antibacterial activity of curry leaves (*Murraya koenigii*) against *Escherichia coli*

Table 6 and Figure 6 clearly indicated that the ZOI for methanolic extract of *Murraya koenigii* was lowest against *Escherichia coli* and ethanolic extract showed minimum ZOI against *Escherichia coli* whereas acetonic extract showed maximum zone of inhibition against *Escherichia coli*. DW (control) showed no ZOI.

Table 6: Antibacterial activity of curry leaves (*Murraya koenigii*) against *Escherichia coli*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
M. koenigii	16 ± 1.0	18 ± 1.0	20±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

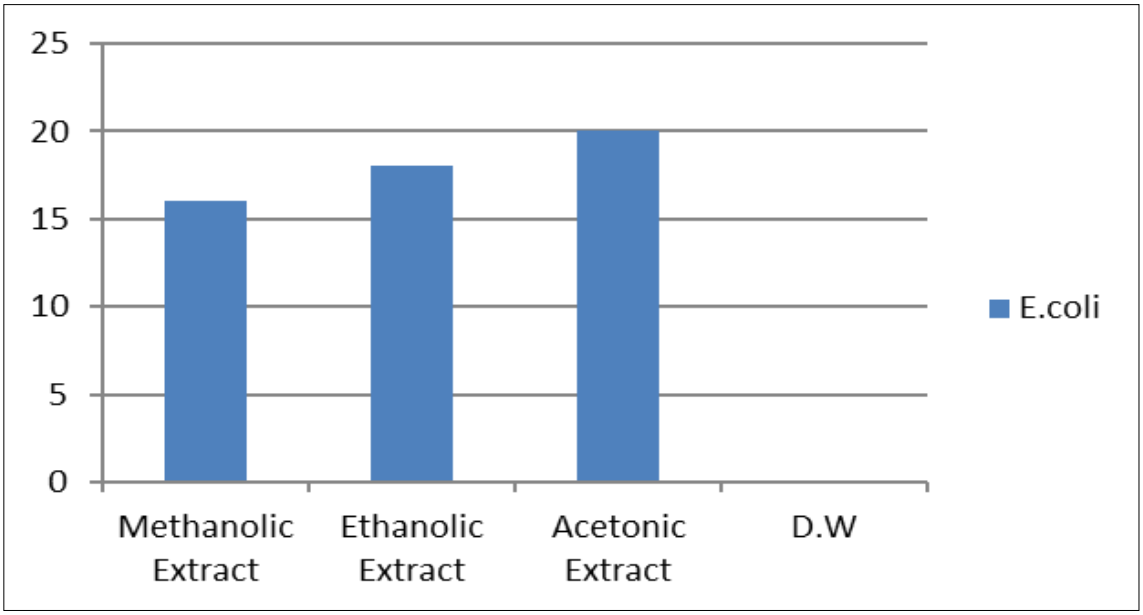

Fig 6: Antibacterial activity of curry leaves (*Murraya koenigii*) against *Escherichia coli*

(C.) Antibacterial activity of curry leaves (*Murraya koenigii*) against *Salmonella typhi*

Table 7 and Figure 7 clearly indicated that the ZOI for methanolic extract of *M. koenigii* was lowest against *Salmonella typhi* and ethanolic extract showed minimum ZOI against *Salmonella typhi* whereas acetonic extract showed maximum zone of inhibition against *Salmonella typhi*. DW (control) showed no ZOI.

Table 7: Antibacterial activity of curry leaves (*Murraya koenigii*) against *Salmonella typhi*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
M. koenigii	15 ± 1.0	16 ± 1.0	22±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

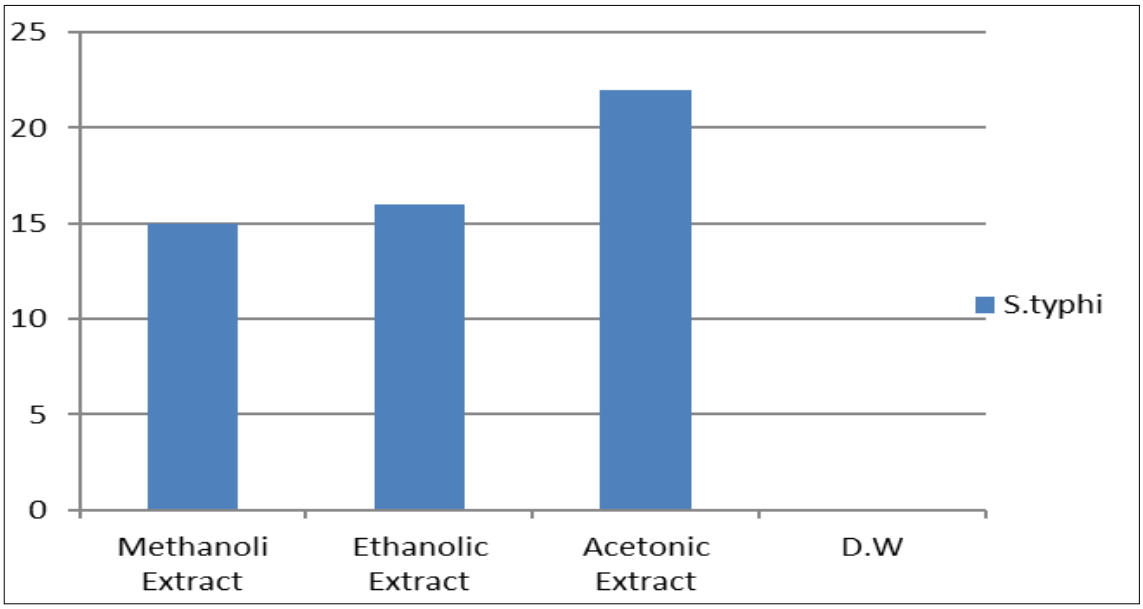

Fig 7: Antibacterial activity of curry leaves (*Murraya koenigii*) against *Salmonella typhi*

(D.) Antibacterial activity of curry leaves (*Murraya koenigii*) against *Staphylococcus aureus*

Table 8 and Figure 8 clearly indicated that the ZOI for methanolic extract of Curry leaves (*Murraya koenigii*) was lowest against *Staphylococcus aureus* and ethanolic extract showed minimum ZOI against *Staphylococcus aureus* whereas acetonic extract showed maximum zone of inhibition against *Staphylococcus aureus*. DW (control) showed no ZOI.

Table 8: Antibacterial activity of curry leaves (*Murraya koenigii*) against *Staphylococcus aureus*

Solvent / Plant Species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
M. koenigii	10± 1.0	12 ± 1.0	14±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

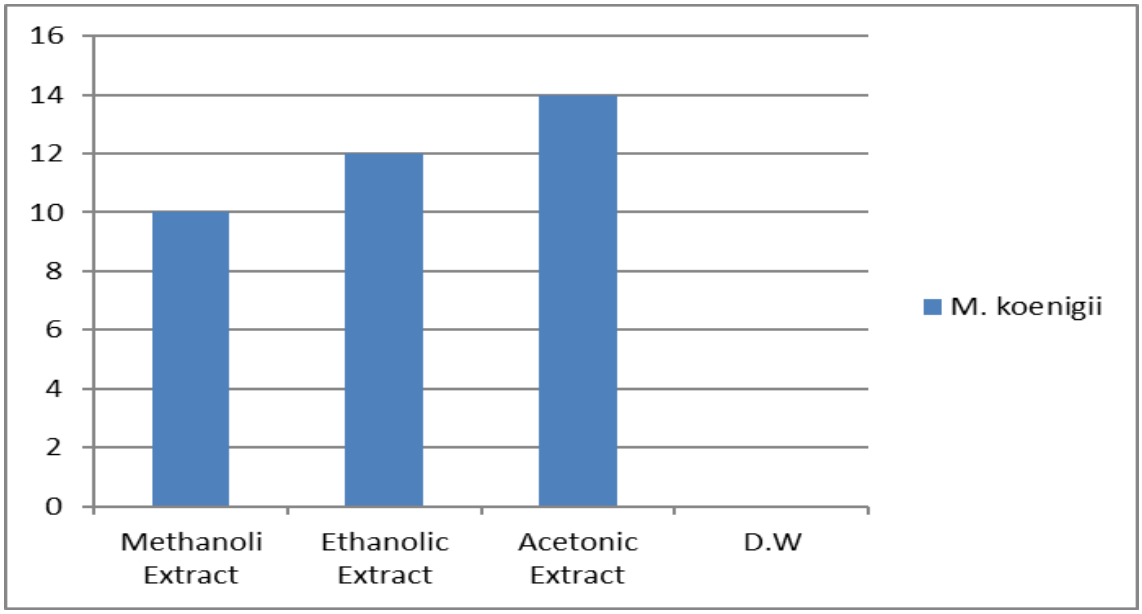

Fig 8: Antibacterial activity of curry leaves (*Murraya koenigii*) against *Staphylococcus aureus*

Results for total flavonoid content (TFC)

The contents of total flavonoid compounds in crude methanolic, ethanolic and acetonic extracts obtained from curry leaves (*Murraya koenigii*) is presented in Table 9. The results were reported as Quercetin Equivalents (QE) mg/g extract.

Table 9: Total flavonoid content in methanolic, ethanolic and acetonic extracts of *Murraya koenigii* sample under study

Plant material	Plant part used	Concentration of plant extract (mg/ml)	O.D. at 415 nm (Methanolic extract)	O.D. at 415 nm (Ethanolic extract)	O.D. at 415 nm (Acetonic extract)	Total flavonoid (mg QE/g extract)
Murraya koenigii	Leaves	1.0	0.992	1.306	0.885	0.30

References

1. Berthold-Pluta A, Puta A, Debevere J. The effect of selected factors on the survival of *Bacillus cereus* in the human gastro-intestinal tract. Microbial Pathogenesis. 2015; 82:7-14.
2. Blumenthal CZ. Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*: Justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. Toxicology and Pharmacology. 2004; 39:214-228.
3. Heyman D. *Control of communicable diseases manual.* 18th Edn. American Public Health Association, Washington DC, 2004.
4. Kubicek CP, Harman GE. *Trichoderma* and *Gliocladium*, vol. 1-2, Taylor & Francis, London: 1998; 278:393.
5. Morena E, Mishra RP, Arshad M, Sami A. Antibacterial properties of *Rosa indica* stem leaves and flowers. Electronic Journal of Biology. 2000; 12(15):4564-4573.
6. Nelson BD, Hansen JM. Reaction of soybean cultivares to isolates of *Fusarium solani* from the Red River Valley. *Plant Disease*s. 1997; 81:664-668.
7. Pang T, Levine MM, Ivanoff B, Wain J, Finlay BB. Typhoid fever important issues still remain. Trends in Microbiology. 1998; 6:131-133.
8. Pitt JI. The genus *Penicillium* and its teleomorphic states Eupenicillium and Talaromyces. Academic Press, London, 1979.
9. Shah AS, Wakade AS, Juvekar AR. Immunomodulatory activity of methanolic extract of *Murraya koenigii* leaves. Indian Journal of Experimental Biology. 2008; 46(7):505-509.
10. Wulf MW, Markestein A, van der Linden FT, Voss A, Klaassen C, Verduin CM. First outbreak of methicillin resistant *Escherichia coli* ST398 in a Dutch Hospital, June 2007. European Survey. 2008; 28:13-39.