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Assessment of Phytochemical Constituents and Antimicrobial Activity of *Lantana Camara* L.

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In present study the phytochemical constituents such as total phenol, total flavonoid contents and antibacterial activity against four gram negative and two gram positive isolates *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogens*, *Proteus vulgaris, Lactobacillus, S. aureus, Bacillus subtilis* and antifungal activity against four fungal strains *Penicillium, Aspergillus niger, T. mentagrophytes, Microsporum fulvum* of petroleum ether, diethyl ether, chloroform and acetone extract of leaves and flowers of *Lantana camara L* were evaluated. Maximum zone of inhibition was recorded in the presence of free flavanoid fraction of the plant extract against *Trichophyton mentagophytes* and *Microsporum fulvum* which was the most susceptible fungus for all the extracts tested. The extract also compared favourably with streptomycin which serves as a positive control. Minimum inhibitory concentration (MIC) was recorded for all bacteria and fungi in which highest MIC was of *B. subtilis* and *M. fulvum*. The UV-Vis and FTIR spectroscopic analysis also revealed the presence of different active groups and bonds. *L. camara* contains phytochemical compounds with antibacterial & antifungal activities. Moreover, the chloroform & acetone leaf & flower extracts of *L. camara* are active against pathogenic microorganisms.

Keywords: Antimicrobial activity, insecticidal, Larvicidal, *Lantana camara* and verbenaceae.

In ancient times, plants have been utilized as an important source of medicines as they are a reservoir of chemical agents with antimicrobial properties. Medicinal plants, which form the backbone of traditional medicine, in the last few decades, have been the subject for very intense pharmacological studies. *Lantana camara* L., a member of family Verbenaceae, is an evergreen, aromatic weed, native to tropical America, but it is now cultivated in many other parts of the world (Raghu *et al.,* 2004). Almost all parts of this plant have been used traditionally for treatment of several ailments due to their multiple biological activities such as antihelmintic (Patel *et al*., 2011), anti-leukemia (Badakhshan *et al*., 2009), larvicidal (Kumar & Maneemegalai 2008), antioxidant (Bhakta & Ganjewala, 2009), antibacterial, antiproliferative (Gomes-de Melo *et al.*, 2010), antiulcerogenic (Thamotharan *et al*., 2010), haemolytic (Kalita *et al*., 2011), antimutagenic activity, antihypertensive (Kaur *et al.*, 2010) and hepatoprotective activities (Abou El-Kassem *et al*., 2012). Most importantly, the flower extracts of *L. camara* are used in folk medicine for the management of several disorders including cancers, asthma, tumors, bilious fevers, chicken pox, eczema, measles, ulcers, swellings, high blood pressure, catarrhal infections, rheumatism, tetanus, malaria and abdominal viscera (Ghisalberti, 2000; Day *et al*., 2003). *Lantana camara* Linn is a flowering ornamental plant belonging to family verbenaceae. *Lantana camara* is also known as lantana, wild sage, Surinam tea plant, Spanish flag and West Indian lantana. *L. camara* is a well known

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medicinal plant in traditional medicinal system. *L. camara* contains lantadenes, the pentacyclic-triterpenes which is reported to possess a number of useful biological activities.

Recently, there is a revival of interest in the use of plants as natural remedy for medication of several health disorders due to the reason that they possess multiple biological activities, compatibility with system biology, potential physiological functions and protective role against several degenerative diseases (Suhaj, 2006; Tadhani *et al*., 2007, Espin *et al*., 2007; Wolfe *et al*., 2009; Lifschitz, 2012; Gawe, 2012). The extraction of antioxidant components from a plant material is a crucial step so as to accomplish further fractionation, isolation, purification and characterisation of biologically active compounds. A variety of extraction techniques such as orbital shaker, stirring, accelerated solvent extraction, microwave assisted extraction and supercritical fluid extraction etc., are in use to recover antioxidant and nutraceutical components from plant. All techniques have some advantages and disadvantages over others, but none of these is claimed to be perfect in all aspects. In view of the above-mentioned reports, this study was planned to explore the availability of potent antimicrobial agents of *L. camara* flowers using different solvents.

MATERIALS AND METHODS

Collection of Plant materials & test organisms

The plant materials used are leaves and flower of *Lantana camara* Linn. The samples were collected, at Turari campus, ITM University, Gwalior (MP) India.

The microbial cultures used in this study, were *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 8165), *Bacillus subtilis* (MTCC 1143), *Lactobacillus* sp*., Proteus vulgaris* (MTCC-1771), *S. aureus* (MTCC *3160), Enterobacter aerogens* (MTCC-7325) *Penicillium* sp*., Aspergillus niger* (MTCC 9652)*, T. mentagrophytes* (MTCC 7687) and *Microsporum fulvum* (MTCC 2837). These cultures were collected procured from Microbial Type Culture Collection Centre, Chandigarh. Bacterial isolates were inoculated into Nutrient broth as well as fungus and dermatophytes were inoculated into potato dextrose agar then incubated at 37^0 C for 24 hours and at 27^0 C for 3-5 days for fungi respectively. Penicillin antibiotic (1 mg/ml) was used as positive control for the test bacterial strains. Sterilized distilled water and Dimethyl sulfoxide (DMSO) were used as negative control.

Processing of plant materials

The leaves and flowers of *Lantana camara* L were dried in the hot air oven at 40ºC until all the water contents dried off. The dried samples were ground separately into fine powder. About 180 g each of the powdered specimen was soaked in the solvents of acetone, chloroform, petroleum ether, diethyl ether by using soxhlet extraction method for the extraction of pure solvent until the colour of solvent became white.

Antimicrobial sensitivity of crude extract of leaves & flowers of *Lantana camara* L. Antibacterial sensitivity test by well agar diffusion method

The crude extract was screened for antimicrobial activity using agar well diffusion method as described by Russell & Furr (1977). Muller Hinton Agar (MHA) was prepared in Mac. Carthney bottle and cooled to 45^0 C and inoculated with loopful culture. MHA medium was poured into a well-labelled Petri dish and allowed to set. Holes or wells were then bored into the set inoculated MHA using sterile cork borer. Using sterile syringe, extracts at concentration of 25 mg/ml were transferred aseptically into the wells bored. The plates were left on the bench for about one hour to allow proper diffusion of the extract into the MHA. This procedure was repeated for each bacterial isolate. The plates were incubated right way up at 37^0 C for 24 hours. After 24 hours the plates were observed for clear zone of inhibition, which indicate the relative susceptibility of the bacteria to the extract. The diameter of the zones of inhibition was measured and recorded in mm.

Antifungal sensitivity test by biomass reduction method

The crude extract was screened for antifungal activity using biomass reduction method. The medium used was Sabouraud's dextrose (SD) broth. 50 ml of each flask containing sterile molten SD broth was inoculated with a loopful culture with four different extracts of leaves, flower and also antifungal agent (cycloheximide) and kept in incubator shaker at 27^0 C for 48 hrs. After 48 hrs,

the broth culture was filtered through pre-weighted Whatman filter paper. Filter paper was dried inside an oven at 50ºC for overnight. After drying, the biomass was further weighted and percentage reduction in biomass was calculated using the following formula-

$$\text{Percentage reduction of biomass} = \frac{(w_t) - (w_0)}{(w_0)} X100$$

Where, w_0 is Initial wt. of filter paper and w_t final wt. of filter paper

Phytochemical analysis of crude extracts

Phytochemical screening of the *L. camara* was performed to detect the presence of different classes of constituents, such as alkaloids, steroids, flavanoids, reducing sugar and tannin (Naz, 2013).

Thin Layer chromatographic analysis

Each of the aforesaid two extracts was, to begin with, checked by Thin Layer Chromatography (TLC) on analytical plates over silica gel (TLC-grade; Merck India). For each extract of leaf and flower of *Lantana camara* one solvent system was used as developing systems as petroleum ether: chloroform: water (6:2:2) with boiling point 40-60ºC. In each case, the spots were visualized by exposure of plates to iodine vapour.

Instrumental analysis

UV-Vis spectrophotometer analysis

The extracts were examined under visible and UV light for proximate analysis. For UV-Vis spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1filter paper. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

Fourier transforms infrared spectrophotometer (FTIR) analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

RESULTS

Antibacterial activity of *L. camara* leaf extract

Figure 1 shows that *L. camara* leaf extracts have strong antibacterial activity. Extracts were prepared in petroleum ether, diethyl ether, chloroform and acetone. However only chloroform extract was found to be the most effective against all the bacteria except *Pseudomonas* and *E. coli*.

Antibacterial activity of *L. camara* flower extract

L. camara flower extracts also possess strong antimicrobial activity. The extracts were prepared in petroleum ether, di ethyl ether, chloroform and acetone. However only chloroform extract was found to be the most effective against all the bacteria except *E. coli* and *Pseudomonas.*

Percentage inhibition of fungal biomass using leaf extracts of *L. camara*

L. camara leaf extracts possess strong antifungal activity. The extracts were prepared in petroleum ether, diethyl ether, chloroform and acetone. Only acetone was found most effective against *Aspergillus niger* whereas in case of *Penicillium*, the chloroform showed the poor activity. Maximum effectiveness was also observed with acetone against *T. mentagrophytes*. The plant extract showed the more effective result as compared to cyclohexamide antibiotic.

Percentage inhibition of fungal biomass using flower extract of *L. camara*

L. camara flower extracts possess strong antifungal activity. The extracts were prepared in petroleum ether, diethylether, chloroform and acetone. However, only petroleum ether found to be the most effective against all the tested fungi**.**

Preliminary phytochemical screening

Qualitative test detected around five common secondary metabolites in four different extracts of leave and flower of *L. camara* such as alkaloid, and other minor compounds like soluble starch, flavanoid test, tannins, reducing sugar, alkaloid however could not be detected in flower .reducing sugar could not be detected in leaf.

TLC analysis of leaves and flower crude extracts of *L. Camara*

The TLC chromatogram of chloroform extracts of both flower and leaf is presented in Table 2. The spots were characterized by RF value and colour were visualised after spraying with iodine vapour. In case of chloroform extracts of leaves the RF value is 0.45 and the colour of the spot is yellow which shows the presence of flavanoid-glycoside compound.

Minimum Inhibitory Concentration (MIC) of the chloroform solvent extract

Minimum inhibitory concentration of the chloroform extract showed in Table 3 for the test bacterial cultures which reveal that the MIC value is 0.195 mg/ml for Gram negative bacterial culture except *E. coli* while no MIC was recorded against *S. aureus* & *E. coli*.

Minimum inhibitory concentration of the chloroform extract showed in Table 4 for the test fungus cultures. Table 4 reveals that the MIC value is 0.390 mg/ml for *A. niger* & *Penicillium* species.

UV-Vis & FTIR spectrum for the chloroform leaf extract of *L. camara*

Chloroform extracts of leaf of *L. camara* exhibited a characteristic band at 1084cm-^{1} indicating the presence of (OH group) and at 1389.34cm-^{1} (C=H group) and 1465.05cm^{-1} for (C-H stretching) and 17.34.99 cm^{-1} for (C=O carbonyl group) 2918.99cm^{-1} for (OH group) and 3553.78cm^{-1} for (OH group) (Figure 5a & b).

UV-Vis & FTIR spectrum for the chloroform flower extract of *L. camara*

The extracts of flower of *L. camara* exhibited a characteristic band at 1405.45 for (C-H stretching) and 1739.85 cm^{-1} for (C=O keto) and 2822.40 cm^{-1} for (C-H stretching) and 2853.34cm^{-1} for (C-H stretching) and 33402 cm^{-1} for (OH group) (Figure 6a & b).

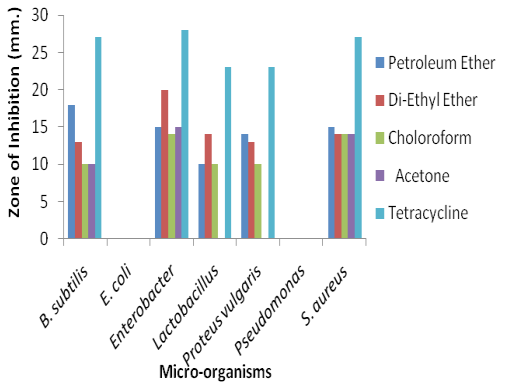

Fig. 1. Antibacterial activity of *L. camara* leaf extract against different bacteria

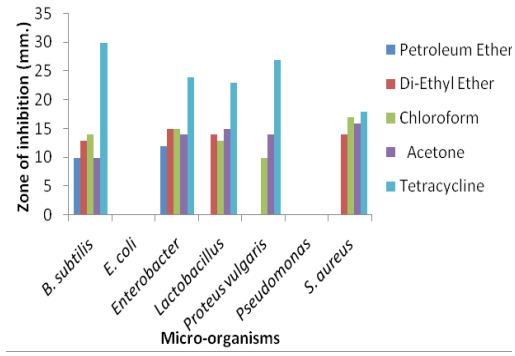

Fig. 2. Antibacterial activity of *L. camara* flower extract against different bacteria

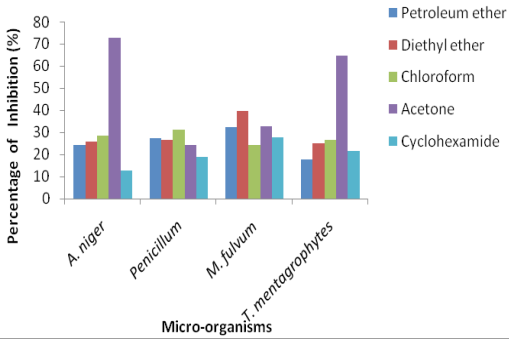

Fig. 3. Percentage inhibition of fungal biomass using leaf extract of *L. camara*

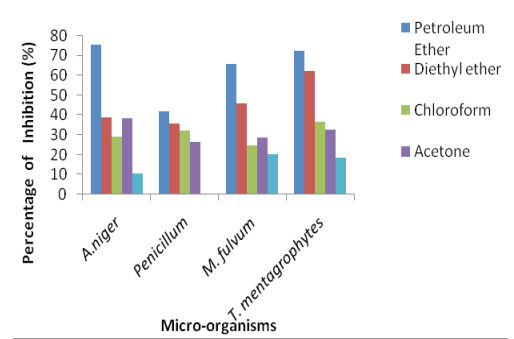

Fig. 4. Percentage inhibition of fungal biomass using flower extract of *L. camara*

DISCUSSION

In present work the biochemical composition of *L. camara* flower and leave extracts were studied. However, the biochemical as well as the chemical compositions of the concerned plants parts is often influenced by different origins, environmental and seasonal factors. Previously reported seasonal changes in the chemical composition of essential oils in more than seventy *L. camara* from different parts of the world. Very recently, Bhakta and Ganjewala (2009) reported the effects of leaf position on the level of secondary metabolites in *L. camara*. These studies have clearly suggested the geographical, developmental stage of the plant and or biochemical compositions in *L. camara*.

L. camara has been studied extensively for their antibacterial properties (Mello *et al*., 2005; Verma & Verma, 2006). *L. camara* possess many important biological activities. Lantadenes present in all *L. camara* is believed to be responsible for almost all the biological activities (Barre *et al*., 1997). In addition, other secondary metabolites such as alkaloids, terpenoids, and phenolics could be held partially responsible for some of these biological activities (Barre *et al*., 1997). However, constituents like 1, 8-cineole, sabinene, and caryophyllene and other minor constituents viz., E-nerolidol, bicyclogermacrene, and pinene identified in leaf essential oils were also found to be responsible for the biological activities of essential oils (Chowdhury *et al*., 2007; Sonibare & Effiong, 2008). Therefore, antibacterial activities of *L. camara* leaf and flower extracts reported here might be due to the presence of some of these chemical constituents particularly lantadenes and theveside in the extracts.

Table 1. Phytochemical analysis of extracts of Leaves and Flower of *Lantana camara*

Phytochemicals	Leaf				Flower			
	P. ether	Diethyl ether	Chloroform	Acetone	P. ether	Diethyl ether	Chloroform	Acetone
Alkaloid	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Reducing Sugar	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Flavanoid	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Soluble Starch	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
Tanin	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve

Table 2. Thin layer chromatogram of crude extracts of *L. Camara*

Extract	Solvent system	Result
Chloroform extracts of leaves of *L. camara*	Petroleum ether : chloroform : water - 6:2:2	0.45
Chloroform extracts of flower of *L. camara*	Petroleum ether : chloroform : water - 6:2:2	0.54

Table 3. Minimum inhibitory concentration (MIC) of chloroform extract of *L. camara* on test bacterial cultures

Bacteria isolates	MIC (mg/ml)	
	Leaf Extract	Flower extract
B. subtilis	3.125	3.125
S. aureus	0	0
E. coli	0	0
Enterobacter aerogens	0.195	0.195
Proteus vulgaris	0.195	0.195

Table 4. Minimum inhibitory concentration (MIC) of chloroform extract of *L. camara* on test bacterial cultures

Bacteria isolates	MIC (mg/ml)	
	Leaf Extract	Flower extract
Aspergillus niger	0.390	0.390
Penicillium species	0.390	0.390
M. fulvum	0.781	0.781
T. mentagrophytes	0.485	0.431

Though, the mechanism of the action of these chemical constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the type of solvent used. Perhaps it is one of the reasons behind differences in the antibacterial activities of the plants. These differences in the susceptibility of the test organisms to the different extracts might be due to the variation in the rate at which active ingredients penetrate their cell wall and cell membrane structures. In conclusion, *L. camara* plant with flower & leaf extracts have displayed variable antibacterial & antifungal activities most probably due to the differences in the biochemical and phytochemical composition.

The extracts of *L. camara* exerted a broader spectrum of inhibitory activity on Gram positive bacteria than Gram negative bacterial strains. However, *Staphylococcus aureus* was found to be resistant to the extract (Ganjewala *et al*., 2009). These extracts of leaves and flower also show activity against fungus and dermatophytes. Chloroform extract of leaves and flower shows highest activity against fungus and dermatophytes.

Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extracts and the one that predominates over the others (Enwuru *et al*., 2008). It may also be used to search for bioactive lead agents that could be used in the partial synthesis of some useful

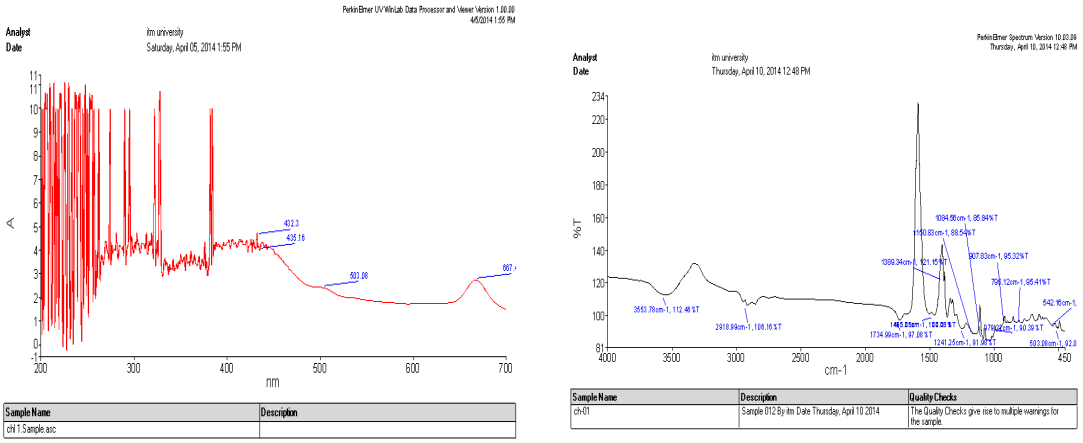

Fig. 5a & b. UV-Vis & FTIR spectrum for the chloroform leaf extract of *L. camara*

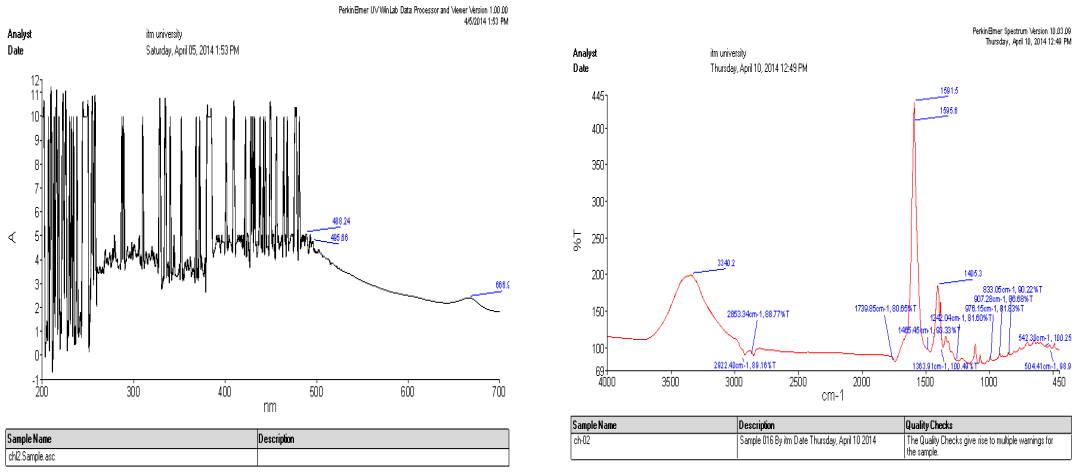

Fig. 6a & b. UV-Vis & FTIR spectrum for the chloroform flower extract of *L. Camara*

drugs. Phytochemical screening of *L. camara* leaf extracts revealed the presence of Alkaloid, Tannins ,Flavonoids and soluble starch as major active secondary metabolite while reducing sugar were absent but the extracts of flower of *L. camara* revels the presence of reducing sugar, tannins, flavanoid and soluble starch while Alkaloid was absent. This is similar to phytochemical study of aerial parts of *Lantana camara*. Many researchers have also shown the presence of flavonoids in the leaf and flower extract of *L. camara* (Sathish & Maneemegalai, 2008).

CONCLUSION

The ultimate conclusion of this study supports the traditional medicine use of different plant extracts in treating different infections caused by pathogenic bacteria & fungi in India. This study showed that *L. camara* contains phytochemical compounds with antibacterial & antifungal activities. Moreover, the chloroform & acetone leaf & flower extracts of *L. camara* are active against pathogenic tested microorganisms. It also suggests that a great attention should be paid to medicinal plants which are found to have plenty of pharmacological properties that could be sufficiently better when considering a natural food and feed additives to improve human and animal health.

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