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Screening of phytoconstituents and antibacterial efficacy of leaves extracts of *Lantana camara*

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Abstract
In the present study an attempt was made to identify the presence of phytochemicals in the Aqueous, Ethanol, Methanol and Petroleum ether extracts of leaves of *Lantana camara*. The leaves extracts were studied qualitatively to ascertain the presence of phytochemicals such as Alkaloids, Phenols, Tannins, Saponins, Terpenoids, Flavonoids, Oils, Resins, Gums, Carbohydrates and Amino acids by adopting standard methods. The antibacterial activities of *L.camara* leaves extracts were tested against pathogenic bacteria *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Salmonella paratyphi A* by using the agar disc diffusion method. Among the solvents used, Methanolic extracts of *L. camara* demonstrated higher antibacterial activities against *E.coli* (2.63±.033 mm) in 500 µl concentration. Fractions were separated from the extracts of hexane and ethyl-acetate by using the column chromatography and thin layer chromatography for further analysis.The study provided referential information for the identification of the leaves extracts of *L. camara*.

Keywords: *Lantana camara*, extracts, phytochemical constituents, antibacterial activity, column chromatography and thin layer chromatography

1. Introduction
In ancient period, the plants and its by-products were used as medicine to treat various ailments and found to be one the effective medicine without side effects. Now a day, drugs are also required for the production of crops to meet out food requirement of people, because of increasing pests and high loss agriculture yield. Aquaculture is one of the other major sources of animal food productionand the major growing sectors of agriculture and it is dominated by fish culture both fresh and marine water environments for food production (Ravi *et al.*, 2007) [19]. Billions of people from 58 countries across the world depend on fish food *i.e.*, animal protein as their primary source (FAO, 2007) [6]. During cultivation, the food fishes are prone to get various diseases and eventually lead to great losses *i.e.*, both in terms of quantity and quality.

In order to alleviate the economic losses from these deadly pathogens it becomes indispensable to select and use fish strains which are resistant to various pathogens. Besides this, extracts of the medicinal plants are used to mitigate the ill effects of pathogens on the food fishes, however, only limited attempts have been made on food fishes to test their efficacies. The extracts of medicinal plants are in use both developing and developed countries of the world (Alanis *et al*., 2005) [1]. On the other hand, the allopathic medicines used against the pathogens cause problems such as allergy, side effects, etc on the fishes. Since the pathogens evolve rapidly with multidrug resistant properties, there is an urgent need to develop new and safer drugs from herbal products. The development of therapeutics from herbs and medicinal plants are promising products with low cost for treatment and greater accuracy without causing toxicity (Madhuri *et al.*, 2012) [15].

The bacteria that can infect fishes are Gram negative, including *Aeromonas hydrophila, Flavobacterium columnare, Vibrio* sp and *Pseudomonas* sp. Gram positive bacteria such as *Staphylococcus aureus* found in the fishes and other aquaculture-reared organisms are widely responsible for mortality worldwide (Lavilla-Pitogo *et al*., 1998 [14]; Chen *et al.*, 2000) [3].

In addition to this *Pseudomonas sp., Proteus vulgaris, Serratia sp., Staphylococcus sp, Salmonella sp.*, are some of the normal opportunistic pathogens affecting both fresh and marine aquaculture causing tissue infections, haemorrhages in the internal organs, virulence and immunity reduction in host. When the normal environmental conditions are changed, the disease often leads to mortality, thus causing heavy economic losses (Swain *et al.*, 2007) [23]. Among the known pathogenic bacteria *Klebsiella sp.* is found to be infectious to various organisms including fish.

Several factors influence the risk of microbiological contamination in aquaculture. *Salmonella* in freshwater fishes has been usually related to the faecal contamination of water from where fishes were harvested (Mhango *et al*., 2010) [17]. Fish feed is considered as a major source of *Salmonella* infection in commercial fish farms. The environmental factors such as water quality play a significant role in the incidence of *Salmonella* in fish and a great risk for those consuming fish caught in contaminated waters without sanitary control (Santiago *et al*., 2013) [21].

In India the leaves of this plant *L. camara* are boiled and the decoction is used as a medicine against cough. Further, it is used in the treatment of wounds and powdered leaves are applied to cuts, ulcers and swellings (Verme, 2006) [25]. The activities of *L. camera* leaves' such as antibacterial, antifungal, anti-inflammatory, analgesic, anti-tumour, and sedative have been reported by Jasim Uddin Chowdhury *et al*., 2007 [9]. Therefore, in the present investigation was made to find the antibacterial efficacy of the leaves extracts of *L. Camara.*

Materials and Methods

Plant Collection

L. camara leaves were collected from Musiri, Tiruchirappalli District, Tamil Nadu and identified with the help of Botanist, Research Department of Botany, St. Joseph College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

Extraction

The collected plant leaves were washed thoroughly in running tap water to remove debris and dust particles. The rinsed leaves in distilled water were shade dried and powdered in a mechanical grinder. The powered leaves were stored in an air tight container for further use. 50 g of powdered leaves was used for the extraction using four different solvents such as Aqueous, Ethanol, Methanol and Petroleum ether, respectively. The separated extracts were then filtered by using Whatman No.1 filter paper. The filtrates were transferred to airtight bottles, labelled and stored at 4ºC until further analysis was performed.

Preliminary phytochemical analysis

The phytochemical screening of the *L. Camara* extracts was performed, individually, to detect the presence of different classes of constituents such as alkaloids, phenols, flavonoids, terpenoids, steroids, saponins, oils, resins, carbohydrates, proteins, tannins, glycosides, gums and amino acids (Kumar *et al*., 2011 [13]; Hamid and Aiyelaagbe, 2011) [8]. All the samples were run in triplicates.

Column Chromatography

Column chromatography was used to get the fraction of leaves extracts (Davies Don *et al*., 2007) [4], respectively in aqueous, ethanol, methanol and petroleum ether. 2.0g of extract was subjected to column chromatography on Silica gel (60 -120) mesh packed and eluted with mixture of hexane and ethyl acetate in the ratio of 50:50 of increasing polarity to obtain fractions. The eluted fractions were collected in vials. The individual compounds were collected as column fractions and analysed further using with TLC chromatogram and the Rf values were determined.

Test for microorganisms

The test microorganisms used in this investigation were *S. aureus, E. coli, P. aeruginosa, K. pneumonia* and *S.paratyphi A.* Separate sterile nutrient agar slant were prepared and the bacterial strains were individually inoculated into separate slants under aseptic conditions and incubated at 37°C at 24 hrs.

Antibacterial Activity

Antibacterial activities of the aqueous, ethanol, methanol and petroleum ether extracts of *L. camara* leaves were determined by using the agar-well diffusion method (Naz and Bano, 2012) [18]. The filter paper disc was impregnated with leaf extraction on Muller Hinton Agar medium were prepared which test organisms swabbed were made in each of these plates using sterile cork borer. The test solution was prepared by dissolving leaves extracts of 100, 250 and 500 µl concentrations. Chloramphenicol (30 µg) was used as the positive control. All the plates were incubated at 37°C at 24 hrs. The zone of inhibition appearing around the discs were measured and recorded in millimeter. The experiments were repeated three times.

Statistical Analysis

All values were expressed as Mean ± SD values and the same are presented in Tables. All these analyses were done by using SPSS (Statistical Package for Social Sciences) program version 16.0 for windows.

Results and discussion

The preliminary phyto-chemical screening of leaf extracts of *L. camara* revealed that the presence of ten phytoconstituents in aqueous extracts such as Alkaloids, Phenols, Flavonoids, Steroids, Saponins, Proteins, Tannins, Resins, Gums and Aminoacids, and also the same phytoconstitutents were present in Ethanol extracts of *L. camara* including Terpenoids. The methanol extracts of *L. camara* shown eleven phytoconstituents like Alkaloids, Phenols, Flavonoids, Steroids, Terpenoids, Saponins, Proteins, Tannins, Oils, Resins and Aminoacids. But, the petroleum ether extracts of *L. camara* present revealed only five such as Alkaloids, Phenols, Flavonoids, Steroids and Resins. It indicated the presence of effective phyto-chemical compounds in the extract of methanol (Table.1). The presence of flavonoids, saponins and tannins might be a major active secondary metabolite in the leaf extract. Similarly, many researchers have also reported that the presence of flavonoids in the leaf extract of *L. camara* (Sathish Kumar and Maneemegalia, 2008 [22]; Kensa, 2011) [10].

The antibacterial activities of aqueous, ethanol, methanol and petroleum ether extracts of *L. camara* leaves were studied against five bacterial pathogens such as *S. aureus, E. coli, P. aeruginosa, K. pneumonia* and *S. paratyphi A* respectively. The calculated effective dose of 100, 250, 500 µl extract concentrations were used and simultaneously control were also maintained. The high inhibition activity were noticed in all the methanol, ethanol, aqueous and petroleum ether solvent extracts of *L. camara* against *E.coli* (2.63±.033), *K. pneumonia* (2.50±.057), *P. aeruginosa* (2.12±.003) and *E.coli* (1.73±.033) in 500 µl concentrations (Tables 2-5). Among these all the maximum 2.63±.033mm zone of inhibition was obtained against *E.coli*. The inhibition of growth of the bacterial strains while treating with the extract may be the presence of phyto chemical substances such as alkaloids, tannins, flavonoids and saponins etc. which might have produce a definite physiological actions against on pathogen

reproductions (Edeoga *et al*., 2005 [5], Khan *et al*., 2011) [11].
The systematic screening of plant extracts for antimicrobial activity is a continuous effort to find new antibacterial compounds (Mariajancyrani *et al*., 2014) [16]. Previous studies using extracts from Lantana species showed that they were able to inhibit the growth of gram positive and gram negative bacteria strains (Barreto *et al*., 2010) [2]. The presence of these tannins could be the reason why the leaves are used locally for treatment of wound, sores and skin diseases (Kokwaro, 2009) [12]. Similar findings have been reported by Ganjewala *et al*., (2009) [7]. Antimicrobial activity of Lantana species has been evaluated by several researchers (Salada *et al*., 2015 [20]; Barreto *et al*., 2010 [2]; Ganjewala *et al*., 2009) [7] and it has been found that Lantana species are good antimicrobial activity. This confirms the reason for the use of these plants in the treatment of microbial infections (Salada *et al*., 2015) [20]. Thus, it could be explained that higher concentrations of the extract above those used in the experiment would be required to inhibit the growth of the organisms. Among different solvents used in the study the best solvent of Methanol extracts. The methanol extract of *L. camara* inhibited the growth of E.coli (Udayaprakash *et al*., 2011) [24].

Table 1: Qualitative phytochemical screening of the leaves extracts of *L.camara* obtained by four different solvents.

S. No.	Chemical constituent	Aqueous	Ethanol	Methanol	Petroleum ether
1	Alkaloids	+	+	+	+
2	Phenols	+	+	+	+
3	Flavonoids	+	+	+	+
4	Steroids	+	+	+	+
5	Terpenoids	-	+	+	-
6	Saponins	+	+	+	-
7	Carbohydrates	-	-	-	-
8	Proteins	+	+	+	-
9	Tannins	+	+	+	-
10	Oils	-	-	+	-
11	Resins	+	+	+	+
12	Glycosides	-	-	-	-
13	Gums	+	-	-	-
14	Aminoacids	+	+	+	-

+Denotes presence of compound, -Denotes Absence of compound

Table 2: Zone of inhibitions recorded against five bacteria by using three different concentrations of Methanol leaves extracts of *L. camara*.

Organisms	Zone of inhibition (mm)Mean±S.D.			
	100µl	250 µl	500 µl	Control
E. coli	2.65±.025	2.01±.016	2.63±.033	5.74±.108
S. aureus	1.45±.000	1.55±.003	1.69±.000	4.59±.000
K. pneumonia	2.04±.006	1.56±.003	2.33±.003	5.53±.005
P. aeruginosa	1.64±.010	2.12±.003	1.64±.010	5.35±.006
S.paratyphi A	2.75±.003	2.13±.005	2.19±.000	4.68±.096

Table 3: Zone of inhibitions recorded against five bacteria by using three different concentrations of Ethanol leaves extracts of *L. camara*.

Organisms	Zone of inhibition (mm)Mean±S.D.			
	100 µl	250 µl	500 µl	Control
E. coli	0.45±.003	0.45±.003	0.90±.00	2.56±.033
S. aureus	1.13±.033	1.33±.033	1.40±.000	1.76±.033
K. pneumonia	1.16±.066	1.26±.033	2.50±.057	2.86±.033
P. aeruginosa	1.13±.033	1.16±.066	1.10±.000	2.33±.066
S. paratyphi A	1.26±.033	1.43±.033	1.40±.000	2.76±.033

Table 4: Zone of inhibitions recorded against five bacteria by using three different concentrations of Petroleum ether leaves extracts of *L. camara*.

Organisms	Zone of inhibition (mm)Mean±S.D.			
	100 µl	250 µl	500 µl	Control
E. coli	1.13±.033	1.26±.033	1.73±.033	2.04±.006
S. aureus	1.16±.066	1.33±.033	1.26±.033	1.55±.003
K. pneumonia	1.10±.000	1.16±.066	1.40±.000	1.76±.033
P. aeruginosa	0.45±.003	0.90±.00	1.13±.033	2.01±.016
S. paratyphi A	1.56±.003	1.69±.000	1.43±0.03	1.26±0.03

Table 5: Zone of inhibitions recorded against five bacteria by using three different concentrations of aqueous leaves extracts of *L. camara*.

Organisms	Zone of inhibition (mm)Mean±S.D.			
	100 µl	250 µl	500 µl	Control
E. coli	0.90±0.00	1.13±.033	1.45±.000	1.53±0.03
S. aureus	1.26±0.03	1.45±.000	1.70±0.05	2.13±.005
K. pneumonia	1.30±0.00	1.56±.003	1.64±.010	1.76±.033
P. aeruginosa	0.86±0.03	1.73±0.03	2.12±.003	2.04±.006

S. paratyphi A	1.33±0.03	1.64±.010	1.43±.033	2.43±0.03

Mean±S.D. values were obtained from three individual observations.

Table 6: The observed results of Thin Layer Chromatography and their Rf values of *L. camara* leaves extracts.

S. No	Sequential extracts	Solvent phase	Solvent run (cm)	Rf values	Colors of peaks
1	Ethanol	Ethyl acetate: Hexane	3.5	0.77	Green
2	Ethanol	Ethyl acetate: Hexane	-	-	-
3	Ethanol	Ethyl acetate: Hexane	-	-	-
4	Methanol	Ethyl acetate: Hexane	2.0	0.4	Yellow
5	Methanol	Ethyl acetate: Hexane	2.7	0.6	Green
6	Methanol	Ethyl acetate: Hexane	3.5	0.77	Thick green
7	Petroleum ether	Ethyl acetate: Hexane	3.2	0.71	Green
8	Petroleum ether	Ethyl acetate: Hexane	-	-	-
9	Petroleum ether	Ethyl acetate: Hexane	-	-	-
10	Aqueous	Ethyl acetate: Hexane	2.5	0.5	Yellow
11	Aqueous	Ethyl acetate: Hexane	3.4	0.75	Green
12	Aqueous	Ethyl acetate: Hexane	-	-	-

Conclusion

Phyto-chemicals present in the leaves of *L. camara*, indicate their potential as a source of novel medicines. The results of present study suggest that the leaves of *L. camara* has the antibacterial activities against different bacteria. It concludes that the *L. camera* leaves extract thus provide safe, easy, effective and practical solutions to every day ailments leaving behind no toxins and creating a clean, pleasant atmosphere. The overall results indicated that promising baseline information for the potential uses of methanol leaves extract of *L. camera* in the treatment of infectious organisms. This study provides antimicrobial activity of *L. camara* that may be used to control pathogenic bacteria and fungi of fishes.

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