Phytochemical screening, antioxidants and antimicrobial potential of *Lantana camara* in different solvents

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**ABSTRACT**

**Objective:** To evaluate the antioxidant activity, hydrogen peroxide radicals scavenging activity, reducing power, the total phenolic and flavonoids contents, and antimicrobial and antifungal activities of methanol, ethanol and water extracts of leaves of *Lantana camara* (*L. camara*).

**Methods:** Methanol, ethanol and water extracts were evaluated against four Gram positive and Gram negative bacterial isolates (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*) and two fungal strains (Aspergillus fumigatus and Aspergillus flavus). Methanol extract at different concentrations was tested for antioxidant potential and phytochemicals were determined by using spectrophotometric method.

**Results:** The total phenolic content was (40.859 ± 0.017) mg gallic acid/g in the leaves of *L. camara*, while the flavonoids was (53.112 ± 0.199) mg/g dry weight. Methanol leaf extract of *L. camara* showed maximum antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and was also effective against other bacterial strains as compared to ethanol and aqueous extracts of leaves. The methanol leaf extract of *L. camara* exhibited significant inhibition (71%) and (66%) against *Aspergillus fumigatus* and *Aspergillus flavus* respectively.

**Conclusions:** The methanol extract of the *L. camara* leaves is effective against selected bacterial and fungal strains. Its phytochemical contents have broad antimicrobial properties and the plant might be a novel source of antimicrobial drug.

**KEYWORDS**

MIC, Antioxidants, Phytochemicals, Antimicrobial, *Lantana camara*

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**1. Introduction**

Resistance to antimicrobial agents is a major global public health problem[1]. Infectious diseases account for approximately one–half of all death in tropics. Despite the progress made in the understanding of microorganisms and their control in industrialized nations, incidents due to drug resistant microorganisms and the emergence of unknown disease causing microbes, posed enormous public health concern[2]. *Streptococcus pyogenes*, *Staphylococcus aureus* and *Streptococcus pneumoniae*, the organisms that causes respiratory and cutaneous infection, as well as *Pseudomonas* and members of *Enterobacteriaceae*, causing diarrhea and urinary tract infections and sepsis, are now resistant to virtually all of the known antibiotics[3]. This resistance is largely due to indiscriminate use of antimicrobial drugs commonly used for the treatment of infectious diseases[4]. Furthermore some antibiotics have serious undesirable side effect which limit their application, so there is serious need to develop new antimicrobial agents that are very effective with minimal unwanted side effect. Plants represent a potential source of novel antibiotic prototypes[5].

*Lantana camara* (*L. camara*) is mainly used as a herbal medicine and in some areas as firewood and mulch[6]. It is also used for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high

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blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxyl of abdominal visceral[7]. Extracts from the leaves exhibit antimicrobial, insecticidal and nematicidal activity and also contain verbascoside, which possesses antimicrobial, immunosuppressive and antitumor activities[8]. Several previous reports have described antifungal, anti proliferative and antimicrobial activities of L. camara[9-13]. The present study aims to investigate the phytochemicals, free radical scavenging activities and antimicrobial potential of leaves extract of the commonly used medicinal plant L. camara.

2. Materials and methods

2.1. Plant materials

Fresh leaves of L. camara were collected from Margalla Hills of Quaid-i-Azam University Islamabad, Pakistan. The plants were identified by the National Herbarium, Department of Plant Sciences, Quaid-i-Azam University Islamabad.

2.2. Processing of the plant material

Plant leaves were washed thoroughly with distilled water. The leaves were dried under shade at room temperature. The dried leaves of L. camara were finely ground using electrical grinder and stored in air tight containers for further use. A total of 250 g of the pulverized plant material was extracted for 4 d in methanol, ethanol and sterile water[14]. The separated extracts were then filtered through Whatman’s No. 1 filter paper and the methanol and ethanol filtrate were then separately condensed to dryness using rotary evaporator. The thick extracted mass was then dried at room temperature. Dried extract was collected in an air tight container and stored at 4 °C for further analysis.

2.3. Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH), methanol, ferric chloride, chloroform, H2SO4, HCl, benzene, NH4OH, potassium ferrocyanide, sodium chloride, ethanol, Folin-Ciocalteau, Na2CO3, gallic acid, hydrogen peroxide and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.4. DPPH radical scavenging activity

The antioxidant activity was assessed in DPPH radical scavenging system using gallic acid and ascorbic acid as a positive control, and the decrease in absorbance was determined at 517 nm in a spectrophotometer (HITACHI Model: U-1100 573×415). Absorbance of the hydrogen peroxide activity was recorded at 570 nm in a spectrophotometer (HITACHI Model: U-1100 573×415). Hydrogen peroxide scavenging activity (in triplicate) was calculated by following equation:

Hydrogen peroxide scavenging activity (%) = 1− A1/A0×100

Where A0 was the absorbance of the control and A1 was the absorbance of the sample.

2.5. Hydrogen peroxide–scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method described by Ruch et al[19]. Absorbance of the hydrogen peroxide activity was recorded at 230 nm in a spectrophotometer (HITACHI Model: U-1100 573×415). Hydrogen per oxide scavenging ability (in triplicate) was calculated by following equation:

Hydrogen peroxide scavenging activity (%) = 1− A1/A0×100

Where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample[18].

2.6. Reducing power assay

The reducing powers of the extracts were determined according to the method described by Chung et al[20]. The extract which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferrie ferrous complex that has an absorption maximum at 700 nm in a spectrophotometer (HITACHI Model: U-1100 573×415). Potassium ferricyanide+Ferric chloride potassium Antioxidant Ferrocyanide+Ferrous chloride

2.7. Preliminary phytochemical screening

Phytochemical screening of the L. camara was performed to detect the presence of different classes of constituents, such as alkaloids, phenolics, flavonoids, tannin, saponins, terpenes, phlobatannins and coumarins[21,22].

2.8. Quantitative analysis of phytochemicals

2.8.1. Total phenolic contents (TPC)

TPC of L. camara were determined by the Folin-Ciocalteau colorimetric method using gallic acid as a standard, and the absorbance was measured at 765 nm in a spectrophotometer (HITACHI Model: U-1100 573×415). Results were expressed as gallic acid equivalent (GAE) mg/g of dried extract. Data for plant extract was recorded in triplicate[15,23].

2.8.2. Determination of the total flavonoids content

Total flavonoids content was determined according to the protocol of Sakaknaka et al[24]. The absorbance was measured immediately at 510 nm in a spectrophotometer (HITACHI Model: U-1100 573×415). Flavonoids were estimated as mg/g of dried fraction. All samples were run in triplicate.

2.8.3. Determination of total tannins

Total flavonoids content was determined according to the protocol of Sakaknaka et al[24]. The absorbance was measured immediately at 510 nm in a spectrophotometer (HITACHI Model: U-1100 573×415). Flavonoids were estimated as mg/g of dried fraction. All samples were run in triplicate.

2.8.4. Determination of total tannins

Tannin content was determined by using Van–Burden and Robinson WC method[25]. Absorbance at 120 nm was recorded in a spectrophotometer (HITACHI Model: U-1100 573×415) within 10 min and tannins contents were expressed as mg/g of the dried fraction.
2.9. Test microorganism

The test microorganisms used in this investigation included bacteria *Staphylococcus aureus* ATCC 6538 (*S. aureus*), *Pseudomonas aeruginosa* ATCC 7221 (*P. aeruginosa*), Klebsiella pneumoniae (*K. pneumoniae*) and *Bacillus subtilis* ATCC 6059 (*B. subtilis*); and fungi Aspergillus fumigatus (*A. fumigatus*) and Aspergillus flavus (*A. flavus*). The bacterial isolates were first sub cultured in a nutrient broth (SIGMA) and incubated at 37 °C for 18 h, while the fungal isolates were sub cultured on a Sabouraud dextrose agar (MERCK) for 72 h at 25 °C.

2.10. Positive and negative control

Penicillin (1 mg/mL) was used as positive control for the test bacterial strains. Sterilized distilled water and dimethyl sulfoxide were used as negative control.

2.11. Antibacterial activity

Antibacterial activity of the methanol, ethanol and aqueous extract of *L. camara* leaf extract was determined by using the agar–well diffusion method. The bacterial strains were first cultured in a nutrient broth for 18 h prior to use and standardized to 0.5 McFarland standards (10⁶ CFU/mL).

Nutrient agar medium was prepared by adding nutrient agar 2.3 g in 100 mL of distilled water; pH was adjusted at 7.0 and was autoclaved. It was allowed to cool up to 45 °C. Petri plates were prepared by pouring 75 mL of seeded nutrient agar and allowed to solidify. Wells were bored into the agar using a sterile 6 mm diameter cork borer. Approximately 100 µL of the crude extract at 12 mg/mL were added into the wells, allowed to stand at room temperature for about 2 h and incubated at 37 °C. Controls were set in parallel in which case the respective solvents were used to fill the well. The plates were observed for zones of inhibition after 24 h. The effects were compared with those of penicillin at a concentration of 1 mg/mL.

2.12. Determination of relative percentage inhibition

The relative percentage inhibition of the test plant extract with respect to positive control was calculated by using the following formula:

\[\% \text{ inhibition} = 100 \times \frac{100 - X}{Y}\]

Where, X is total area of inhibition of the test extract, Y is total area of inhibition of the solvent, and Z represents total area of inhibition of the standard drug. The total area of the inhibition was calculated by using area = πr²; where, r=radius of zone of inhibition.

2.13. Assay for antifungal activity

The agar tube dilution method was used for the determination of antifungal activity of *L. camara* leaves extract in different solvents. The samples were prepared by dissolving 12 mg extract in 1 mL of dimethyl sulfoxide. Culture media was prepared by dissolving 6.5 g of Sabouraud dextrose agar (MERCK) per 100 mL distilled water (pH 5.6). The 10 mL of Sabouraud dextrose agar was dispensed in screw capped tubes or cotton plugged test tubes and was autoclaved at 121 °C for 21 min. Tubes were allowed to cool at 50 °C and Sabouraud dextrose agar was loaded with 67 µL of extract pipetted from the stock solution. The tubes containing the media were then allowed to solidify in slanting position at room temperature. Three slants of the extract sample were prepared for fungus species. The tubes containing solidified media and plant extract were inoculated with 4 mm diameter piece of inoculum, taken from seven days old culture of fungus. One test tube of each extract was prepared, used as positive control. Slants without extract were used as negative control. The test tubes were incubated at 28 °C for 7 d. Cultures were examined twice weekly during the incubation. Reading was taken by measuring the linear length (mm) of fungus in slant and growth inhibition was calculated with reference to negative control.

Percentage inhibition of fungal growth for each concentration of compound was determined as

\[\% \text{ inhibition of fungal growth} = 100 \times \frac{\text{Linear growth in test}}{\text{Linear growth in control}}\]

3. Results

3.1. DPPH radical–scavanging activity

Results shown in Table 1 indicates the relative activities against ascorbic acid and butylated hydroxytoluene (BHT). The activity of 0.8 mg/mL BHT was the highest followed by ascorbic acid and *L. camara* leaf extracts respectively. Leaves extracts of the plant quenched DPPH in a dose dependent manner; [\(R^2=0.9837\)] for *L. camara* against the control (ascorbic acid and BHT).

| Plant and drugs | % inhibition±SD | Logarithm equation | IC₅₀ (mg/mL) |
|----------------|-----------------|--------------------|--------------|
| *L. camara*    | 0.2             | y=19.87ln(x)+43.994 | ≥0.2         |
|                | 0.4             | R²=0.9837           |              |
|                | 0.6             | y=64.83ln(x)+40.44  |              |
|                | 0.8             | 73.13±0.42          |              |
| Ascorbic acid  | 0.2             | y=15.853ln(x)+61.372| ≤0.2         |
|                | 0.4             | R²=0.9999           |              |
|                | 0.6             | y=78.63±0.40        |              |
|                | 0.8             | 83.47±0.46          |              |
| Gallic acid    | 0.2             | y=14.029ln(x)+69.801| ≤0.2         |
|                | 0.4             | R²=0.9795           |              |
|                | 0.6             | y=78.80±0.40        |              |
|                | 0.8             | 83.93±0.38          |              |

3.2. Hydroxyl radical–scavanging activity

The scavenging abilities of selected plant leaves fraction extracts on hydroxyl radical inhibition by the 2-deoxyribose
oxidation method are shown in Table 2. The results are indicated as the inhibition rate. Leaf extract of L. camara showed good hydroxyl radical scavenging activities (45.8%–73.3%) at a concentration of 0.2–0.8 mg/mL in the reaction mixture. Leaf extract showing hydroxyl radical-scavenging activity was increased with increasing concentration of the extract sample. Leaf fraction of L. camara had higher activity but lower than that of ascorbic acid and BHT (Table 2).

### Table 2
Analysis of hydroxyl radical scavenging activity of leaf extracts of L. camara.

| Plant and drugs (mg/mL) | % inhibition±SD | Logarithm equation | IC₅₀ (mg/mL) |
|-------------------------|-----------------|--------------------|-------------|
| L. camara               |                 | y=16.35ln(x)+54.281 | 0.2         |
| Ascorbic acid           | 0.2             | y=18.22ln(x)+67.989  | 0.2         |
| Gallic acid             | 0.4             | y=12.58ln(x)+78.234  | 0.2         |

#### 3.3. Reducing power

The reducing power of the selected plant leaf extract increased with increase in concentration. At 0.8 mg/mL concentration, the leaves extracts of L. camara showed absorbance of 0.888 (Table 3). Thus, the plant leaves extracts exhibited a lower reducing ability than the standard. Also, the reducing ability was found to be the concentration dependent. With increasing concentration, the reducing ability of all the leaves extracts was found to increase. In this case, at 0.8 mg/mL, the reducing power was found to be at maximum value.

### Table 3
Analysis of reducing power ability of leaf extracts of L. camara.

| Plant and drugs (mg/mL) | % inhibition±SD | Logarithm equation | IC₅₀ (mg/mL) |
|-------------------------|-----------------|--------------------|-------------|
| L. camara               | 0.2             | y=0.1053ln(x)+0.7014 | 0.2         |
| Ascorbic acid           | 0.4             | y=0.1485ln(x)+0.7812  | 0.2         |
| Gallic acid             | 0.2             | y=0.3147ln(x)+0.7722  | 0.2         |

#### 3.4. Qualitative analysis of phytochemicals

Results of phytochemical screening presented in Table 4 reveals moderate concentration of alkaloids, phenolics, flavonoids, tannin, saponin, terpenoids, phlobetin and coumarine.

### Table 4
Qualitative phytochemical analysis of leaf extracts of L. camara.

| Constituents | Presence |
|--------------|----------|
| Alkaloids    | Mayer’s test +++ |
| Phenolic     | ++        |
| Flavonoids   | +++       |
| Tannin       | ++        |
| Saponin      | ++        |
| Terpenoids   | –         |
| Phlobetin    | –         |
| Coumarin     | –         |

#### 3.5. Quantitative analysis of phytochemicals

##### 3.5.1. TPC

TPC was determined in comparison with standard gallic acid and the results were expressed in terms of mg GAE/g dry sample of plant. TPC values for L. camara was (40.859±0.017) as mg GAE/g dry sample.

##### 3.5.2. Total flavonoid content

Total flavonoid content was determined in comparison with standard rutin and the results expressed in terms of mg RU/g of dry sample of plant. Total flavonoid content values for L. camara (53.112±0.199) was mg RU/g dry sample.

##### 3.5.3. Estimation of tannin

The results of total tannin were presented in Table 5. The amount of tannin for L. camara (0.860±0.038) mg/g of the dried fraction in dry sample.

### 3.6. Antibacterial activity

The antibacterial activity of methanol, ethanol crude extracts and water extracts of leaves of L. camara was investigated against two gram positive (B. subtilis and S. aureus) and two gram negative (P. aeruginosa and K. pneumoniae) bacterial strains (Figure 1). The methanol and ethanol extracts of L. camara exhibited the maximum zone of inhibition 21.7 mm and 19.7 mm against gram positive strain S. aureus and 20 mm and 17.7 mm zone of inhibition against gram negative strain P. aeruginosa and showed minimum antibacterial activity against B. subtilis and K. pneumoniae. The results revealed that aqueous leaf extracts of L. camara has minimum antibacterial activity against four bacterial strains as compared to methanol and ethanol solvents.
ethanol and water extracts of leaves tissue of *L. camara* showed maximum relative percentage inhibition (63%) against *S. aureus*, while water extract showed maximum inhibition of 60% against *S. aureus* (Figure 2).

3.8. Minimum inhibitory concentration (MIC)

Results showed in Table 5 revealed the MIC of methanol, ethanol and water extracts from leaves tissue of *L. camara*. Methanol leaf extract of *L. camara* ranged between 5–8 mg/mL, ethanol 6.5–12 mg/mL and water extract was recorded 6 mg/mL. MIC range of penicillin (standard drug) was recorded between 0.05–0.25 against four bacterial strains.

| Test bacteria | Methanol (mg/mL) | Ethanol (mg/mL) | Water (mg/mL) | Penicillin (mg/mL) |
|---------------|------------------|-----------------|---------------|-------------------|
| *S. aureus*   | 5.0              | 6.5             | 8.0           | 0.05              |
| *B. subtilis* | 8.0              | 10.0            | nd            | 0.20              |
| *P. aeruginosa* | 5.0            | 8.0             | 10.0          | 0.05              |
| *K. pneumoniae* | 8.0              | 12.0            | nd            | 0.25              |

4. Discussion

From the present study the maximum radical scavenging and antimicrobial activities of methanol extract of leaves of *L. camara* was investigated.

The phytochemical compositions of *L. camara* reported previously[29]. With regards to reducing power, higher reducing activities can be attributed to higher amounts of polyphenolics and the reducing capacity of a compound may reflect its antioxidant potential[30]. It has been reported that the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[31].

*L. camara* has been studied extensively for their antibacterial properties. *L. camara* possess many important biological activities *viz.*, antipyretic, antimicrobial, antiinflammatory, antimicrobial, fungicidal, insecticidal, nematocidal and others[32]. Lantadene which present in all medicinal plants have been documented[33].

Therefore, antibacterial activities of *L. camara* leaf and flower extracts might be due to the presence of some of these chemical constituents particularly lantadene and theveside in the extracts. The presence of polyphenolics, anthocyanins and proanthocyanidins in *L. camara* leaves which could also be responsible for the antibacterial properties of the *L. camara* have been documented[33].

3.9. Antifungal activity

Antifungal activity of methanol and water leaf extracts of *L. camara* was investigated against two fungal strains *viz.* *A. fumigatus* and *A. flavus* (Table 6). Methanol leaf extract of *L. camara* showed 71.4% and 66.4%, while water extract showed 61.5% and 57.8% inhibition against *A. fumigatus* and *A. flavus* respectively (Table 6).

Table 6

| Test fungi | Linear growth (cm) | Percentage inhibition (%) |
|------------|-------------------|---------------------------|
|            | Methanol | Water | Methanol | Water |
| *A. fumigatus* | 2.8     | 4.0    | 71.4     | 61.5  |
| Control    | 9.8     | 10.3   | –        | –     |
| *A. flavus* | 3.33    | 4.3    | 66.4     | 57.8  |
| Control    | 9.9     | 10.2   | –        | –     |
have been reported\[35\]. Methanolic extracts of root and shoots of the herb *Heracleum candicans* wall (Apiaceae), showed antifungal effect against *Pythium* and *Aspergillus* species\[36\]. The value of medicinal plants lies in phytochemical constituents that cause definite pharmacological action on the human body\[37\]. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavonoids, phenolics and polyphenols, tannins, terpenoids, sesquiterpenes *et al.*, are effective antimicrobial substances against a wide range of microorganisms\[37\].

The study showed that the methanol leaf extract of *L. camara* had more antimicrobial potential than ethanol and water extract. The antimicrobial activity of *L. camara* may be attributed to the various phytochemical constituents present in the crude extract. The purified compounds may have even more effectiveness with respect to inhibition of bacterial and fungal strains. The work carried was a basic approach to find out the antimicrobial activity of *L. camara* in different solvents. We therefore suggest more work should be required to isolate, identify and characterized the active components and also possibly their mechanism of biological action and thus, find its way into the arsenal of antimicrobial drugs.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**Comments**

**Background**

Plants have been used to treat a variety of ailments and the introduction of orthodox medicine did not affect their use. *L. camara* belongs to Verbenaceae family and is reported to be used in traditional medicine system for the treatment of itches, ulcers, cuts, swellings, eczema, bilious fever and cataract. The literature reported that different parts of the plants are used in the treatment of cold, whooping cough, bronchitis, chicken pox and eye injuries.

**Research frontiers**

Research was performed on leaves of *L. camara* in different solvents to determine the most efficient solvent system against microbial infections. The leaf extract in three different solvents have been studied against both Gram negative and Gram positive bacterial strains and also against two fungal species (*A. fumigatus* and *A. flavus*). Antioxidants and phytochemical screening of *L. camara* leaves have also been studied.

**Related reports**

Our results of efficient methanol extract are in agreement with the previous work which showed that in plants most of the compounds having antimicrobial potential (Verma *et al.*, 2006). Methanol leaf extract is more effective against pathogenic bacterial strains than ethanol or water extracts (Naz *et al.*, 2011). Previous studies using extracts from *Lantana* species showed that they were able to inhibit the growth of Gram–positive bacteria strains (Júnior *et al.*, 2005).

**Innovations & breakthroughs**

Data regarding potential of leaf extract of *L. camara* against Gram positive and Gram negative bacterial strains, against fungal *Aspergillus* species in different solvents and also about antioxidants and phytochemicals is scarce. This study has shown that methanol leaf extract had a significant higher antimicrobial potential than ethanol and water leaf extracts.

**Applications**

The present study indicated that the antibacterial and antifungal activities vary with the different solvents of plant leaf material used. *L. camara* was effective even at low concentration against bacterial and fungal pathogens. The solvent extracted leaves extracts of *L. camara* contains some highly potential phytochemicals, which could be characterized and effective against bacterial and fungal infections.

**Peer review**

This is a good study in which the authors evaluated the antimicrobial potential of *L. camara* leaf extracts in different solvents and also analyzed the antioxidant and phytochemicals in leaves. The results are interesting and suggest the presence of potential phytochemicals, antioxidants and active compounds which could be identified and thus, find its way into the arsenal of antimicrobial drugs.

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