IJPSR (2020), Volume 11, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 January 2020; received in revised form, 03 April 2019; accepted, 04 April 2020; published 01 May 2020

EVALUATION OF *IN-VITRO* ANTI-BACTERIAL AND CYTOTOXIC ACTIVITY OF *LEEA MACROPHYLLA*

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Keywords:

Leea macrophylla, Antibacterial activity, Brine shrimp lethality assay

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ABSTRACT: Studies have confirmed the medicinal potential of the *Leea macrophylla* mentioned in traditional medicine. While the effects of the *Leea macrophylla* extract on some bacteria and Brine shrimp lethality using their different concentrations has not been previously explored. The present study shows that the standardized aqueous and ethanolic extract of *Leea macrophylla* exhibited antibacterial and cytotoxic activity. The findings of the present work provide promise for the development of new molecules of treat microbial infections and cancer.

INTRODUCTION: *Leea macrophylla* (Roxb.) (Family: Leeaceae) is a herb or herbaceous shrub with a very big size leaf like an elephant-ear. The plant parts of Leea macrophylla are used by tribal people in the cold, cough, headache, tetanus, *etc.* [1, 2] It also has ethnobotanical uses in goiter, gastric tumor, lipoma body pain and rheumatic pain [2-5]. Although *Leea macrophylla*. *Leea macrophylla* has various ethnopharmacological uses; the plant have not been investigated for antimicrobial and cytotoxic activity against prominent gram-positive and gram-negative human pathogenic bacterial strains. Besides, cytotoxic activity screening of the extracts was also carried out with view to assess the presence of antitumor activity of different extracts.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(5).2448-50

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(5).2448-50

In-vitro lethality test has been successfully used as a preliminary study of cytotoxic and antitumor agents.

MATERIALS AND METHODS:

Preparation of Extracts: The plant species *Leea macrophylla* [Roxb.ex Hornem] belonging to Family: Leeaceae was collected from Kalgaon village Taluka. Patan, District- Satara and authenticated at Botanical department of Yashwantrao Chavan College of Science, Karad. The plant was dried under sunlight and fine powder of the plant was prepared by using a hand grinder.

Preparation of *Leea macrophylla* Aqueous Extract (LMAE): powder was mixed with 30 ml distilled water boiled for 30 min in round bottom flask attach with a reflux condenser. The material was filtered Whatman filter paper no 40, and filtrate was collected.

Preparation of *Leea macrophylla* Ethanolic Extract (LMEE): powder was mixed with 30 ml

alcohol and 10 ml distilled water boiled for 30 minutes in round bottom flask attach with reflux condenser. The material was filtered Whatman filter paper no 40 and filtrate was collected. The filtrate was collected in porcelain dish. Alcohol was evaporated and then added 4 ml distilled water. Both the extract was used in the concentration range of 10, 50, 100, 150, 300 µg/ml.

Antibacterial assay: The antibacterial assay was carried out by employing 24 h cultures of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Klebsiella pneiumonieae*. The activity of aqueous and ethanolic extracts of *Leea macrophylla* was tested separately using Agar well diffusion method. The medium was sterilized by autoclaving at 120 ºC (15 lb/inch square). About 30 ml of the Agar medium with the respective strains of bacteria was transferred aseptically into each sterilized Petri plate. The plates were left at room temperature for solidification. A well of 5 mm diameter was made using a sterile cork borer. The standard drug and extracts were placed in 6mm diameter well. Antibacterial assay plates were incubated at 37 ± 2 ºC for 24 h. The standard disc 5 mm diameter with ciprofloxacin (100 µg/disc) was used as a positive control for antibacterial activity [6, 7].

Brine Shrimp Lethality Bioassay / Cytotoxicity Assay: Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds. The bioassay was carried out against a simple zoological organism, brine shrimp nauplii. The brine shrimp lethality bioassay was carried out on the aqueous and ethanolic extracts of *Leea macrophylla* using standard procedure. Briefly, brine shrimp (*Artemiasalina leach*) eggs were hatched in a hatching chamber filled with fresh seawater. The chamber was kept under illumination using a fluorescent bulb for 48 h for the eggs to hatch into shrimp larvae. 30 mg of each extract were separately dissolved in 3 ml of DMSO, and from these 300, 150, 100, 50, and 10 µg/ml were prepared by serial dilution. Each concentration was tested in triplicate, giving a total of 15 test-tubes for each sample. A control containing 5 ml of DMSO solvent was used for each solvent. The final volume of the solution in each test-tube was made up to 5 ml with seawater immediately after adding shrimp larvae. The test-tubes were maintained under illumination. Survivors were counted after 24 h, and the percentage death at each dose was determined, and LC_{50} values were calculated. After 24 h of incubation, the test tubes were inspected using a magnifying glass, and the number of survivors was counted. The concentration-mortality data were analyzed statistically for the determination of LC_{50} values [6, 7].

RESULTS:

Antibacterial Assay: The antibacterial activities of the aqueous and ethanolic extracts of *Leea macrophylla* obtained by the cup plate method are presented in **Table 1**. The extracts showed varying zones of inhibition at two concentrations (150 and 300 µg/ml) against two gram-positive and two gram-negative bacteria.

The ethanolic extract showed a significant zone of inhibition against *B. subtilis*, *E. coli*, *S. aureus*, and *K. pneumonia* with respect to the standard. The maximum zone of inhibition was obtained for the LMAE at both concentrations against *S. aureus*.

TABLE 1: ANTIBACTERIAL ASSAY OF *LEEA MACROPHYLLA* EXTRACTS

Sample name	Dose in µg/ml	Zone of inhibition in mm			
		S. aureus	***B. subtilis***	***E. coli***	***K. pneumonia***
Control (DMSO)	0.1	NA	NA	NA	NA
LMAE	150	6.230 ± 0.24	-	-	-
	300	12.7330 ± 0.77	8.960 ± 0.5774	-	-
LMEE	150	9.0012 ± 0.0001	9.3360 ± 0.5774	6.760 ± 0.5774	7.4590 ± 0.674
	300	16.0102 ± 1.0000	13.360 ± 0.5774	9.5600 ± 0.5774	12.43 ± 0.2974
Standard (Ciprofloxicin)	100	54.6670 ± 0.5774	53.660 ± 0.5774	45.0001 ± 1.0000	40.0020 ± 1.0000

(-) No zone of inhibition detected. Values are means ± SEM from three readings

Brine Shrimp Lethality Bioassay (Cytotoxicity Assay): Both the extracts of the *Leea macrophylla* showed positive results indicating that the test samples are biologically active. Following the procedure of Meyer, the lethality of *Leea macrophylla* extracts of the brine shrimp was

evaluated. The results of the brine shrimp lethality after 24 h exposure to all the samples and the positive control, vincristine sulphate is summarized in **Fig. 1.** Plotting of log of concentration (log C) versus percent mortality (% Mortality) for all test samples showed an approximately linear correlation shown in **Fig. 2**. In this bioassay, aqueous and ethanolic extracts of *Leea macrophylla* revealed prominent cytotoxicity with the LC_{50} values of 256.77 ug/ml and 180.66 µg/ml respectively.

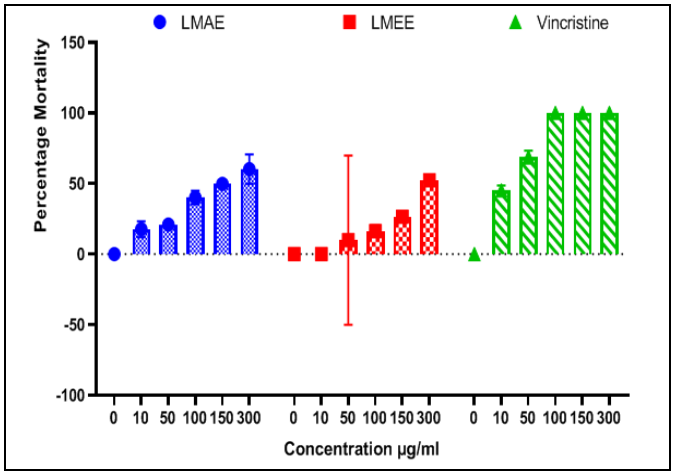

FIG. 1: *IN-VITRO* CYTOTOXIC ACTIVITY OF *LEEA MACROPHYLLA* EXTRACTS Values are means ± SEM from three readings

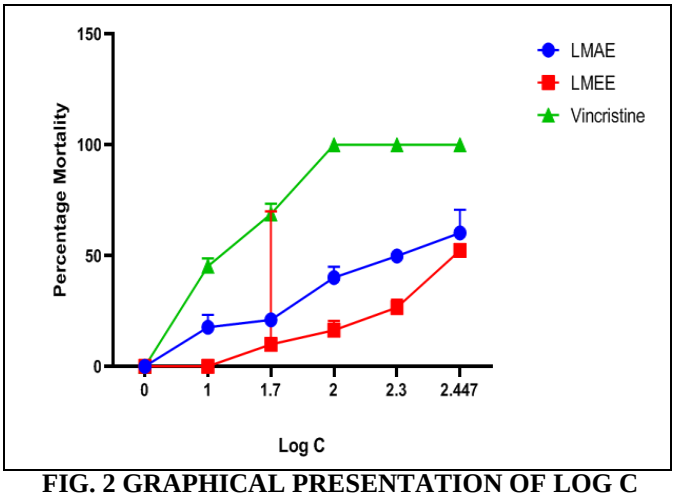

FIG. 2 GRAPHICAL PRESENTATION OF LOG C VERSUS PERCENT MORTALITY OF *LEEA MACROPHYLLA* EXTRACTS

CONCLUSION: The *Leea macrophylla* extracts have the potential to be a candidate for the investigation of cytotoxic compounds. The findings of the present work provide us preliminary information on the most promising plant species that could be used as a basis for the development of new tools of great therapeutic importance to overcome microbial infections and cancer.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: Nil

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How to cite this article:
Somade PM, Chopade AR, Patil PA and Kengar SB: Evaluation of *in-vitro* anti-bacterial and cytotoxic activity of *Leea macrophylla*. Int J Pharm Sci & Res 2020; 11(5): 2448-50. doi: 10.13040/IJPSR.0975-8232.11(5).2448-50.