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Original Article

A study on antimicrobial and cytotoxic activities of two common weeds

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ABSTRACT

The purpose of this study was to evaluate two common weeds causing detrimental effects in our environment. *Erigeron bellidioides* Benth. is a relatively unexplored plant causing a lot of distress and *Lantana camara* Linn. well established as a notorious weed in Nepal. Both the plants were studied for antibacterial activity against selected microorganisms and cytotoxic activity was also assessed. The plants were extracted in three solvents viz. hexane, ethyl acetate and methanol by soxhlet extraction. The extracts were subjected to antibacterial assay against two gram positive and two gram negative microorganisms using cup-diffusion method. The extracts possessing antimicrobial activity were then subjected to the determination of minimum inhibitory concentration. The cytotoxic activity was assessed by performing the Brine Shrimp Lethality Assay. The present study therefore, lays out a basis for further research upon these plants.

Keywords: *Erigeron bellidioides*, *Lantana camara*, Brine Shrimp Lethality Assay, antibacterial activity

INTRODUCTION

Nepal is a country where most of the population depends upon traditional medicines for treatment purposes. Traditional medical treatment, based upon the use of medicinal plants relies upon native knowledge gained from inherited experience. However this knowledge is scientifically un-documented and the only means by which it is communicated is verbally.[1]

Considerable benefits have been verified by abundant natural substances derived from plants regarding the prevention, treatment and management of various forms of cancer. However, only a restricted amount have been screened for their prospective role in cancer prevention or their cancer inhibition efficiency using *in vitro* or *in silico* models among the numerous natural constituents found in plants.[2] The chemical range of such secondary plant metabolites that result from plant evolution may be equal or even superior to that found in synthetic conjugational chemical libraries.[3]

Pathogenic bacterial strains achieve multidrug resistance owing to their plain/plastic genomes and related DNA trades with different strains. Ultimately, the MDR (Multi Drug Resistance) strains are spread around the world, bringing about unbalanced morbidity and mortality.[4] This issue influences the economic and social/public health factors greatly, prompting the need of a critical quest for antimicrobials from alternating sources. Plants provide an overt source of drugs and serve as an alternative for synthetic antimicrobials.

In Nepal, *Erigeron bellidioides* has been established as a challenging and bothersome weed. Known to considerably reduce crop yields, thorough research on this herb is in its formative years. Locally, it has been reported to be toxic to cattle and goats and used as bug repellant. Indigenously used as a blood purifier[5], it is very pertinent that a large amount of research remains to be performed on this unexplored, underutilized and neglected weed, *E. bellidioides.*.

L. camara is a remarkable weed, having around 650 varieties in more than 60 nations. It is recognized and intensifying in numerous locales of the world, regularly as an after-effect of clearing of forest for timber or farming. It affects seriously on agriculture and in addition to natural ecosystems. A native of South America, *L. camara* has a wide variety of traditional uses everywhere throughout

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the world. In Central and South America, the leaves were made into a poultice to treat wounds, chicken pox and measles. Fevers, colds, stiffness, asthma and hypertension were treated with arrangements from the plant. In Ghana, an imbuement of the entire plant was utilized for bronchitis and the powdered root in milk was given to kids for stomach-ache.[6-8]

L. camara is a well-established invasive alien plant which apart from being toxic in nature is very harmful for the native plants as well. Biological invasion of weeds has acquired the spotlight for intense management and research activities worldwide for more than the past half century.[9] Earlier studies on weed control have largely suggested that the substitution of chemical methods to control such environmental atrocity by any other means physical or biological is not as effective. Such weeds are not only a nuisance to our surroundings but harm our natural biodiversity too. By assessing its potential medicinal properties the status of this plant can be elevated. This shall help in the proper management of local resources which have been posing as a nuisance and give way to further necessary research.[10]

MATERIALS AND METHODS

Plant material: The aerial parts of plant *E. bellidioides* was collected from Shankenjung Village Development Committee - 4, Illam (4000 ft), Nepal during the month of July whereas, the aerial parts of the plant, *L. camara* was collected from Gairidhara-2, Kathmandu (4000 ft) during the month of June and was duly identified at the National Herbarium Center and Plant Laboratory, Godawari, Lalitpur, Ministry of Forest and Soil Conservation, Government of Nepal.

Chemicals and apparatus: The laboratory reagents were provided by National Model College for Advance Learning. Equipments and materials used during this work are listed in Table 1 and 2 respectively.

Organisms: Pure culture of *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (Clinical isolate), *Escherichia coli* (ATCC 25922), *Salmonella typhii* (Clinical isolate) The microorganisms were obtained by the courtesy of microbiology laboratory, National College. *Artemia salina* (brine shrimp eggs) was provided by National Model College for Advance Learning.

Plant processing: The plant material was cut into pieces and shade dried at room temperature. Dried sample was crushed into powder by the help of an electric grinder. The plant powder was stored in an air-tight container for further evaluation.

Physicochemical evaluation: Total ash value was determined by following the protocol of World Health Organization.[11]

Extraction: The plant material was shade dried at room temperature. The dried sample was crushed into powder using an electric grinder and was stored in an air-tight container for further use. The powder was then weighed, 18 gm at a time and was subjected to extraction in soxhlet apparatus by using non-polar to polar solvents (hexane, ethyl acetate and methanol).The so obtained liquid extracts were then subjected to evaporation using a rotary evaporator under vacuum at the temperature of 37±5 ºC until the solid mass was obtained. The extracts were then weighed and kept in glass vials and stored in refrigerator at 4°C for further analysis.

Qualitative antimicrobial test: Preliminary antimicrobial test of the extracts were carried out by cup diffusion method. The procedure for antimicrobial test from agar well diffusion was adapted from Joshi et.al.[12]

Preparation of agar plates: Mueller-Hinton agar was prepared from commercially available dehydrated base according to the manufacturer's instruction as per the label of the pack. 38.6 grams of the agar powder was suspended in 300ml distilled water in a conical flask. It was then heated to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. It was allowed to cool and 25ml of freshly prepared and cooled medium was poured into glass, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of 4mm. The agar plates were allowed to cool at room temperature for solidification.

Preparation of standard culture inoculums of test organism: The inoculating loop full of growth after touching the top of isolated colonies of test bacteria in the agar plates was transferred into a tube containing Mueller Hinton broth medium. The broth culture was incubated at 37±2 °C for 2 hours to obtain the turbidity optically comparable to that of the 0.5 McFarland standards resulting in a suspension containing approximately 1 to 2 × 10^8 CFU/ml of the test organism.

Inoculation of the plates: Inoculation of the prepared agar plates was carried out by dipping a sterile cotton swab into the tube inoculums, removing excess inoculums by pressing and rotating the swab firmly against the side of the tube

above the liquid level, followed by streaking all over the agar medium surface in the Petri dish, rotating the dish through an angle of 60° after each application. Finally, the swab was passed around the edge of the agar surface. The inoculums were left to dry for 3 to 5 minutes at room temperature with the lid closed.

Preparation of the well: With the help of sterile cork-borer having a diameter of 6 mm, wells were made in the agar plates and labeled appropriately. Using a micropipette, 50 µl of 100 mg/ml of each extract and same volume of positive and negative control sample, i.e. 0.01% ofloxacin and DMSO respectively were placed in different cups. Plates were left for a while, until the extracts diffused in the medium with the lid closed. Then, the plates were incubated at 37°C for 24 hours. After overnight incubation, the diameter of each zone (including the diameter of the well) as judged by the unaided eye was measured with the help of a ruler held on the back of the inverted plate and the measurements were recorded in mm. The extracts presenting with a zone of inhibition of 20 mm or more were considered to be highly active and those having a zone of inhibition of <20 mm were considered to be moderately active.[4]

Determination of minimum inhibitory concentration: A dilution test was carried out by adding dilutions of ethyl acetate extract to series of broth. The MIC values were determined for the ethyl acetate extract of both the plants against *Bacillus cereus* and *Salmonella typhii*. Concentration of test sample taken was 100mg which was serially diluted in 10 test tubes containing nutrient broth and standardized inoculums of the test organisms. The test tubes were incubated at 37ºC for 24 hours and turbidity was observed for.

Brine Shrimp Lethality Assay: The brine shrimp lethality assay was performed by following the method of Moshi et al.[13] The brine shrimp bioassay is based on the concentration of sample under consideration which kills 50% of laboratory–breed *Artemia salina* within 24 hours under the specified appropriate condition. This concentration is known as LC_{50} and is expressed in µg/ml. Cytotoxicity was evaluated in terms of LC_{50} (lethality concentration). After, hatching the brine shrimps eggs for 24 hours in a beaker containing artificial seawater, a needle less syringe was used to remove the hatched brine shrimp eggs. The test and control samples were prepared of three extracts of the plants viz., ethyl acetate and methanol. A stock solution (1000 ppm) of the plant extracts was prepared. Six different concentrations namely, 500 ppm, 250 ppm, 125 ppm, 75 ppm, 50 ppm, 25 ppm were prepared by serial dilutions from the stock solution in sea water using volumetric flasks. An aliquot of each concentration (10ml) was transferred, in triplicate, into clean test tubes. Ten shrimp nauplii were transferred to each test tube. DMSO in seawater was used as negative control. The test tubes were then incubated for 24 hours at 27°C. After the completion of 24 hours, the number of survivors in each test tube was counted against illuminated background and percentage mortality at each dose level and control was calculated using following formula:

$$\%mortality = \frac{number\ of\ dead\ shrimps}{number\ of\ dead + alive\ shrimps} \times 100$$

The criterion for toxicity of plant extracts were taken as: LC_{50} values > 1000 µg/ml (non-toxic), ≥ 500 µg/ml ≤ 1000 µg/ml (weakly toxic) and < 500µg/ml (toxic)

Statistical analysis: All the quantitative tests were conducted in triplicate. The data was presented in the form of mean ± SD (standard deviation). The results were analyzed statistically by the help of Microsoft excel 2007.

RESULTS & DISCUSSION

This study was performed to assess the biological activity of two common and problematic weeds, *E. bellidioides* and *L. camara. E. bellidioides* is a troublesome weed, locally causing death in cattle after ingestion and affecting the biodiversity of the land. It has not been studied intensively and its medicinal properties if present have not been verified. This study was conducted in order to test any biological activity present in this plant. Investigation of such plants does not only verify its properties but also helps us create a better use of such nuisance plants as well as its availability in abundance initiates economic utility.

L. camara is another common weed in Nepal. Over time, it has found a wide range of traditional uses. Indigenous to tropical and subtropical America, it has been used as a medicinal plant in various parts of the world. Its usage in folk medicine ranges from topical application for cuts and wounds, to treat infectious diseases such as malaria, chicken pox, tuberculosis and measles, it is also used to treat bilious fever, rheumatism, ulcers, tetanus, typhoid fever, influenza and bronchitis.[14] All these traditional uses add more fuel to the fact that this plant must have some medicinal properties, which can only be verified by proper scientific research. Although *L. camara* is widely used in traditional medicine it has garnered a reputation for being a nuisance weed in Nepal. The total ash

value of *L. camara* was determined to be 16.3% and for *E. bellidioides* it was determined to be 8.26%. The extractive values of the plant material with different solvents were calculated and tabulated as shown in Table 3.

E. bellidioides presented moderate anti-bacterial activity against, *Escherichia coli, Bacillus cereus* and *Salmonella typhii.* The ethyl acetate extract was found to be more effective than hexane and methanol extracts in terms of anti-bacterial activity. It presented minimum inhibitory concentration at 6.25 mg/ml for *Bacillus cereus* and *Salmonella typhii.* The antimicrobial activity is shown is table 4. The obtained values for minimum inhibitory concentration are presented in Table 5.

The ethyl acetate and methanol extracts of *E. bellidioides* were toxic according to the brine shrimp lethality assay with LC_{50} value at 21.82µg/ml and 2.62µg/ml respectively.

The results of preliminary antimicrobial activity of *L. camara* extracts revealed that methanolic and hexane extracts possess low activity against *Bacillus cereus* and no antimicrobial activity against *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus.* However, ethyl acetate extract showed moderate activity against *Bacillus cereus* and *Salmonella typhii* and low activity against *Staphylococcus aureus* and *Escherichia coli*. The determination of minimum inhibitory concentration for *Bacillus cereus* and *Salmonella typhii* by ethyl acetate extracts revealed the minimum inhibitory concentration to be 3.12 mg/ml for both *Bacillus cereus* and *Salmonella typhii.* According to a study, its phytocompound (lantic acid) was active against both *S. aureus* and *Salmonella typhi*.[15] Another research shows that, hexane, chloroform and carbon tetrachloride extracts showed significant activity on *Bacillus megaterium*, *Salmonella paratyphi*, *Vibrio mimicus*, *Vibrio parahaemolyticus*, *P. aeruginosa*, *B. cereus*, *B. subtilis* and *E. coli.*[16] Previous research work regarding the antimicrobial activity states that the methanolic extract presented antimicrobial activity against *S. aureus* at 23mm zone of inhibition with MIC value at 5mg/ml.[17] The results of my present study was not similar to previous research done in *L. camara*, this can be attributed to the various factors such as climatic and geographical variations and difference in time collection. Another important factor which may be responsible for poor results is the extraction method and the conditions of extraction such as temperature and total time for extraction.

The brine shrimp lethality bioassay was performed on the methanolic extract of *L. camara*. The lethal concentration (LC_{50}) value of methanolic extract was determined at 53.30 µg/ ml. This indicates that the methanolic extract exhibited toxic behavior against *Artemia salina*. The literature review of brine shrimp lethality bioassay revealed the research performed with ethanolic extracts of *L. camara* and indicated the lethality concentration at 55 µg/ml.[18] The results of my study are quite similar to the research done previously.

CONCLUSION

This study focused on the anti-bacterial activity and preliminary cytotoxic activities of two common weeds, *E. bellidioides* and *L. camara*. It revealed that the ethyl acetate extracts of both the plants presented moderate antibacterial activity. The extracts of both the plants showed cytotoxic activity against brine shrimp, this is due to the fact that both the plant's LC_{50} value is less than 1000 µg/ml. The brine shimp lethality assay is but a preliminary screening method; more specific bioassay should be performed to devise a mechanism of action. Any useful compounds of therapeutic value can be further studied in both the plants.

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Table 1. List of equipments used:

Equipment name	Model no.	Origin
Analytical balance	AR-3130/ OHAUS	Germany
Weighing balance	98-110/Scaltec	Germany
Soxhlet extractor	Borosil	-
UV-Spectrophotometer	UV-2450/Shimadzu	Japan
Rotary vacuum evaporator	ATICO	India
Incubator	-	
Hot air oven	-	

Table 2. List of chemicals used:

S.N.	Chemicals
1.	Hexane
2.	Ethyl acetate
3.	Methanol
4.	Ofloxacin
5.	artificial sea water
6.	Nutrient broth
7.	Mueller-Hinton agar
8.	Dimethyl sulfoxide

Table 3: Extractive values of *L. camara*:

Plant	Extracts	Extractive value
Lantana camara	Hexane	3.33%
	Ethyl acetate	10.51%
	Methanol	11.21%
Erigeron bellidioides	Hexane	3.23%
	Ethyl acetate	7.143%
	Methanol	14.59%

Table 4: Antimicrobial activity of plant extracts:

Microorganisms	Zone of inhibition (mm)						
	Erigeron bellidioides			*Lantana camara*			Control*
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol	
Staphylococcus aureus	0	4	0	0	6	0	19
Bacillus cereus	3	9	4	4	8	5	24
Salmonella typhimurium	0	14	0	0	14	0	16
Escherichia coli	0	9	0	0	4	0	18
*Control = 0.01% ofloxacin							

Table 5.Minimum inhibitory concentration of ethyl acetate extract:

Microorganism	MIC value	
	Erigeron bellidioides	*Lantana camara*
Bacillus cereus	6.25 mg/ml	3.12 mg/ml
Salmonella typhii	6.25 mg/ml	3.12 mg/ml

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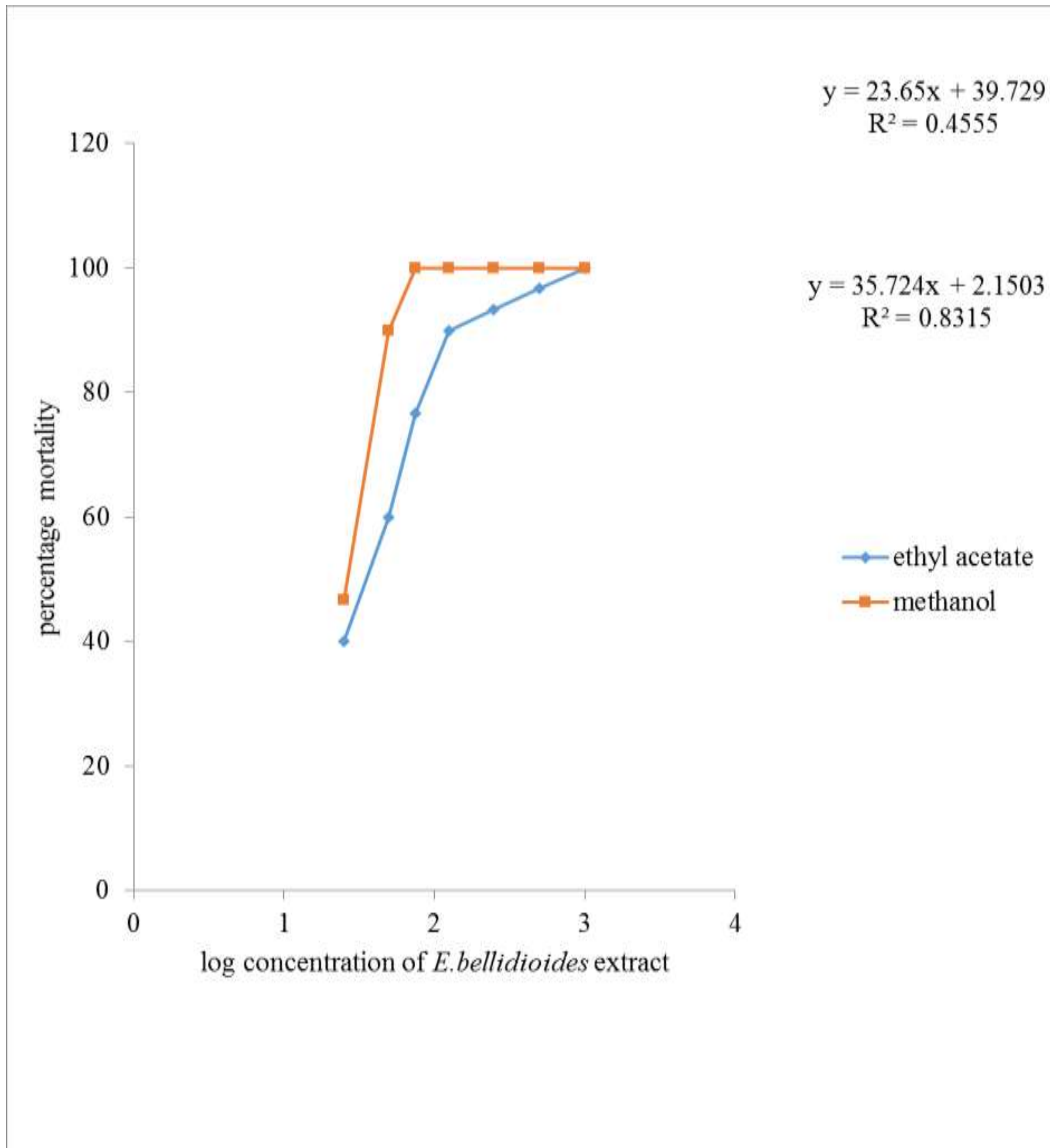

Figure 1. Mortality response of brine shrimp against ethyl acetate and methanol extract of *E. bellidioides.*

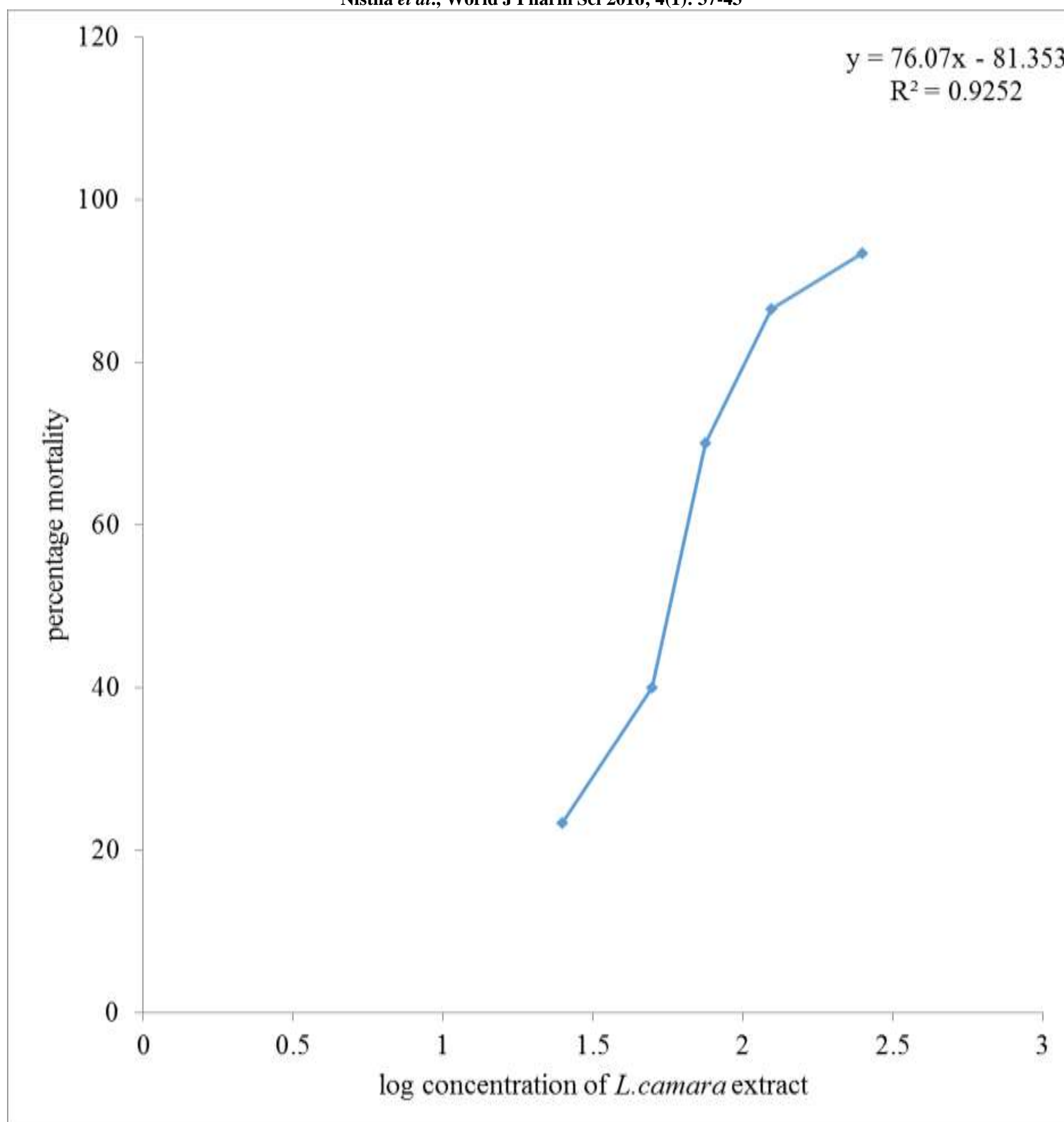

Figure 2. Mortality response of brine shrimp against methanol extract of *Lantana camara.*