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Research Article

ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *LUDWIGIA PARVIFLORA* L. AGAINST STANDARD BACTERIAL STRAINS AND COMPARISON OF ITS ACTIVITY WITH THAT OF STANDARD ANTIBIOTICS

SHIBU GEORGE[1]*, MELVIN JOY[2]

[1]Department of Zoology, St. Aloysius College, (Affiliated to Mahatma Gandhi University, Kottayam, Kerala), Alappuzha, Kerala, India. [2]St. John's Research Institute, Bengaluru, Karnataka, India. Email: shibugeorgepala@gmail.com

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ABSTRACT

Objective: The objective of this study was to evaluate the antimicrobial activity of methanolic extract of *Ludwigia parviflora* L. using standard bacterial strains and compare its activity with that of standard antibiotics.

Methods: The antibacterial activity and antibiotic susceptibility tests were done by disk diffusion method using MTCC bacterial strains.

Results: The study revealed that the methanolic extract of the whole plant of *L. parviflora* L. was effective to inhibit the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Escherichia coli*. Among the tested strains, *S. aureus*, *P. aeruginosa, K. pneumoniae,* and *E. coli* were more susceptible to the methanolic extract of *L. parviflora* than the commonly using antibiotic tetracycline 30 mcg. The activity of methanolic extract was also higher than the activity of gentamicin 10 mcg against the *P. aeruginosa*.

Conclusion: The study concluded that the crude methanolic extract of the whole plant of *L. parviflora* L. is a good source for antibacterial agent against *S. aureus*, *P. aeruginosa*, *K. pneumoniae,* and *E. coli*. Hence, this plant can be used as a natural alternative to the common antibiotics such as gentamicin and tetracycline against common bacterial infections after validating its pharmacological and toxicological activities.

Keywords: Antimicrobial activity, *Ludwigia parviflora*, Antibiotics, Methanolic extract, Disk diffusion method, Gentamicin, Tetracycline.

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INTRODUCTION

Plants have been used as a source of medicine in traditional or folk medicine from time immemorial due to their magical power to cure diseases [1]. They not only contain nutrients but also chemicals for providing health benefits. Anticancer, antioxidant, anti-inflammatory, antihelminthic, and antimicrobial agents are common in many plants [2]. These chemicals called phytochemicals are the by-products of plant metabolism and are having specific physiological effects in the human body [3,4]. Many research activities are currently undergoing in the world to discover the medicinal activities of such chemicals. Nowadays, researchers have given more attention to discover new antimicrobial drugs of plant origin because most of the available synthetic antibiotics are losing their capacity to inhibit the growth of microorganisms [5,6]. This is mainly due to the ability of microorganisms to develop resistance against the continuously using antibiotics [7,8]. Therefore, this study is focused to validate the antimicrobial activity of *Ludwigia parviflora* L. against standard bacterial strains and also to compare its activity with that of standard antibiotics for suggesting a natural alternative to the commonly available antibiotics.

L. parviflora L. belongs to the family Onagraceae. It is commonly known as water primrose. It is commonly seen in wet places, sandy river bed, along streams, rice field, etc. It has been used in traditional medicine to overcome many diseases including fever [9]. Its leaves and roots are also used in the treatment of ulcer, wound healing, etc. [10].

METHODS

Preparation of plant extract

The whole plants of *L. parviflora* L. were collected from different geographical areas of Upper Kuttanad, Alappuzha district, Kerala state. A voucher herbarium specimen was prepared and deposited at the Department of Zoology, St. Aloysius College, Edathua, Alappuzha, Kerala, and the authentic identification of the plant material was done by Mr. Bijeshmon P.P, Botanist, Sreedhareeyam Ayurvedic Research and Development Institute, Koothattukulam, Kerala, India. The collected plant sample was washed thoroughly under tap water, dried under sunlight, and powdered using a mixer grinder. Serial extraction of the powder was made by a Soxhlet extractor using solvents such as petroleum ether, acetone, methanol, and distilled water. The Soxhlet extracts were filtered using Whatman No. 1 filter paper and then concentrated. The concentrate was considered as stock and kept in the refrigerator. From the stock, a concentration of 10 mg/disk was prepared and was used for the preliminary antibacterial screening.

Bacterial strains

Standard MTCC strains purchased from the Institute of Microbial Technology, Chandigarh (IMTECH), India, were used for the study. The strains were as follows:

1. *Staphylococcus aureus* (MTCC Code 3160)
2. *Escherichia coli* (MTCC Code 40)
3. *Pseudomonas aeruginosa* (MTCC Code 4673)
4. *Klebsiella pneumoniae* (MTCC Code 3040)

Antibiotic disks

Standard antibiotic disks purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India, were used for antibiotic sensitivity comparison. The following antibiotics were used for the study.

1. Chloramphenicol (30 mcg)
2. Gentamicin (10 mcg)
3. Penicillin G (10 u)
4. Ciprofloxacin (5 mcg)
5. Tetracycline (30 mcg)
6. Amikacin (30 mcg).

CULTURE MEDIA

The dehydrated Mueller-Hinton Agar medium purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India, was used. The medium was rehydrated and sterilized in an autoclave and was poured into sterilized Petri dishes and allowed to set. The plates were stored at 4–10°C in the refrigerator. Before inoculation, the surface of the petri plates was dried in an incubator.

ANTIBACTERIAL ASSAY BY DISK DIFFUSION METHOD

The antibacterial activity and antibiotic sensitivity test were done by disk diffusion method as described by Kirby *et al.,* in 1966 [11]. The dried plates were inoculated by the test strains uniformly over the surface using a sterile cotton swab. A sterile 6-mm Whatman No.1 filter paper loaded with appropriate extract was placed on the surface of the inoculum and gently pressed by a sterile forceps. Control disks (made up of each solvent such as petroleum ether, acetone, methanol, and water) were also placed on the surface of inoculum. The plates were incubated at 37°C for 16–20 hrs. The zone of inhibition of bacterial growth around the disk was measured in millimeters. Tests were repeated 3 times, and the mean values were calculated (mean fractions were avoided) and recorded. In antibiotic sensitivity test, only the methanolic extract was used because the results of preliminary screening showed that all other extracts (petroleum ether, acetone, and aqueous) were insignificant against the tested strains.

RESULTS AND DISCUSSION

The result of the antibacterial activity of various extracts of *L. parviflora* against standard strains is given in Table 1 and the antibiotic susceptibility test in Table 2.

Repeated experiments with control disks showed that they did not possess any inhibitory effect. This indicated that the solvent alone is ineffective to produce antibacterial activity. Similarly, the petroleum ether extracts and aqueous extracts were ineffective against the tested strains. This may be because the antibacterial principles of the tested plant may be soluble in organic, polar solvents such as methanol or partially polar solvent such as acetone. An activity with an inhibition zone of 6–10 mm is considered a lesser activity and has no significance in antibacterial studies. However, an activity between 11 and 15 mm can be considered as moderate and those of 15 mm and above as high and powerful. An activity with 15 mm or above would be taken into consideration for further studies including purification of the active principles.

The acetone extract of *L. parviflora* showed slight activity against all the tested strains (inhibition zone was <9 mm, not a prominent activity). However, its methanolic extract was very active against *S. aureus* with an inhibition zone of 21-mm diameter. This activity has very much significance in the field of antibacterial studies. However, the activity of the methanolic extract was moderate against *P. aeruginosa*, *K. pneumoniae*, and *E. coli*.

On analyzing the effect of standard antibiotics against the tested strains, it is evident that all the tested antibiotics such as chloramphenicol, gentamicin, ciprofloxacin, tetracycline, and amikacin were active against the four tested strains. Nevertheless, none of the bacterial strains could be inhibited by the antibiotic - Penicillin G which indicates that the Penicillin G is an outdated antibiotic in the field of antibacterial therapy.

Among the active antibiotics, the strength of activity was varying in different bacterial strains. Maximum inhibition against *S. aureus* was given by ciprofloxacin 5 mcg and minimum inhibition by tetracycline 30 mcg. On comparing the results of the preliminary screening, it is clear that the crude methanol extract (10 mg/disc) of the whole plant of *L. parviflora* (inhibition zone of 21 mm) has higher activity than tetracycline 30 mcg. Therefore, the methanolic extract of *L. parviflora* can be taken into consideration for a detailed study to suggest them as an alternative to tetracycline 30 mcg against the treatment of disease caused by *S. aureus*.

Antibiotic activity against the *P. aeruginosa* showed that ciprofloxacin 5 mcg showed the highest activity and minimum by gentamicin 10 mcg. However, tetracycline 30 mcg has been totally resisted by the *P. aeruginosa*. When comparing the tested sample, the crude methanolic extract of *L. parviflora* showed slightly higher activity against *P. aeruginosa* than gentamicin and tetracycline. Therefore, it can be concluded that the methanolic extract of *L. parviflora* (10 mg/disc) is a good alternative for gentamicin 10 mcg and tetracycline 30 mcg against the treatment of infections caused by *P. aeruginosa* (Fig. 1).

Chloramphenicol 30 mcg showed the highest activity against *K. pneumoniae* and least activity by tetracycline 30 mcg. However, the methanolic extract (10 mg/disk) of *L. parviflora* showed increased activity against the *K. pneumoniae*. Hence, it can be concluded that the crude methanolic extracts of *L. parviflora* are an alternative source for tetracycline 30 mcg against the growth of *K. pneumoniae*.

Highest inhibition against *E. coli* was shown by ciprofloxacin 5 mcg and least inhibition by tetracycline 30 mcg. However, the methanolic extract of *L. parviflora* (10 mg/disc) showed more activity than tetracycline 30 mcg against the *E. coli*. Therefore, the methanolic extracts of *L. parviflora* can be considered for a detailed study to suggest them as an alternative to tetracycline 30 mcg in the treatment of diseases caused by *E. coli*.

One report from Selvamuthu *et al.* showed that its leaves contain some antibacterial activity [12]. The present study also suggests that the methanolic extract of the whole plant of *L. parviflora* possesses powerful antibacterial activity against *S. aureus*, *P. aeruginosa, K. pneumoniae*, and *E. coli*. Since the methanolic extract of the whole plant of *L. parviflora* showed promising activity against all the tested bacterial strains, the present study suggests that this plant can be taken into consideration for isolating the active principle to develop a new broad-spectrum antibiotic against the common pathogenic bacteria and would be used as a more powerful alternative to the common antibiotics, tetracycline and gentamicin (Fig. 2).

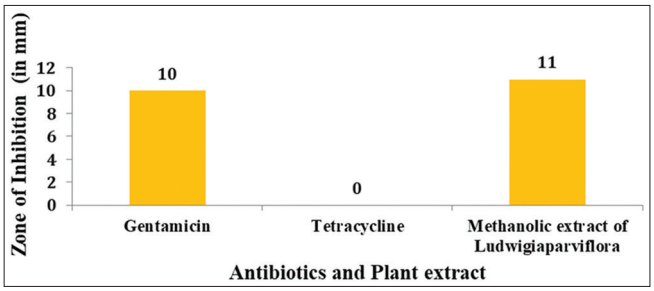

Fig. 1: Activity of methanolic extract of *Ludwigia parviflora*, tetracycline and gentamicin against *Pseudomonas aeruginosa*

Table 1: Antibacterial activity of various extracts of *L. parviflora* against standard bacterial strains

Plant	Bacterial strains tested (zone of inhibition in mm)															
	Staphylococcus aureus				*Pseudomonas aeruginosa*				*Klebsiella pneumoniae*				*Escherichia coli*			
	Pe	A	M	Aq	Pe	A	M	Aq	Pe	A	M	Aq	Pe	A	M	Aq
L. parviflora	0	8	21	0	0	8	11	0	0	9	11	0	0	7	13	0

Pe: Petroleum ether extract, A: Acetone extract, M: Methanolic extract, Aq: Aqueous extract, *L. parviflora: Ludwigia parviflora*

Table 2: Comparison of the activity of the methanolic extract of *L. parviflora* with that of antibiotics against the standard bacterial strains

Bacterial strains	Standard antibiotics tested (zone of inhibition in mm)						
	1	2	3	4	5	6	7
Staphylococcus aureus	25	27	0	35	15	34	21
Pseudomonas aeruginosa	11	10	0	30	0	16	11
Klebsiella pneumoniae	22	16	0	24	10	21	11
Escherichia coli	22	20	0	30	11	25	13

1: Chloramphenicol (30 mcg), 2: Gentamicin (10 mcg), 3: Penicillin G (10 u), 4: Ciprofloxacin (5 mcg), 5: Tetracycline (30 mcg), 6: Amikacin (30 mcg), 7: Methanolic extract of *Ludwigia parviflora*

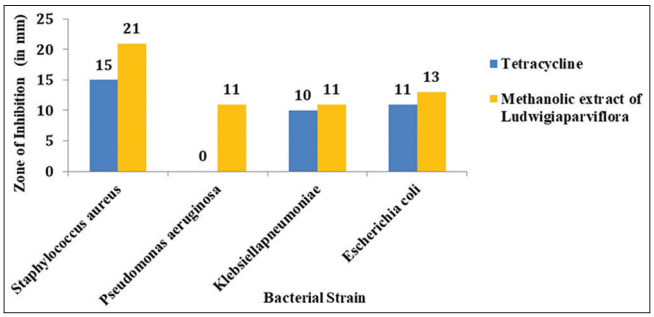

Fig. 2: Activity of methanolic extract of *Ludwigia parviflora* and tetracycline against standard bacterial strains

CONCLUSION

The whole plants of *L. parviflora* collected from various regions of Upper Kuttanad, Alappuzha district, Kerala state, were tested for antibacterial activity against standard MTCC bacterial strains by disk diffusion method. The activity was also compared with the activity of standard antibiotics. The results of the study revealed that the methanolic extract of *L. parviflora* possessed a promising activity against all the tested strains. Its activity was higher than the activity of tetracycline 30 mcg against *S. aureus, P. aeruginosa, K. pneumoniae,* and *E. coli*. It also showed slightly higher activity than the common antibiotic, gentamicin 10 mcg against the *P. aeruginosa*. Therefore, the study can be concluded that the crude methanolic extract of the whole plant of *L. parviflora* with a concentration of 10 mg/disk is equivalent to gentamicin against *P. aeruginosa* and is also more powerful than tetracycline against all the tested strains. Hence, isolation of active antimicrobial principle from *L. parviflora* and its detailed toxicological and other pharmacological studies are suggesting.

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AUTHOR'S CONTRIBUTION

Shibu George was responsible for doing the laboratory work and writing the research paper.

Melvin Joy involved in its result analysis.

CONFLICTS OF INTEREST

Authors have none to declare.

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