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Evaluation of the toxic weed *Lantana camara* as a potent medicinal plant

Ketki Gwalani[1], Nandita Nashikkar[2], Sunita Bundale[3], Avinash Upadhyay[4*]
[1-4] Hislop School of Biotechnology, Hislop College, Temple Raod, Civil Lines, Nagpur, Maharashtra, India

Abstract
Weeds are the plants that invade native ecosystems and are undesirable for the survival of indigenous flora and fauna. Typically these are the plants that grow aggressively and multiply very quickly without any natural controls. These plants display adverse effects through contact or ingestion. One such weed called *Lantana camara* has become one of the most invasive weeds and is known to outgrow native vegetation. Despite the measures taken to eradicate this weed, it is still growing in wide range of habitats as it can grow in extreme harsh conditions also. It is desirable to find some alternative use of this weed as it is very difficult to eliminate. Therefore the present investigation deals with evaluation of this potentially toxic weed as a medicinal plant. With this view in mind, the present study was carried out to evaluate the anti-quorum sensing potential of this weed. Different solvent extracts of the plant were prepared with Soxhlet apparatus and were tested for anti-swarming potential against *Proteus mirabilis* and pigment inhibition in *Serratia marcescens*. P.ether (3.33±0.57), Chloroform (3.66±1.15), and methanol (4±1) extracts of *L. camara* showed maximum inhibition in the swarming pattern of *Proteus mirabilis.* Reduction in the pigment production without inhibiting the bacterial growth in *Serratia marcescens* was observed in the presence of ethyl acetate (28±0.1) and methanol (21±0.1) extract. Results of the study indicate that despite being a toxic weed *Lantana camara* can be alternatively utilized as potent quorum sensing inhibitory plant and can be put for evaluation for diversified applications.

Keywords: quorum sensing, soxhlet apparatus, *Serratia marcescens*, pigment inhibition, swarming inhibition

1. Introduction
Weeds are the plants that are harmful to the naturally occurring flora and fauna. They grow aggressively and multiply very quickly. Typically these are the plants which are considered as "plants in wrong place as they are undesirable in a particular condition. *Lantana camara* is an evergreen shrub native to subtropical and tropical America but a few species are indigenous to tropical Asia and Africa [1]. *Lantana camara* is more popular as a toxic weed in most of the countries because of its adverse effects through contact or ingestion [2]. This plant can grow even in extremely harsh climatic conditions. *Lantana* poisoning has been taking a heavy toll of live stock year after year [3]. It also exerts Allelopathic action on neighboring vegetation [4]. Despite the measures taken to eradicate this weed, it is still growing in the wide range of habitats. It has become a weed of *National Significance* as it is regarded as one of the worst weeds because of its invasiveness, potential for spread and economic and environmental aspects [3]. Taking into consideration all these aspects about this weed, it is desirable to find some alternate uses of this plant. Efforts are being made to find out the therapeutic potential of this plant. Extracts from the *Lantana* leaves exhibit antimicrobial and insecticidal activity [6].The leaves are used in the treatment of itches, cuts, ulcers, swellings, bilious fever, eczema and rheumatism [7]. Many previous studies have described the antifungal, antimicrobial, immunosuppressive and antitumor activities of *Lantana* [8, 9, 10, 11]. Hence the present study aimed to find out some important medicinal properties of this potentially toxic weed. With this view in mind, the study was carried out to evaluate the anti-quorum sensing potential of *Lantana camara*. The problem of multidrug resistant bacteria is becoming a global concern these days. These pathogenic organisms are becoming resistant to a wide range of antibiotics which poses a problem in the treatment of various infectious diseases [12]. There is a need to find out an alternative strategy to combat these pathogens. Quorum sensing is one such phenomenon exhibited by almost all types of pathogenic bacteria. It is a system that controls the virulence of these organisms. So if quorum sensing can be controlled these pathogens are rendered less virulent or avirulent making them unable to establish the infection. Therefore the study was carried out to evaluate the anti-quorum sensing potential of *Lantana camara* against two pathogens *Proteus mirabilis* and *Serratia marcescens*.

2. Materials and Methods
2.1 Collection of plant material
Fresh leaves and stems of *Lantana camara* were collected from the nearby premises. Collected plant material was dried in the shade for 20-25 days.

2.2 Preparation of Extracts
Once dried, the leaves were ground and extracted using a Soxhlet apparatus with solvents in the series of increasing polarity Chloroform, P.ether, ethyl acetate, acetone and methanol. The solvents were evaporated to dryness and further dilutions were made in DMSO to obtain the required concentration. DMSO (Dimethyl sulfoxide) was also taken as solvent control.

2.3 Bacterial strains and culture medium

Proteus mirabilis, a pathogenic strain with a trait of swarming motility that is controlled by quorum sensing and *Serratia marcescens*, again a pathogenic strain having pigment producing capability that is also quorum sensing controlled were used in this study. Both the strains were grown in LB broth and Nutrient Agar medium at temperature ranging from 30° to 37°C.

2.4 Bioassays for anti-quorum sensing

Swarming motility assay and pigment inhibition assay were selected for testing of anti quorum sensing potential of the plant extracts.

2.4.1 Swarming inhibition assay (*Proteus mirabilis*)

Swarming motility assay was conducted with LB media consisting of 0.5% agar. Swarm plates were poured and typically allowed to dry in the incubator before being used. Swarm plates prepared with different concentrations of the extracts were then centrally inoculated with the overnight grown bacterial culture in LB broth. These plated were then incubated for 24 hrs at 37°C. Inhibition in the swarming motility was determined by measuring the diameters of the swarm zones compared to control.

2.4.2 Pigment Inhibition assay (*Serratia marcescens*)

Agar well diffusion assay adopted to detect pigment inhibition in *Serratia marcescens.* The assay was conducted with Nutrient agar medium consisting of 2% agar. Briefly, plates were poured and allowed to solidify. Once completely solidified, wells were made. Then appropriate dilutions of control and plant extracts were loaded into each well according to increasing concentration. Solvent was used as control. Plates were then incubated for 24 hrs to check the inhibition of pigment production around the well.

3. Results

3.1Anti-swarming assay

All the extracts of the plant viz. Chloroform, P.ether, ethyl acetate, acetone, and methanol were tested for ability to inhibit swarming in *Proteus mirabilis.* DMSO was taken as solvent control. The results of this assay were interpreted in two ways: reduction in the number of the swarm zones or rings and reduction in the diameter of the swarm zones as compared to control. Out of the five extracts P.ether, chloroform, and methanol extracts showed maximum inhibition in the swarm zones as shown in Table 1. The number of the swarm rings was significantly reduced in the presence of these extracts as compared to control. Also the pattern of swarming motility was found to be different. The width of the individual swarm rings increased along with a concomitant decrease in the number of rings. Fig.2.A significant reduction in the diameter of swarm zone was observed in chloroform extract with only three zones and a maximum diameter of 28.33±0.81 as compared to solvent control 73.50±0.64.In the presence of P.ether extract the maximum diameter was 26.00±0.42 and that in methanol extract was 21.00±0.28.

3.2 Pigment inhibition assay

Pigment production is one of the phenomenon controlled by quorum sensing regulatory mechanisms. Therefore the extracts were then tested for ability to inhibit pigment production in *Serratia marcescens.* Out of the five extracts, ethyl acetate extract and methanol extracts were found to be the most potent in pigment inhibition assay at varying concentrations as shown in the graph in figure 4. Initially it was assumed that the pigment inhibition was found to be dose dependent. However a slight variation occurred in methanol extract which showed greater inhibition at 60 µl (23±0.26) than 100µl (21±0.2. In ethyl acetate extract treatment the pigment inhibition was observed to be dose dependent. As is evident from Table.3 the ethyl acetate extract exhibited maximum inhibition with zone diameter of 28 ±0.1 and methanol extract showed maximum inhibition with zone diameter of 21±0.2.

The pigment inhibition assay involves testing of the extract for only the pigment inhibition and no growth inhibition. Area of colorless turbid zone (Fig 5) indicates that the extract is allowing inhibition of quorum sensing mechanisms in the organism by only inhibiting the formation of pigment and not the growth of the organism.

4. Tables and Figures

Table 1: Effect of various extracts on swarming pattern in *Proteus mirabilis*

Sr. No.	Extract	Mean Number of Swarm Zones
1.	Control	6.66±1.52
2	P.ether	3.33±0.57
3.	Chloroform	3.66±1.15
4.	Ethyl acetate	4.66±0.57
5.	Acetone	4.66±0.57
6.	Methanol	4±1

(Values are presented as mean±SD for three experiments)

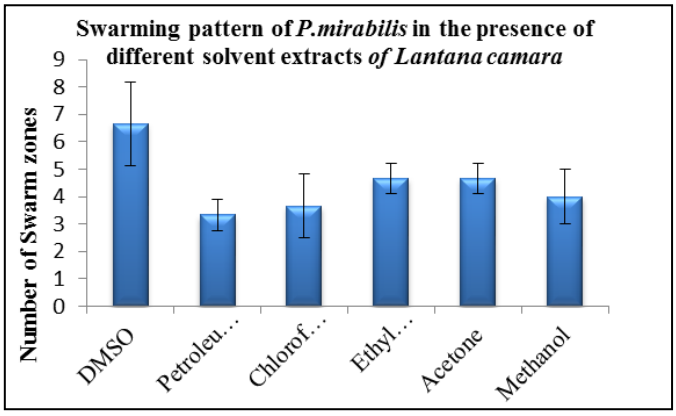

Fig 1: Graph showing reduction in number of swarm zones in the presence of different solvent extracts

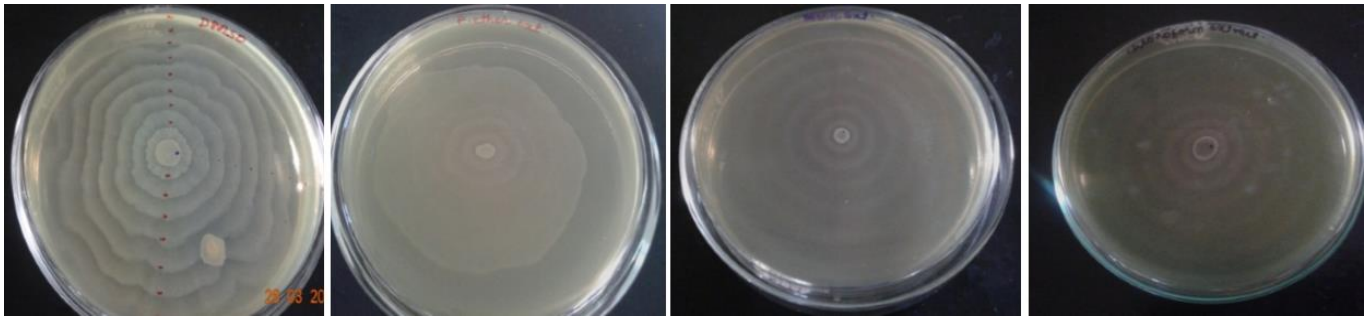

Plate 1 **Plate 2** **Plate 3** **Plate 4**

Fig 2: Pictures showing inhibition in swarming motility in *P.mirabilis*. Plate 1: DMSO control, Plate 2: P. ether extract, Plate 3: Methanol extract, Plate 4: Chloroform extract

Table 2: Anti swarming activity in *P.mirabilis* in the presence of different extracts of *Lantana camara*

Swarm zone inhibition(in mm)						
Number of Swarm zones	**Control**	**P.ether**	**Chloroform**	**Ethylacetate**	**Acetone**	**Methanol**
1	14.67±0.12	10.00±0.26	9.00±0.36	8.33±0.32	5.00±0.36	8.33±0.59
2	26.00±0.26	14.67±0.31	18.67±0.76	16.00±0.52	11.67±0.25	14.00±0.62
3	38.00±0.26	22.67±0.55	28.33±0.81	24.67±0.72	20.00±0.52	24.00±1.32
4	49.00±0.61	26.00±0.42		35.00±1.06	26.67±0.61	21.00±0.28
5	60.33±1.27			34.00±0.14	27.00±0.71	
6	64.50±0.07					
7	73.50±0.64					

(Values are represented as mean±SD for three experiments)

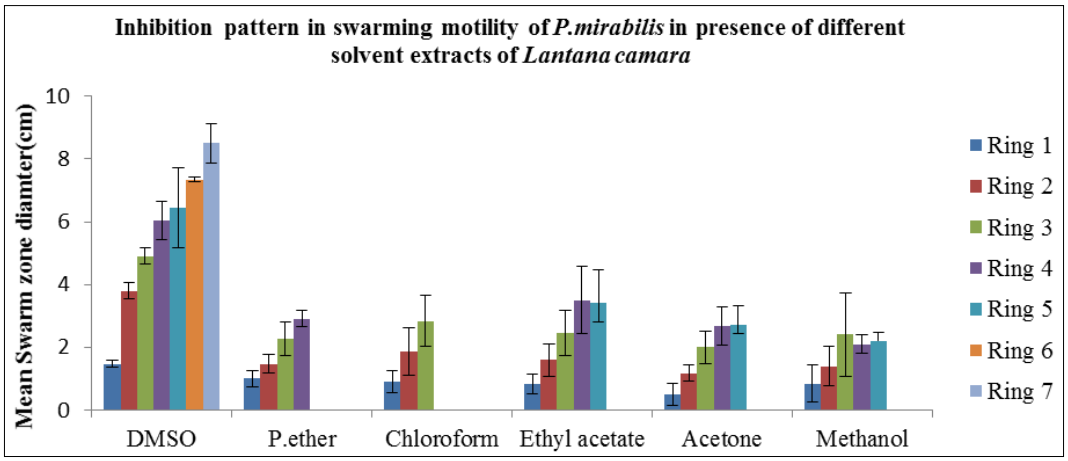

Fig 3: Graph showing significant reduction in the swarming pattern in the presence of extracts

Table 3: Anti-quorum sensing activity of extracts via pigment inhibition in *S.marcescens*

Extract conc. (µL)	**Zone of Pigment inhibition (mm)**	
	Ethyl acetate	Methanol
20	15.33±0.2	20.33±0.41
40	22.33±0.11	23.66±0.2
50	22±0.36	16±0.43
60	25.33±0.05	23±0.26
70	23±0.2	18.33±0.2
100	28±0.1	21±0.2

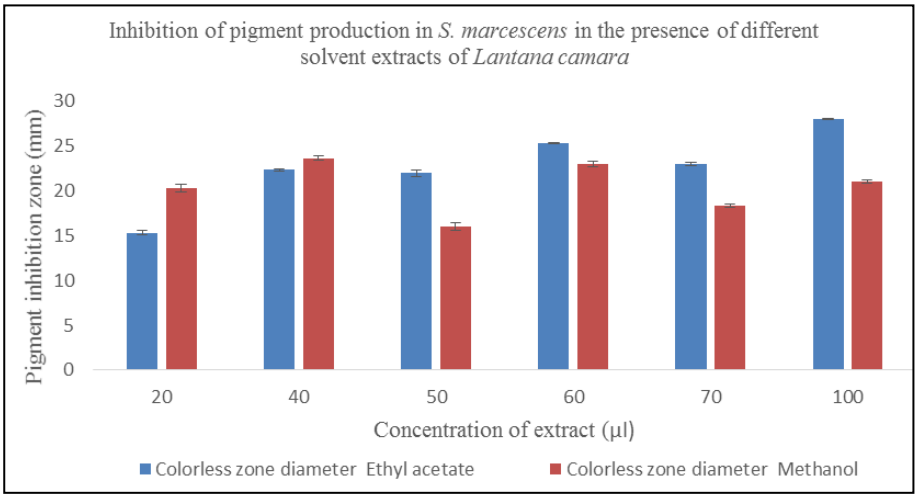

Fig 4: Graph showing reduction in pigment inhibition with increasing concentration

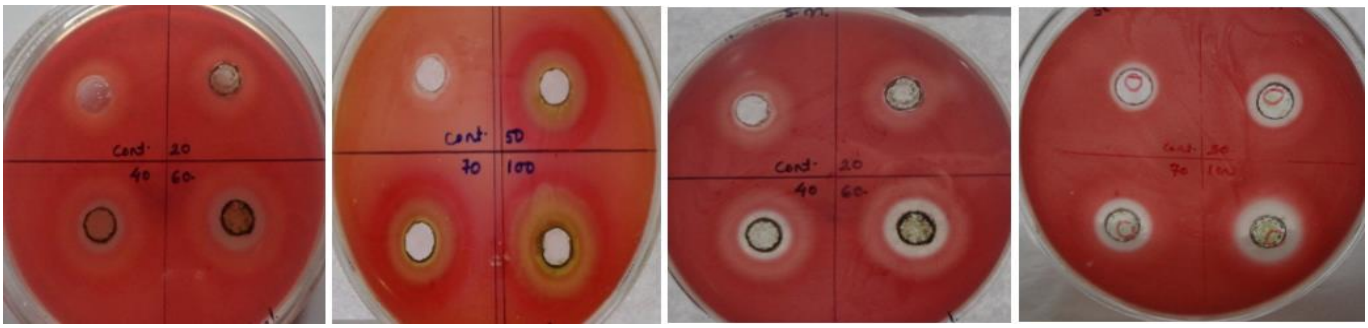

Plate a **Plate b** **Plate: c** **Plate: d**

Fig 5: Pictures showing pigment inhibition in *S. marcescens* in the presence of ethyl acetate and methanol extracts at different concentrations. Plate a: Methanol extract (20, 40, 60) Plate b: Methanol (50, 70,100) Plate c: Ethyl acetate (20,40,60) and Plate d: Ethyl acetate (50,70, 100)

5. Discussion

Lantana camara is regarded as a weed of National significance because of its invasive ability and toxic effects [14]. It is a serious threat to existing biodiversity as it does not allow any other species of plants to grow nearby. Therefore it is immensely important to control the spread of this weed. It is also harmful to other plant species because of its allelopathic potential [15]. Besides its harmful effects, this weed has got some useful features also like anti-inflammatory action and hepatoprotective effects [15]. However to our knowledge very few studies have been undertaken regarding the anti-quorum sensing potential of this weed. With the emerging problem of antibiotic resistance in the pathogenic bacteria, there is a need to find out an alternative strategy to tackle with these multidrug resistant organisms [16]. Therefore the present study focused on studying the quorum sensing inhibitory aspect of the plant. Most of the studies use *Chromobacterium violaceum* strain as an indicator for quorum sensing [17, 18]. But present study is novel in that it utilizes *Serratia marcescens* strain as an indicator of quorum sensing regulation. Significant anti-quorum sensing activity of the extracts of this plant was observed against two potent pathogens *P.mirabilis* and *S. marcescens*. Therefore this potentially toxic weed can be put in the field of research for finding out its alternative medicinal uses, which will ultimately help control the growth of this weed.

5. Conclusion

It is evident from the results of this study that various solvent extracts of *Lantana camara* showed activity against the tested pathogens. The P. ether and methanol extracts at concentration showed significant reduction in the swarming motility and number of swarm rings in the pathogen *P.mirabilis.* As well as the ethyl and methanol extracts showed maximum reduction in pigment production in *S. marcescens* without inhibiting the bacterial growth. Both these results are indicative that this weed has anti-quorum sensing potential. As swarming motility and pigment production, both these aspects are controlled by quorum sensing regulatory genes in these organisms, inhibition of these properties indicate that the quorum sensing genes are also getting inhibited. And further if the quorum sensing mechanisms are inhibited the pathogenicity of the organisms is in turn reduced. Therefore the present investigation clearly indicates the potential of using this toxic weed as a medicinal plant. Further investigations are needed to explore more such properties of this weed. There is a need to find out the active ingredient present in this plant by applying various purification techniques. *Lantana camara* is a species which has invaded most parts of world. There is a need to adaptively manage the species. Results of the study indicate that despite of being a toxic weed *Lantana camara* can be alternatively utilized as potent quorum sensing

inhibitory plant and can be put for evaluation for diversified applications.

6. References

1. Ashish Saraf, Sadaf Quereshi, Kavita Sharma, Noor Afshan Khan. Antimicrobial activity of *Lantana camara* L. Journal of Experimental Sciences. 2011; 2(10):50-54 ISSN: 2218- 1768
2. Indrayani Rawat, Surendra Sutar. Composting of Tropical Toxic Weed *Lantana camara* L. Biomass and Its Suitability for Agronomic Applications. Journal of Compost Science & Utilization. 2014; 22(3).
3. Sharma OP, *et al.* A review of the noxious plant *Lantana camara*. Toxicon.
4. Adiguzel A, Gulluce M, Sengul M, Ogutcu, Sahin F. Karaman I. Antimicrobial effects of *Ocimum basilicum* (*Labiatae*) Extract. Turk. J. Biol. 2005; 29:155-6.
5. Nagumanthri V, Rahiman S, Tantry BA, Nissankararao P, Kumar MP. In vitro antimicrobial activity of *Acacia nilotica*, *Ziziphus mauritiana*, *Bauhinia variegate* and *Lantana camara* against some clinical isolated strains. Iran Journal of Science and Technology. 2012; A2:213-217.
6. Sousa EO, Almeida TS, Rodrigues FFG, Campos AR, Lima SG, Costa JGM. *Lantana montevidensis Briq* improves the aminoglycoside activity against multiresistant *Escherichia coli* and *Staphylococcus aureus*. J Pharmacol. 2011; 43(2):180-182.
7. Mushatq S, Haider MS, Ali A, Javed S, Khokhar I, Mukhtar I. In vitro comparative screening of antibacterial and antifungal activities of some common weeds extracts. Pak J Weed Sci Res. 2012; 18(1):15-25.
8. Ganjewala D, Sam S, Khan KH. Biochemical compositions and antibacterial activities of *Lantana camara* plants with yellow, lavender, red and white flowers. Eur Asia J Bio Sci. 2009; 3(10):69-77.
9. Mohammadpour M, Sadeghi A, Fassihi A, Saghaei L, Movahedian A, Rostam M. Synthesis and antioxidant evaluation of some novel ortho-hydroxypyridine-4-one iron chelators. Res Pharm Sci. 2012; 7(3):171-179.
10. Milin K. Agrawal, Alka Varma and Surendra Goyal. Antibacterial screening of extract of the leaves of *Lantana camara*. Indian J.L. Sci. 2012; 1(2):97-99.
11. Lingayya Hiremath, Pushpa Agrawal and A.H. Manjunatha Reddy. Isolation, purification and characterization of proteins from *Lantana camara* Linn., antagonistic against *Colletotrichum falcatum* Went. International Journal of Engineering Sciences Research-IJESR. 2011; 2(5). ISSN: 2230-8504; e-ISSN-2230-8512.
12. Lavanya G, Brahmaprakash GP. Phytochemical screening and Antimicrobial activity of compounds from selected medicinal and aromatic plants. International Journal of Science and Nature. 2011; 2(2):287-291.
13. Venkata Narasimha Kadali, Tadi Ramesh, Sudhakara Rao Pola, Sandeep BV. Phytochemical analysis and antibacterial activity of Crossandra infundibuliformis Ethanolic stem extract. Int. J. of Research in Pharmacy and Pharmaceutical Sciences. 2016; 1(1):04-07. ISSN: 2455-698X.
14. Weed Management Guide. CRC for Australian Weed Management and Commonwealth Department of Environment and Heritage. ISBN 1-920932-08-9.
15. Rakesh Kumar, Rahul Katiyar, Surender Kumar, Tarun Kumar, Vijay Singh. *Lantana camara*: An alien weed, its impact on animal health and strategies to control. Journal of Experimental Biology and Agricultural Sciences. 2016; 4(3S). ISSN No. 2320-8694.
16. Abdelhakim Bouyahya, Nadia Dakka, Abdeslam Et-Touys, Jamal Abrini, Youssef Bakri1. Medicinal plant products targeting quorum sensing for combating bacterial infections. Asian Pacific Journal of Tropical Medicine. 2017; 10(8):729-743.
17. Ahmed A Zaki, Mona I Shaaban, Nadia E Hashish, Mohamed A Amer, Mohamed-Farid Lahloub. Assessment of anti-quorum sensing activity for some ornamental and medicinal plants native to Egypt. Sci pharm. 2013; 81:251-258.
18. Aparna Y, Lalitha Narayanan, Sarada J. Quorum quenching ability of dietary spice *Cinnamomumverum* on pathogenic bacteria. IJPSR. 2014; 5(12). E-ISSN: 0975-8232; P-ISSN: 2320-5148.