DOI: http://doi.org/10.4038/vingnanam.v17i1.4188

Comparative *In Vitro* antimicrobial activity of selected four medicinal plant leaves

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Abstract – In the treatment of diabetes mellitus, the traditional medical system of Sri Lanka has demonstrated to be far more effective, with less side effects and lower costs than modern synthetic medications. Ethnobotanical research is also necessary to learn about the world's traditional usage for treating chronic illness. Therefore, the present evaluation was aimed to compare the antimicrobial activity between the ethanolic leaves extracts of *Murraya koenigii, Tinospora cordifolia, Enicostemma littorale* and *Gymnema sylvestre* which are commonly found in Jaffna District against three different bacterial species such as *Staphylococcus* sp., *Enterococcus* sp. and *Escherichia coli* sp. by determining zone of inhibition using agar well diffusion method and compared with standard antibiotics. All experiments were conducted in triplicate. The statistical analysis was carried out using one-way Analysis of variance and values were expressed as the mean ± standard deviation. Results showed all the tested leaf extracts showed promising antibacterial activity against all the bacterial strains which was comparable with positive control Streptomycin. *E.littorale* showed the highest zone of inhibition diameter against *Enterococcus* sp. (14.08±2.46 mm). *M.koenigii* showed the highest zone of inhibition diameter against *Staphylococcus* sp. (8.96±2.49 mm) and *E.coli* (9.67±0.996 mm). Present study determined that the ethanol leaf extracts of *Murraya* sp. and *Enicostemma* sp. showed the highest antibacterial activity than other two species against all tested bacteria. Therefore, *E.littorale* and *Enicostemma* sp. can be used for developing therapeutic agents for the treatment of infectious diseases caused by microbes in future.

Keywords **-** Agar well diffusion, Antibacterial activity, Inhibition zone, Medicinal plant leaves

1. INTRODUCTION

Plants have been a source of inspiration for new medicinal molecules since ancient times, and plant derived medicines have made a significant contribution to human healthiness and well-being. It is critical to investigate medicinal plants with a folkloric reputation in more depth for novel medications (Roja and Rao, 2000; Ali *et al.*, 2001; Nitta *et al.*, 2002).

The presence of essential phytochemicals makes the plant useful against a variety of infectious diseases, and it has a high potential for producing useful human medications. Phytochemicals are secondary metabolites which considered as a part of a plant's multicomponent defense mechanism (Harborne and Baxter, 1995; Harvey *et al.*, 2015) and that are produced in small amounts because the plant does not require them. They occur naturally in all sections of the plant's body, including the bark, leaves, stem, and root (Tiwari *et al.*, 2011) and may differ from one part to another (Lahlou, 2004). Therefore, these secondary metabolites are very important to prevent many bacterial infections.

Bacterial infections cause a considerable number of deaths in the human population today. Antibiotics are commonly used in response to serious bacterial infections, and hazardous microorganisms acquiring antibiotic resistance is common as a result of indiscriminate antibiotic

usage. Treatment of antibiotic-resistant dangerous bacteria will become a big issue (Mahinda and Mohan, 2006). As a result, finding new antibacterial medications from natural herbal plants has become a necessity.

Numerous studies have been found that the medicinal plants which are used in traditional medicinal practices were inhibit growth and virulence of various microbes against infections that is potential antibacterial activity (Ahmad *et al.,* 1998; Ahmad and Beg, 2001; Kumar *et al*., 2006; Bibi *et al.,* 2011; Cioch *et al.*, 2017) and few natural products have been approved as new antibacterial drugs (Kameshwara Rao, 1998; Subramani and Goraya, 2003). However, due to the widespread use of antibiotics, an increase in the incidence of antibiotic-resistant bacteria may render current antimicrobial medicines ineffective in controlling some bacterial infections. As a result, there is a pressing need for research into new chemicals that are effective against human diseases (Shahidi and Karimi Nik, 2004).

The objective of the present study was to predict the comparative antimicrobial activity between the ethanol leaf extracts of *Murraya koenigii*, *Tinospora cordifolia*, *Enicostemma littorale* and *Gymnema sylvestre* which are used in traditional medicine in Jaffna, Sri Lanka against selected three bacterial species namely *Staphylococcus aureus, Enterococcus faecalis* and *Escherichia coli.*

Murraya koenigii (Linn) Spreng (Rutaceae family) commonly known as curry leaf (Sinhala name: *Karapincha* and Tamil name: *Kaariveppirai*) which is an important leafy vegetable and distributed in India and the Southeast Asian Region. It is a natural flavoring agent with a number of important health benefits such as diabetes, diarrhea and anemia, etc. (Bhandari, 2012; Bonde *et al.*, 2011). Gas Chromatography-Mass Spectroscopic study found that the essential oil obtained from curry leaves were exhibited potent inhibition against antibiotic resistant bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and a few other bacteria (Rajendran *et al.,* 2014).

Gymnema sylvestre R. Br. (Asclepiadaceae family) is a large tropical liane native to central and western India and found in tropical Africa and in Australia (Stocklin, 1969). It (Sinhala name: *Masbedda* and Tamil name: *Kurincha*) possesses many bioactive properties such as hypolipidemic, anticancer, antimicrobial, antiviral, larvicidal, antioxidant activity. Also, studies have been found that it can be a potential source for development of metabolites against important bacterial species (Arora and Sood 2017; David and Sudarsanam, 2013; Ramalingam *et al.,* 2019).

Enicostemma littorale Blume (Gentianaceae family) is a perennial glabrous medicinal herb and it (Sinhala name: *Maja-Makka booti* and Tamil name: *Vellarugu*) can be found throughout India, but is especially widespread around the shore. This plant has a lot of antibacterial properties. Because leaf extract of this plant consists of many secondary metabolites such as alkaloids, triterpinoids, catechins, sterols, saponins, phenolic acids, flavonoids and xanthones (Hitesh *et al.,* 2009).

Tinospora cordifolia (Thunb) Miers (Menispermaceae family) which has a diverse range of bioactive components and has been demonstrated to be a medicinally useful plant has received little scientific attention. It (Sinhala name: *Rasakinda* and Tamil name: *Seenthil*) is large, deciduous, climbing shrub found throughout India. *T. cordifolia* extract has been shown to inhibit bacterial growth and increase neutrophil phagocytic and intracellular bacterial capabilities in mice (Sengupta *et al.*, 2009). Although a large amount of research has been done on the antibacterial activity of these selected endangered medicinal plants which are commonly found in Jaffna District, more research

Table 1 : Medicinal plants tested for their antibacterial activity in the study

Botanical name	Family name	Common name	
		Sinhala	Tamil
Murraya koenigii	Rutaceae	*Karapincha*	*Kariveppillai*
Enicostemma littorale	Gentianaceae		*Vellaruku*
Gymnema sylvestre	Apocynaceae	*Masbedda*	*Kurincha*
Tinospora cordifolia	Menispermaceae	*Rasakinda*	*Seenthil*

is needed to confirm its effectiveness against disease-causing microbes. Therefore, this study was planned to comparatively investigate antibacterial activity among these plants against tested bacteria. It will become a great opportunity to create novel antibiotics against many infectious pathogens.

2. MATERIALS AND METHODS

Collection of medicinal plants: The Fresh leaves of four selected different medicinal plants such as *M.koenigii, T.cordifolia, E.littorale* and *G.sylvestre* were collected from Jaffna District.

Identification and authentication of plants: Healthy *M.koenigii, T.cordifolia, E.littorale* and *G.sylvestre*plant materials were botanically authenticated by a Curator and voucher specimens with reference numbers were placed in the National Herbarium Center, Department of National Botanic Garden, Peradeniya, Sri Lanka (Table 1).

Processing of medicinal plants: Leaves of the plants were washed in tap water several times to remove the soil and dust particles. Then they were dried in a shaded place for five days and blended to form a fine powder and stored in airtight containers at room temperature (31±3 ^{0}C).

Preparation of plant extracts: Twenty grams of properly washed dried and milled leaves of above plants were soaked in 100 mL of absolute ethanol (99.98 %) for 5 successive days separately at room temperature (31±3 ^{0}C). The supernatant was filtered through What man filter paper No.1. After the extraction filtrates were concentrated until the complete removal of the solvent on a rotating evaporator (BUCHI) at 52 ^{0}C. Crude extracts were kept under refrigeration at 4 °C.

Test microorganisms: Three bacterial strains from three different species (*Staphylococcus auereus, Enterococcus faecalis* and *Escherichia coli*) provided by the Department of Botany, Faculty of Science, University of Jaffna were used for the antimicrobial tests, according to Table 2. All the test strains were maintained on nutrient agar slants at 4 ^{0}C and sub-cultured on to nutrient broth for 24 hours prior to testing. These bacteria served as test microorganisms for antibacterial activity assay.

Table 2: Test microorganisms used for antimicrobial activity of medicinal plants

Name	Type	ATCC No
Escherichia coli	Gram negative	25922
Enterococcus faecalis	Gram positive	29212
Staphylococcus aureus	Gram positive	29213

Preparation of bacterial suspensions: Each culture to be tested was streaked onto a nutrient agar medium to obtain isolated colonies inside the laminar air flow chamber. After incubation overnight at 37 ^{0}C, a few isolated colonies were selected with asterile inoculating loop. Loopful of bacterial cultures (0.01 ml) of target microorganisms were inoculated into 2ml of sterile water. It was stirred well and bacterial suspensions were prepared.

Assay of Antimicrobial activity using Agar well diffusion method: About 22.68g of Nutrient Agar (NA) powder was dissolved in 810ml of distilled water. Then 15 ml parts of the NA medium were poured into boiling tubes. Medium which was contained in the boiling tubes were autoclaved at 121 ^{0}C for 20-30 minutes. Then 15 ml of sterilized nutrient agar was mixed with 100 µl of bacterial suspensions inside the laminar air flow chamber. The mixture was stirred well and it was poured into sterile petridishes separately. After the solidification the wells were punched over the agar plates using sterile cork-borer (5mm in diameter) and 15 µl of plant extracts were added to the wells separately. The plates were incubated for 24 hours at 37 ^{0}C. Distilled water and Streptomycin (100µg/µl) were used as the negative and the positive control respectively. After incubation the diameter of the formed inhibitory zones formed around each well were measured (mm) in four different fixed directions and recorded. Triplicates were maintained for each test.

Data analysis: Data were statistically analyzed by Analysis of Variance (ANOVA) and Tukey's multiple comparisons at probability value ($P<0.05$) using a SAS statistical package (version 9.1.3) and mean separation was done by Least Significance Difference (LSD).

3. RESULTS AND DISCUSSION

The investigation was based on antibacterial properties of ethanolic extracts of *Murray a*sp., *Enicostemm a*sp., *Gymnem a*sp. and *Tinospor a*sp. against three selected common bacterial species (Table 3). Based on the results obtained *M.koenigii* showed the highest antibacterial activity against all three bacteria such as *Enterococcus faecalis* (9.67±0.996), *Escherichia coli* (9.65±0.996 mm) and *Staphylococcus aureus* (8.96±2.49 mm). *E. littorale* exhibited the highest antibacterial activity against *Enterococcus faecalis* (14.08±2.46 mm) than *Escherichia coli* (4.67±0.626 mm) and *Staphylococcus aureus* (5.79±0.928 mm). *G. sylvestre* showed the highest antibacterial activity against *Staphylococcus aureus* (6.96±1.639 mm). Further, *T. cordifolia* showed the lowest antibacterial activity against all three selected bacteria compared with other three plant extracts. The results obtained from this study revealed that the tested extracts were least effective against *Escherichia coli*, but most effective to *Enterococcus faecalis* and *Staphylococcus auereus.* Therefore, the extracts were found to be more active against selected Gram (+) bacteria than selected Gram-negative bacteria at 37 ^{0}C. When considering positive and negative controls,

Table 3 : Bacterial growth inhibition zones of ethanolic extract of plant leaves and antibiotic against bacterial species tested by agar disc diffusion assay (Results are expressed as Mean ± SD and statistical significance was evaluated by ANOVA.)

	Diameter of the inhibition zones (mm)		
Plant species	**25922**	**29212**	**29213**
M.koenibgii	9.65±0.996^b	9.67±0.996^c	8.96±2.49^b
G.sylvestre	5.46±0.914^c	5.13± 0.627^d	6.96±1.639^c
T.cordifolia	0.29±0.698^d	0.81±1.247^e	1.31±1.449^d
E.littorale	4.67±0.626^c	14.08±2.46^b	5.79±0.928^c
Positive control	19.541±1.355^a	25.458±1.177^a	21.083±0.258^a
Negative control	0± 0^e	0±0^e	0 ±0^d

Streptomycin exhibited the highest inhibitory effect against *Enterococcus faecalis* and the lowest inhibitory effect against *Escherichia coli*. In negative controls there were no inhibitions zones identified.

For the pharmaceutical sector, plants are the repository for a wide range of phytochemical compounds. Herbal medicine has been proved to have true utility in most populated countries, and around 80% of the rural population trusts it for prime healthcare (Daniel *et al.* 2020). Infectious diseases caused by some bacteria pose a severe hazard to public health around the world (Egglest *et al.*, 2010). Antibiotics are the recommended treatment for bacterial infections; however, the emergence of antibiotic resistance and toxicity concerns have reduced their usage (Malini *et al.*, 2013; Zhang *et al.*, 2006). Antibiotic safety and efficacy constraints complement biological research on the antibacterial role of plants due to similar toxicity and efficacy (Alviano and Alviano, 2009). To combat the existing infectious diseases, novel alternative antimicrobial medications are required. Higher plants' potential as a source of novel pharmaceuticals is yet mostly untapped and herbal medicines have been stated to be safe and have the least side effects, particularly when compared to synthetic pharmaceuticals (Ezekiel *et al.*, 2009). Several herbs used in traditional medicine have been shown to be useful in treating bacterial and viral illnesses. According to the results the active inhibitory effect was detected on Gram positive rather than on Gram negative bacteria. It's possible that the difference in susceptibility between the studied bacterial isolates is attributable to differences in cell wall structure and composition (Hauser, 2015).

Results of the present study could be comparable with the previous studies which were stated that the ethanol extracts of leaves of *E. littorale* showed the highest antimicrobial activity against tested bacterial strains (Deore *et al*., 2008; Rita *et al*. 2010; Mathur, 2013); ethanol extracts of leaves of *M. koenigii*have antibacterial effects (Irfan *et al.*, 2016, Akula *et al.*, 2016, Abeysinghe *et al.*, 2021); ethanol leaf extracts of *G. sylvestre*also have shown a antimicrobial potential (Satdive *et al.*, 2003; Janarthanam and Sumathi, 2010; Irimpan *et al.*, 2011; Gupta and Singh, 2014); ethanolica and methanolic leaf extract of *T. cordifolia* showed greater antibacterial action (Shanthi and Nelson, 2013; Prajwala *et al.*, 2018; Mahesh and Satish, 2008). *T. cordifolia* exhibited antimicrobial properties with the maximum activity (40 µl) at 2% concentration (Agarwal *et al.*, 2019).

Further, chloroform extract of *E. littorale* has antimicrobial activity higher than methanol and acetone extracts (Pitchamuthu *et al.*, 2012); the isolated compounds and different extracts of the whole plant of *E. littorale* have shown antibacterial activity equivalent to that of standard against the gram positive and negative organisms (Pillai et al., 2020); the leaf extracts of *M. koenigii* was found to have high antibacterial activity than anti-fungal activity (Kumar and Simon, 2016); *T. cordifolia* exhibited antimicrobial properties with the maximum activity (40µl) at 2% concentration (Agarwal *et al.*, 2019); ethanol extract of *T. cordifolia* did not show much inhibitory activity against microbes (Prajwala *et al.*, 2018); leaf aqueous extract of *T. cordifolia* exhibited maximum zone of inhibition against selected four bacteria (Gunda and Kommidi, 2020); the aqueous and methanol leaf extract of *G. sylvestre* showed significant antibacterial activity (Beverly and Sudarsanam, 2013) and Methanol extract of *G. sylvestre* showed good antibacterial activity with the high inhibition zones (Kishor Naidu *et al*., 2013). Present study results were slightly different from previous studies of these selected plants. These differences may be due to variations in the preparation procedure, concentrations, and/or storage method, seasonal or geographical variations in the environment from which plant materials were collected, or extraction method used.

Alkaloids, flavonoids, glycosides, terpenoids, steroids, phenols, coumarins, and a number of other

chemical compounds have been discovered in different portions of the plant and are responsible for a variety of biological functions, including antimicrobial and antioxidant properties (Phuyal *et al.*, 2019). Plants high in tannins, for example, have antibacterial potential due to their ability to react with protein to generate stable water-soluble chemicals, causing the bacteria to die by directly harming its cell membrane (Alofolayan, 2003). Flavonoids are a class of phenolic chemicals known for their antiviral (Chiang *et al.*, 2003) antimicrobial (Bastos *et al.*, 2009) and spasmolytic properties (Amor *et al.*, 2005). Among the secondary metabolites studied, alkaloids and polyphenols have shown strong antimicrobial activity (Othman *et al.*, 2019). Phytochemical studies have denoted that high tannin, alkaloids flavonoids and phenolic contents were present in Leaves of *M. koenigii* (Rupali and Kusum, 2019; Victoriya and Manimekalai, 2016) and *E. littorale* (Subasini *et al.* 2010; Vishwakarma *et al.* 2010; Vidyadhar *et al.* 2010). Therefore, these plant leaves have a great influence in controlling infectious diseases due to their effective antibacterial activity.

4. Conclusion

In brief summarizing the above results, it is well cleared that all the tested leaf extracts of *M. koenigii, T. cordifolia, E. littorale* and *G. sylvestre* showed promising antibacterial activity against all the bacterial species tested such as *Enterococcus faecalis*, *Staphylococcus auereus* and *Escherichia coli*. Ethanol extract of *M. koenigii* showed the highest antibacterial activity against all three bacterial species followed by *E. littorale* than other medicinal plants which support their traditional use against infectious diseases. Therefore, *M. koenigii* and *E.littorale* can be used for developing therapeutic agent for the treatment of infectious diseases caused by microbes in future. At the same time, the antimicrobial potential of these selected plant materials should be evaluated in various types of plant extracts with diverse concentrations against different bacterial species to be found their potential antimicrobial effects in future.

5. References

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