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Antibacterial potential of flower extracts of a noxious weed *Lantana camara* L.

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Abstract
The present research work focuses on the bioactive potential of some flower extracts *viz*. benzene, chloroform and acetone extract of a noxious weed *Lantana camara* L. at various concentrations against pathogenic bacteria *Escherichia coli*, *Bacillus cereus*, *Alkaligenes faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Klebsiella pneumoniae*. The percentage yield (w/w) of these extracts was 2.6%, 0.28% and 6.58% respectively and these values correspond to 1.3g, 0.14g and 3.29g of the soluble principles in fractions respectively. Benzene extract was active against *Bacillus cereus* and *Klebsiella pneumoniae* only and did not inhibit the growth of other test bacteria. Chloroform extract was found to be active against 6 of the 8 tested bacteria and was found to be inactive against *Proteus vulgaris* and *Salmonella typhimurium* whereas acetone extract was inactive against *Proteus vulgaris* only. The inhibitory values were quite significant as compared to the control at all the concentrations used and the Critical Difference (CD) values at 5% level of significance were in the range of 0.10 to 0.18. The results suggest the antibacterial potential of the plant which needs to be tapped keeping in view the increased prevalence of the various harmful and infectious diseases caused by these pathogenic bacteria and the side effects related to the synthetic antibiotic therapy with a concern leading to drug resistance among bacteria.

Keywords: Bioactive, pathogenic bacteria, critical difference (CD), antibiotic, resistance

Introduction
Diseases are as old as mankind and have been a major concern since time immemorial. Throughout the globe widespread occurrence of resistance among microbes is quite common these days leading to various health issues as the common antibiotics becomes ineffective against them (Golkar *et al*., 2014) [9]. This is due to rapid changes in the genetic constitution of these microbes and especially those leading to infectious diseases worldwide. The best example is of *S. aureus* found to be multi-drug resistant (Styers, 2006) [17].

However, nature has provided complete storehouse of remedies to cure all ailments of mankind. Herbalism is the practice of healing with medicinal plants and medicinal plants are those plants that have healing properties. Plants possess healing properties due to the presence of anti-microbial substances called secondary metabolites which they develop *in vivo* for self-defense (Evans *et al*., 1986) [6]. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al*., 1999) [12]. Systematic screening of these may result in the discovery of novel effective compounds (Tomoko *et al*., 2002) [18].

Since our body rarely treat these herbal medicines as foreign and are easily accepted by body because of their association with natural biological entities that is proteins, lipids and carbohydrates etc., they do not produce side or after effects and can be used for therapeutic purposes (Chaudhary and Ranjit, 1994) [1]. Also the developmental costs of producing these orally active medicines are likely to be much lower than with bio-technological products or with most compounds produced to date from combinatorial chemistry and that the natural products have greater structural diversity than synthetic compounds, this diversity must be assessed efficiently and effectively (Harvey, 2000) [11].

Many compounds are present in plants which provide them flavour or taste or odours and are being used by humans in their diet and includes flavonoids, terpenoids etc and these have medicinal value (Cowan 1999; Dixon 2001; Kyaw *et al*., 2012) [2, 5, 13]. One such plant is like a common weed *Lantana camara* L. which possess such medicinal compounds aromatic as well as non aromatic in nature.

As such, an attempt has been made to check the antibacterial potential of flower extracts of a noxious weed *Lantana camara* L. with the objectives of extraction of various flower extracts using various solvents and calculation of yield, screening for antibacterial potential and calculation of critical difference values to check for significance of work.

Material and Methods

Fresh flowers of plant *Lantana camara* L. of family Verbenaceae were collected, washed, shade dried at room temperature, converted into powder form using mixer and mortar pestle. 50 g of the sample was loaded in the soxhlet apparatus for extraction. Soxhlet extraction was carried out using solvents with increasing order of polarity in sequential manner for extraction of active ingredients (Harborne, 1984) [10].

Solvent used	Boiling point
Benzene	80 °C
Chloroform	61 °C
Acetone	56 °C

Distillation

It was carried out to separate the residues and solvent from liquid solvent extract (Vogel, 1989) [20]. Thus the semisolid extracts were placed over a water bath for further drying. Percentage yield was calculated and the dried residues were preserved in sealed vials for further use. Dimethyl suphoxide (DMSO) was used for dissolving various extracts for testing antimicrobial efficacy,

Use and Maintenance of microbial cultures

In the present study, various pathogenic bacteria both gram-positive and gram-negative *viz*. *Escherichia coli*, *Bacillus cereus*, *Alkaligenes faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Klebsiella pneumonia* were selected as the test microorganisms. The cultures were maintained on Nutrient Agar slants and stored at 4 °C. Stock cultures were subcultured at regular intervals (Aneja 1996).

Testing of inhibitory efficacy of extracts

Agar wells were prepared in plates with the help of cork borer, 100mL (0.1 ml) of DMSO was added in wells and kept overnight in incubator to test diffusibility and it did well. Three different concentrations of extracts *viz*. 200, 100 and 50 mg/ml were prepared by dissolving dried residues in DMSO for testing. Cup (well) Assay Method (Agar Diffusion Method) was used, with one well as control having 0.1 ml (100mL) of pure solvent (DMSO).

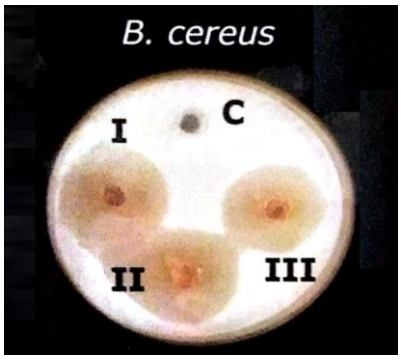
Microbial growth inhibition measurement

It was determined as diameter of inhibition zone around wells at an average of 4 measurements per well at 4 different directions. Zone of inhibition (ZOI) value was measured in mm. The results were analyzed based on statistical analysis – analysis of variance (ANOVA) test to omit the results if obtained due to errors and to check the test of significance and for that the critical difference (CD) values were also calculated at 5% level with respect to the control (Fischer, 1936) [7].

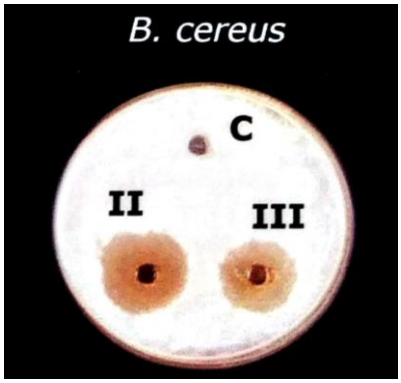
Results and Discussion

After collection and processing of flower sample, soxhlet extraction followed by distillation was carried out and percentage yield was calculated as given in table 1. Highest yield was recorded with acetone followed by benzene and chloroform with a total percentage yield of 9.46% (4.73g w/w).

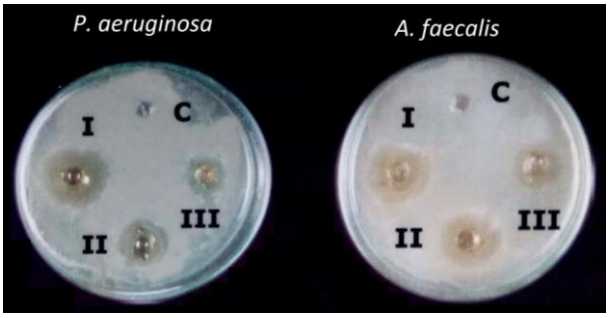
After extraction and calculation of percentage yield, antibacterial potential of the three extracts was assessed and the results obtained are given in table 2. In case of benzene extract it was found that the extract was active against two bacteria only out of the seven bacteria tested and also that the extract was effective at high concentration of 200 mg/ml against *Klebsiella pneumoniae* and lower concentrations were not able to restrict the growth of bacteria. Very good values of zone of inhibition were obtained against the bacteria *Bacillus cereus* which was 30, 30 and 28 mm at the respective concentrations which suggested the presence of active principles in this extract. Also, the values obtained were quite significant based on the CD values (Photograph 1). Chloroform extract was found to be inactive against only two bacteria and the values of ZOI varied from 27 mm being highest against *Bacillus cereus* and 09 mm being lowest against *Pseudomonas aeruginosa* (Photograph 2). Acetone extract was found to be ineffective only against *Proteus vulgaris* and was quite effective against the rest test bacteria was ZOI values ranging from 08 mm to 25 mm. the CD values were quite significant here too as in chloroform extract (Photograph 3, 4). The values suggested the presence of secondary metabolites effective against the test pathogenic bacteria.



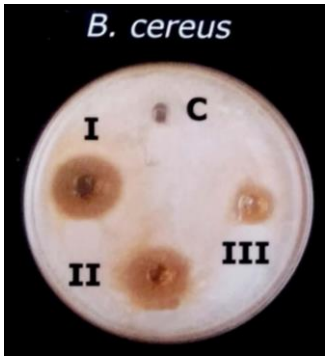
Photograph 1: Effect of benzene extract of *Lantana camara* L. Flower C- control, concentrations 1, 2, 3-200, 100 and 50 mg/ml.



Photograph 2: Effect of Chloroform extract of *Lantana camara* L. Flower C- control, concentrations 1, 2, 3-200, 100 and 50 mg/ml.



Photograph 3: Effect of Acetone extract of *Lantana camara* L. Flower C- control, concentrations 1, 2, 3-200, 100 and 50 mg/ml.



Photograph 4: Effect of Acetone extract of *Lantana camara* L. Flower C- control, concentrations 1, 2, 3-200, 100 and 50 mg/ml.

Darokar *et al*. (1998) [3] carried out antibacterial tests of floral petals of 51 plant species belonging to 26 families against a strain of *Escherichia coli* (CA 8000). Disc diffusion assay method was applied wherein discs were either cut from petals of large flowers or whole petals for small flowers were used as such. The plant *Lantana camara* was found to be ineffective against the selected strain of *Escherichia coli* with no zone of inhibition. Saleh *et al*. (1999) [15] in their study isolated the known triterpenoids that is lantic acid, camaric acid, camarinic acid and lantanilic acid from *Lantana camara* L. cultivated in Egypt. Antibacterial activity of lantic acid was carried out using bioautography assays for gram-positive and gram-negative bacteria. Lantic acid was found to possess strong antibacterial activity against *Escherichia coli* and *Bacillus cereus* for which the minimum inhibitory doses were 0.08 and 0.1 µg, respectively as compared to chloramphenicol for which the minimum inhibitory doses were 0.05 and 0.005 µg, respectively. The results indicated that lantic acid has broad spectrum antibacterial activity and may hold potential as a non-selective antimicrobial agent.

Davidson *et al*. (1999) [4] carried out a study on oleic acid which has been found to have fungistatic activity against a wide range of saprophytic yeasts and molds. Of the eleven fungi tested, best inhibition zones of 8.0 ± 0.2 mm and 9.0 ± 0.2 mm were observed respectively for *Aspergillus* species and *Candida albicans*. It was thus concluded that oleic acid can be used with combination of other compounds in routinely preservation of cakes and pastries easily prone to spoilage. Oleic acid is not highly reactive and easily miscible with lipophilic preparations and also no interference with preservative effect of other chemicals as well as easily metabolized *in vivo* in same manner as fatty acids found in food. Ross (1999) [14] prepared an exhaustive list of chemical constituents present in different parts along with the various pharmacological activities and clinical trials carried out using *Lantana camara* L. It was observed that apart from various constituents, oleic acid was also present in the aerial parts of the plant.

Table 1: Yield of various extracts from flowers of *Lantana camara* L.

S. N.	Extract	Flowers	
		Soluble Principles In Fractions (G)	Percentage Yield (W/W)
1	Benzene	1.30	2.60%
2	Chloroform	0.14	0.28%
3	Acetone	3.29	6.58%
Total		4.73	9.46%

Weight of Flower sample loaded – 50g

Table 2: Inhibitory activity of various flower extracts against bacteria

Extract		Zone Of Inhibition (Zoi) In Mm Against Bacteria								S.Em	C.D. (5%)
		E. c.	*B. c.*	*A. f.*	*P. a*	*P. v.*	*S. t.*	*S. d.*	*K. p.*		
Control		-	-	-	-	-	-	-	-	-	-
BEN.	I	-	30	NT	-	-	-	-	21	± 0.03	0.09
	II	-	30		-	-	-	-	-	± 0.02	0.07
	III	-	28		-	-	-	-	-	± 0.02	0.07
CHL.	I	NT	NT	NT	NT	NT	NT	NT	NT	-	-
	II	15	27	16	12	-	-	17	15	± 0.05	0.14
	III	10	24	12	09	-	-	12	11	± 0.05	0.16
ACT.	I	20	21	22	25	-	20	23	15	± 0.05	0.14
	II	16	19	19	20	-	19	17	14	± 0.05	0.16
	III	14	12	16	14	-	15	15	08	± 0.06	0.18

Note:

1) Roman numerals I, II, III stands for concentrations 200 mg/ml, 100 mg/ml, 50 mg/ml respectively.
2) "–" denotes No Zone of Inhibition, "NT" denotes Not Tested.
3) CONTROL – Pure DMSO – dimethyl sulphoxide.
4) S.Em denotes standard error of mean values.
5) C.D. 5% - critical difference at 5% level of significance.
6) BEN. (Benzene), CHL. (chloroform), ACT. (Acetone).
7) *E. c.* (*Escherichia coli*), *B. c.* (*Bacillus cereus*), *A. f.* (*Alkaligenes faecalis*), *P. a.* (*Pseudomonas aeruginosa*), *P. v.* (*Proteus vulgaris*), *S. t.* (*Salmonella typhimurium*), *S. d.* (*Shigella dysenteriae*) and *K. p.* (*Klebsiella pneumoniae*).

Various plants such as *Cassia auriculata, Calotropis gigantea, Clerodendrum infortunatum, Morinda tinctoria* and *Lantana camara* have been found to be effective against the gram-negative bacterium *Escherichia coli* (Valsaraj *et al*., 1997; Samy and Ignacimuthu, 2000) [19, 16]. Ghisalberti (2000) [8] reported the bioactivity of the various metabolites/compounds of different types present in *Lantana camara* L. Of the pentacyclic triterpenes, ursolate acetate (30 µg/disc) was found to be active against *Staphylococcus aureus* and *Salmonella typhi* with an average antimicrobial index of 0.95 and 0.55 whereas chloramphenicol for *Staphylococcus aureus* and tetracycline for *Salmonella typhi* had an index of 1.6 and 0.8 at the same concentration. A number of furanonaphthaquinones have been reported to possess antimicrobial activity against gram-positive bacteria and fungi, inhibitory effects on the Japanese encephalitis virus and pronounced activity against *Trypanosoma* parasites. Verbascoside (a phenylethanoid glycoside) was found to possess antibacterial activity against *Escherichia coli* and was active against Aujeszky virus. Verbascoside as well as isoverbascoside have been found to possess better antiviral activity against respiratory syncytial virus (RSV) *in vitro* than ribavirin (approved drug for RSV). Apart from the above many other antibacterial, antifungal, antiviral and antimutagenic compounds have been reported to be present in *Lantana camara* with quite good efficacy.

Literature is flooded with the weed nature of the plant *Lantana camara* L. Most of the scientific community across the globe is trying to control the growth of this noxious weed which has encroached upon agricultural and forest areas throughout the world. So far, not much breakthrough has been made in this direction. At this juncture it would be quite pertinent to look at the other side of the coin that is the beneficial role of *Lantana camara*. Since micro-organisms are increasingly showing resistance to the antibiotics used, plant extracts could be used as an alternative to the antibiotics which may provide useful pharmacological active compounds to which micro-organisms are not resistant. As we have found substantial inhibitory activity of the various extracts of *Lantana camara* against a wide spectrum of selected bacterial pathogens, it would be wise to go for further *in vivo* testing. If positive results are found during *in vivo* testing regarding efficacy coupled with least or no side effects, then various extracts of flower of *Lantana camara* L. would find use as therapeutic agents against many infectious diseases. This work provides much needed insight into the broad spectrum antimicrobial potential of the plant *Lantana camara* L.

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