Phytochemical Analysis and Biological Activity of Lantana Camara from Iraq

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Abstract
Today, scientists all over the world are studying medicinal plants. Since people have been using medicinal plants for a long time, they are an important and cheap source of medicines. Due to a lack of technology, most ancient medicines came from plants, and it has been shown that using plants as medicine works. *Lantana camara* is a member of the Verbenaceae family. The present study was carried out on the phytochemical investigation and antimicrobial activities of the extracts of *Lantana camara* from Iraq. The aerial part of the *Lantana camara* herb was sequentially extracted with organic solvents: n-Hexane, ethanol, and methanol. Chemical compositions were analyzed by gas chromatography-mass spectrometry (GC/MS). *L. camara* has been found to contain several important phytochemicals, such as triterpenoids, flavonoids, alkaloids, saponins, steroids, and tannins. It is also known as a plant that makes essential oil, which is sold on the market as *Lantana oils*. Researchers need a full report on the economic and medical benefits of *L. camara* as well as its ethnobotanical, phytochemical, and biological activity. This review will be helpful for people studying medicinal plant phytochemical analysis, antibacterial activity, and anti-fungal activity of *Lantana camara*.
Keywords: Lantana camara, n-Hexane extract, Ethanolic extract, Methanolic extract.

1. Introduction

Medicine plants have a lot of chemicals that have important medical uses. Herbal medicine has been used to treat a wide range of health problems since ancient times. When these plants are looked at in a systematic way, a variety of bioactive compounds that could be used to make new medicines are found. In recent years, people have become more interested in the pharmacological study of many plants that are used in traditional medicine. In the last few decades, a lot of traditionally used plants have been studied in depth using cutting-edge scientific methods and found to have a wide range of medicinal properties, such as anticancer, anti-inflammatory, anti-diabetic, anthelmintic, antibacterial, antifungal, hepatoprotective, antioxidant, larvicidal, and other properties (1-6).
Lantana Camara is a very important plant that has been used in traditional medicine for a long time. In different parts of the world, it has been used to treat many health problems. Leaves are used to treat cuts, rheumatism, ulcers, catarrhal infections, tetanus, malaria, cancer, chicken pox, asthma, ulcers, swelling, eczema, tumors, bilious fever, and ataxy of the abdominal viscera, sores, measles, fevers, the common cold, and hypertension. In Ghana, the whole plant is boiled and drunk to treat bronchitis. The powdered root is mixed with milk and given to children with stomachaches or to get rid of worms. Lantana oil is used to treat skin irritations and keep wounds from getting worse. Leprosy and scabies were treated with decoctions that were put on the skin (7–12).
During the past few decades, L. camara phytochemical profile has been the subject of extensive research. Essential oils, phenolic compounds, flavonoids, proteins, carbohydrates, alkaloids, glycosides, iridoid glycosides, oligosaccharides, phenyl ethanoid, saponins, steroids, quinine, triterpenes, sesquiterpenoides, and tannin are all reported to be present in various parts of L. camara (13-16).
The antibacterial properties of L. camara plants' leaves and flowers have been reported for multiple cultivars. There was strong antibacterial activity against E. coli, Bacillus subtilis, and Pseudomonas aeruginosa in three solvent extracts of leaves and flowers from four different L. camara varieties, but only weak antibacterial activity against Staphylococcus aureus (17).
Antibacterial activity in ethanol extracts of L. camara leaves and roots has been reported. Microdilution assays were used to examine antibacterial activity in vitro. Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, V. cholareae, Escherichia coli, and two multiresistant strains of E. coli and S. aureus were all killed by the extracts (18).
The agar well diffusion method and the broth micro dilution method were used to test methanolic extracts of various L. camara parts for antimicrobial activity against ten bacteria and five fungi. Both Gram-positive Bacillus cereus and Gram-negative Salmonella typhi were inhibited by L. camara leaf extract (19).
The potential antifungal effects of L. camara were tested against Alternaria sp., a fungal pathogen that causes a wide variety of plant diseases, especially in vegetables. Three concentrations of extract (10 mg/ml, 15 mg/ml, and 20 mg/ml) were tested for their antifungal activity using the food poison plate method. L. camara displayed significant antifungal

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activity against Alternaria sp. at a 20 mg/mL dose. (20)
ethanol and hot water extracts of L. camara were tested for their ability to inhibit white rot and brown rot in wood. While the ethanol extract was highly effective at such a low concentration (0.01%), the other extract was also effective against white and brown rot fungi (21).

Taxonomy

Kingdom: Planate; Division: Magnoliophyta; Class: Magnoliopsida; Order: Lamiales; Family: Verbenaceae; Genus: Lantana; Species: Lantana camara.

2. Material and method

2.1. Collection of plant

Fresh aerial parts of Lantana camara were taken from a garden in Baghdad from August 1 to December 30, 2021. First, the wing parts were washed with tap water. Then, they were washed again with deionized water. Finally, they were left at room temperature to dry on their own. The dry plant was then cut into small pieces and stored in a dry, dark place until it was time to make an extract.

2.2. Chemical solvents

Maceration is one of the simplest extraction methods in which coarse and powdered plant material is soaked in solvents like n-Hexane, ethanol and methanol. It is one of the most common and inexpensive ways to get bioactive compounds out of plant matter. Polar compounds are extracted with methanol and ethanol, whereas nonpolar compounds are extracted with n-Hexane and other nonpolar solvents [22-24].

Table (1): Chemical solvent use in maceration method

No.	Solvents	Company	Percentage
1	n-Hexane	India	99%
2	Ethanol	India	99.99%
3	Methanol	India	99.8%

2.3. Preparation of Extracts by Maceration method

The aerial part of L. camara were extracted by maceration, using different solvents with increasing polarity. Briefly, 100 g of powder was treated with 800 mL of each solvent of n-Hexane, 95 g of powder was treated by 800 ml ethanol and 90 gm was treated by 500 ml of methanol. After 48 h at 25 °C in the dark, the supernatant was recovered by filtration. The process was repeated, and the solvent in the supernatant was evaporated in a vacuum rotary evaporator, to partially separate the bioactive compounds in the extracts (25).

2.4. GC-MS analysis

The specifications of the GC-MS are as follows:
1-The type of detector (MS), injection technique split (80.1), injector temperature (260 °C), injection volume (1µl), carrier gas (helium), flow rate (1 ml/min), auxiliary temperature (280 °C), Mode: scans (50-550).
2- Columns were used: type of column (capillary, HP-5MS), length (30 m), diameter (0.25 mm), film thickness (0.25µm).
3-Temperature Program: 60 °C for 4 min, then 3 °C/min to 100 °C for 2 min, then 4 °C/min to 260 °C for 5 min.
4-Mass Spectrometer Detector: Scan Range (50-500), EM (70 eV).
5-The relative percent amount of each component was determined by comparing its average peak area to the total area. The MS solution software provided by the supplier was used to control the system and acquire the data.

2.5. Bioassay techniques

The antibacterial and antifungal activities of *Lantana camara* essential extracts are evaluated as shown below:

2.5.1. Preparation of microorganism

1. The pathogenic bacteria were obtained from the BPC laboratory analysis center.
2. Pathogenic fungi were obtained from the BPC laboratory analysis center.

Table (2): Microorganisms used in antifungal and antibacterial activity of lantana camara extracts.

Gram positive bacteria	Gram negative bacteria	Fungi
Streptococcus mutans Bacillus subtilis Staphylococcus aureus	Escherichia coli Klebsiella pneumonia Pseudomonas aeruginosa proteus vulgaris	candida albicans Rhizopus microspores

2.5.2. Preparation of Potato Dextrose Agar (PDA)

Suspend 39.0 grams in 1000 ml of distilled or purified water. Bring the medium to a boil to dissolve it in the medium. Autoclave at 15 psi of pressure (121 °C) for 15 minutes. Before dispensing or using vials, allow to cool to 45-50 °C. When a pH of 3.5 is required for a particular task, the medium is acidified with sterile 10% tartaric acid. Approximately 1 mL of acid is required for both sterile and cooled medium. The medium does not heat up after adding the acid (26).

2.5.3. Preparation of Petri dishes

PDA agar medium was boiled, cooled at 45 °C, and poured into Petri dishes (8 cm in diameter) for use in sub-cultivation and for further testing. The test organism (a fungus) was incubated at room temperature (37 °C) for three days (27).

2.5.4. Preparation of Mueller agar media

Mueller agar media was prepared with 38.5 grams of media and one liter of distilled water. The mixture was boiled in a water bath until the agar melted, the pH was adjusted to 7.1–7.5, and then the mixture was distributed into conical flask covered with cotton plugs and aluminum foil. Then they were adjusted in an autoclave at 121 °C and The pressure 15 P.S.I for

15 minutes (26).

2.5.5. Preparation of Petri dishes

Mueller agar media was boiled, cooled at 45 °C, and Pour into Petri dishes (8 cm in diameter) for use in sub-cultivation and for further testing. The test organism (bacteria) was incubated at room temperature (37°C) for one day (27).

2.5.6. Agar well diffusion method

1. Place PDA or MHA in a petri dish and leave it to solidify.
2. Add 0.1 mL of 72-hour-old fungal culture or 24-hour-old bacteria culture.
3. Spread the fungal inoculum onto the agar surface using a sterilized spreader
4. Make 4 holes into an agar surface by using a crock borer.
5. Fill the 4 holes with 25, 50, 75, and 100 [µg/ml] of each extract.
6. Incubation at 37 °C for 24-72 hrs.
7. Observation of different sizes of inhibition zones around each extract
8. Using a light source and holding the plate upright, place the metric ruler across the zone of inhibition at its widest diameter and measure from one edge of the zone to the other. Inhibition zones around each extract are of varying sizes.
9. If no zone is reported, report a negative.
10. Zone diameters are reported in milliliters (28).

3. Results

3.1. Maceration method

The air-dried whole plants of (100 g) were extracted with n-Hexane, Ethanol and Methanol. Evaporation of the organic solvent under reduced pressure at 30 – 40 oC yielded a crude extract. As shown in the table (3) below:

Table (3): Results of maceration extract method			
No.	Extract	Weight	Colour
1	n-Hexane extract	2 gm	Light green
2	Ethanol extract	3.5 gm	Dark green
3	Methanol extract	3 gm	Dark green

3.2. GC-MS of n-Hexane extract

The chromatograms of the aerial part of the *Lantana camara* plant show that there are different compounds with different retention times. Figure (1) shows that the GC-MS chromatogram of the n-Hexane extract has (111) different compounds. The bioactive compound present in the aerial part of *Lantana camara* can contribute to the medicinal quality of the plant.

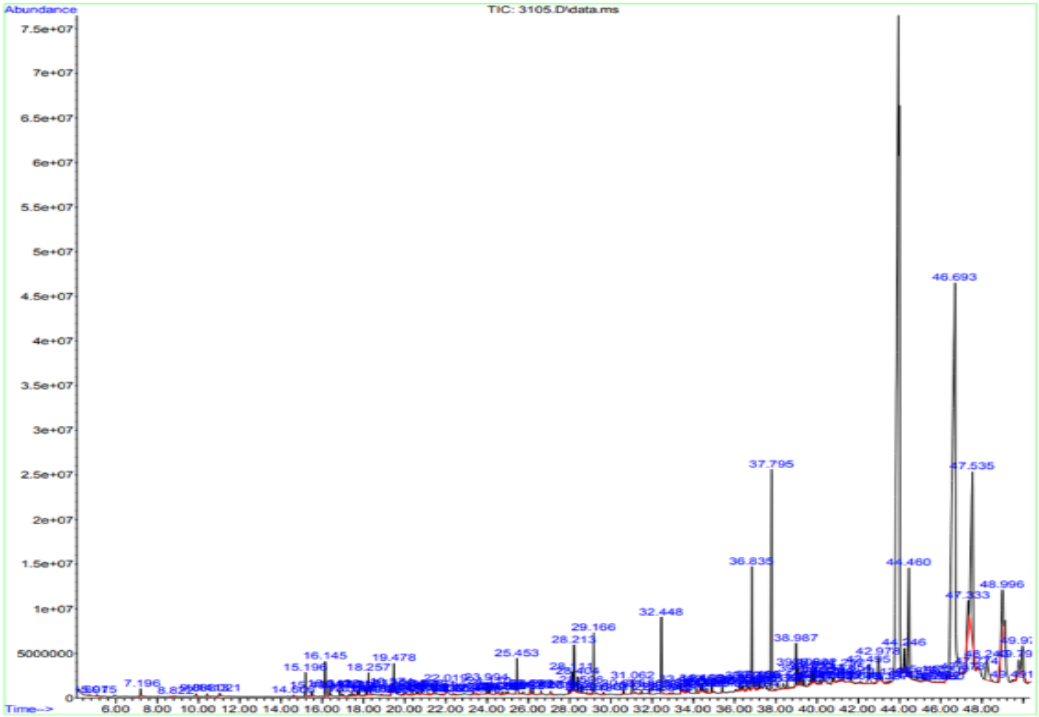

Figure (1): GC-MS of the n-Hexane extract of aerial part of lantana camara.

According to the curve's peak area percentage in Figure (1), the most prevalent phytochemicals in Lantana camara n-Hexane extract are Hexadecanoic acid, methyl ester (1.06%) , 1-Propene-1,2,3-tricarboxylic acid, tributyl ester (1.60 %) , 9,12,15-Octadecatrienoic acid, methyl ester (1.29%) , 1-Propene-1,2,3-tricarboxylic acid, tributyl ester (1.60%) , 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester(3.30%) , Squalene (6.01%) , Camp sterol (1.05%) , Stigma sterol (1.04%) , 2-(3-Ethyl-1H-1,2,4-triazol-5-yl)phenol (4.9%) , 4-Butoxy-2-carbamoylquinazoline (42.65%) , Silane, (4-bromo-3-buten-1-ynyl)trimethyl-, (E)- (9.2%) , 2-(3-Ethyl-1H-1,2,4-triazol-5-yl)phenol (1.81%) , beta –Amyrin (1.87%) , Silane, dimethyl(2-naphthoxy)heptyloxy- (1.25%) .The most important phytochemical compounds found in Lantana camara n-Hexane extract are squalene, beta-amyrin, stigmasterol, campesterol, and Hexadecanoic acid, methyl ester. As shown in Table (4), this chemical has a wide range of biological activities:

Table (4): GC-MS of n-Hexane extract				
No.	Chemical Compounds	Area %	RT (min)	Biological activity
1	Squalene	6.01 %	37.798	Antibacterial, Antioxidant, Antitumor (29,47)
2	Beta-Amyrin	1.87%	48.995	anti-inflammatory antimicrobial (35)
3	Stigma sterol	1.04%	42.976	Anti-osteoarthritis(39) Anti-cancer(40) Anti-Inflammatory (41) Anti-bacterial (42) Anti-fungal (43)
4	Camp sterol	1.05%	42.493	Anti-fungal Anti-amoebic antiviral antibacterial antioxidant (44,45,46)
5	Hexadecanoic acid, methyl ester	1.06%	25.454	Antioxidant, Hypocholesterolemic Nematicide Antiandrogenic (47)

3.3. GC-MS of ethanolic extract

The chromatograms of the aerial part of the *Lantana camara* plant show that there are different compounds with different retention times. Figure (2) shows that the GC-MS chromatogram of the ethanolic extract has (68) different compounds. The bioactive compound present in the aerial part of *Lantana camara* can contribute to the medicinal quality of the plant.

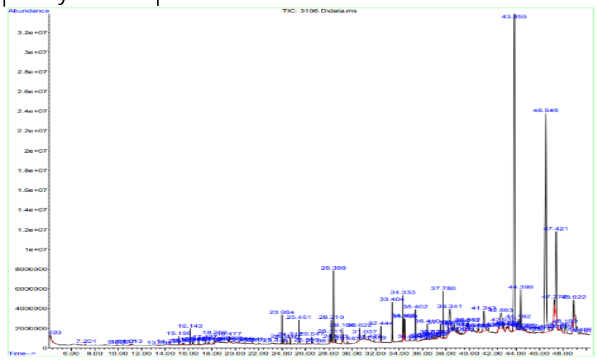

Figure (2): GC-MS of the ethanolic extract of aerial part of lantana camara

According to the curve's peak area percentage in Figure (2), the most prevalent phytochemicals in Lantana camara ethanol extract are Hexadecanoic acid, methyl ester (1.07%) , phytol (3.06%) , Adipic acid, 2-ethylhexyl octyl ester (1.50%) , Hexanedioic acid, dioctyl ester (1,47%) , Hexanedioic acid, bis(2-ethylhexyl) ester (1.18%) , Squalene(1.69%) , dl-alpha-Tocopherol (2.43%), 2-Amino-3-cyano-4-phenyl-5carboethoxy-6-methyl-4H-pyran (2.60%) , Pyrido[3,4-d]pyrimidin-4(3H)-one, 3,6,8-trimethyl- (28.65%) , 2-(3-Ethyl-1H-1,2,4-triazol-5-yl)phenol (2.71%) , 1-(2-Adamantan-1-ylethoxy)-3-(4-benzylpiperazin-1-yl)propan-2-ol (25.32%) , Pyrido[3,4-d]pyrimidin-4(3H)-one, 3,6,8-trimethyl- (6.17%) , 4H-Benz[de]anthracene, 5,6-dihydro- (1.82%).The most important phytochemical compounds found in Lantana camara ethanol extract are phytol, Squalene, DL-alpha-Tocopherol and Hexadecanoic acid, methyl ester. As shown in Table 5, this chemical has a wide range of biological activities:

Table (5): GC-MS of ethanolic extract				
Biological activity	RT (min)	Area %	Chemical Compounds	No.
Anti-nociceptive Antioxidant Anti-inflammatory Anti-allergic (30,31)	28.401	3.06 %	phytol	1
Anti-oxidant(32,47) Anti-bacterial Antitumor	37.782	1.69%	Squalene	2
Anti-oxidant Analgesic Anti-inflammatory Antitumor Anticancer (33,47)	41.243	2.43%	DL-alpha-Tocopherol	3
Anti-oxidant, Hypocholesterolemic Nematicide Antiandrogenic(47)	25.448	1.07%	Hexadecanoic acid, methyl ester	4

3.4. GC-MS of methanolic extract

The chromatograms of the aerial part of the Lantana camara plant show that there are different compounds with different retention times. Figure (3) shows that the GC-MS chromatogram of the methanolic extract has (43) different compounds. The bioactive compound present in the aerial part of Lantana camara can contribute to the medicinal quality of the plant.

Figure (3): GC-MS of the methanolic extract of aerial part of lantana camara

According to the curve's peak area percentage in Figure (3), the most prevalent phytochemicals in Lantana camara Methanol extract are 2-Methoxy-4-vinylphenol (1.11%) , Phenol, 2,6-dimethoxy- (1.5%) , (R*,R*)-5-Hydroxy-4-methyl-3-heptanone (25,05%), Neophytadiene (1.25%), Hexadecanoic acid, methyl ester (1.15%), Phytol (2.02%) , Glycerol 1-palmitate (1.23%) , 4H-Pyran-3-carboxylic acid, 2-amino-5-cyano-6-ethyl-4-(3-pyridinyl)-, methyl ester (3.90%) , 4-.alpha.-Carbethoxy-.alpha.-diethylaminoacetyl]-2-[2-thienyl]-6-chloroquinoline (3.19%) , 2-(Pentafluoropropionyl)oxybenzylidene acetophenone (1.89%) , 2-(3-Ethyl-1H-1,2,4-triazol-5-yl)phenol (21.91%) , 5,5'-Ethylenebis(4-phenyl-2-thiazolamine) (2.16%) , 2-(3-Ethyl-1H-1,2,4-triazol-5-yl)phenol (16.27%) , Benzo[h]quinoline, 2,4-dimethyl- (1,06%) , 1,1,1,3,5,5,5-Heptamethyltrisiloxane (1.24

%).

The most important phytochemical compounds found in Lantana camara methanol extract are neophytadiene, phytol and Hexadecanoic acid, methyl ester .As shown in Table (6), this chemical has a wide range of biological activities:

Table (6): GC-MS of methanolic extract				
No.	Chemical Compounds	Area %	RT (min)	Biological activity
1	Neophytadiene	1.25%	23.99	Anti-bacterial Anti-oxidant (34)
2	Phytol	2.02%	28.395	Anti-nociceptive Antioxidant Anti-inflammatory Anti-allergic (30, 31)
3	Hexadecanoic acid, methyl ester	1.15%	25.448	Anti-oxidant, Hypocholesterolemic NematicideAntiandrogenic (47)

3.5. Agar Well diffusion method

The agar-well diffusion method was used to study the biological activity. The n-Hexane extract of Lantana camara showed activity against six types of bacteria (Streptococcus mutans, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Proteus vulgaris) and two fungi (Candida albicans and Rhizopus microspores).

The ethanolic extract of Lantana camara was more effective against four different types of bacteria (Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis, and Proteus vulgaris) and two different types of fungi (Candida albicans and Rhizopus microspores).

The methanolic extract was more effective against seven different types of bacteria (Streptococcus mutans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris), and two types of fungi (Candida albicans and Rhizopus microspores).

With increasing concentration each extract's biological activity against bacteria and fungi became more potent a concentration of 100 [µg/ml] was more potent than a concentration of less than 75 [µg/ml] and the latter had a greater potency than 50 [µg/ml] and 25 [µg/ml]. The n-Hexane extract was more active in Klebsiella pneumonia. The ethanol extract displayed more activity in Bacillus subtilis .The methanolic extract increased the activity against Proteus vulgaris. All extracts have an effect on the two types of fungi, but the methanolic extract was more effective than ethanol and n-Hexane. Figures 4, 5, and 6 demonstrate this.

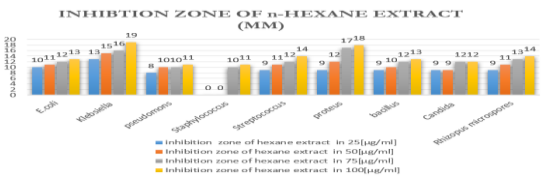

Figure (4): Agar well diffusion method of n-Hexane extract.

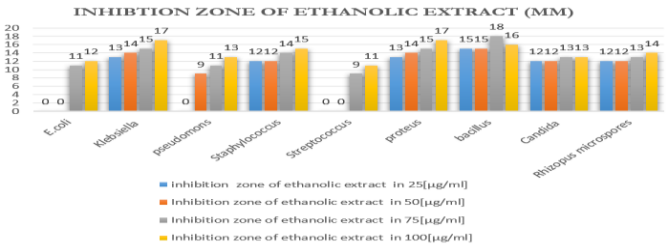

Figure (5): Agar well diffusion method of ethanolic extract.

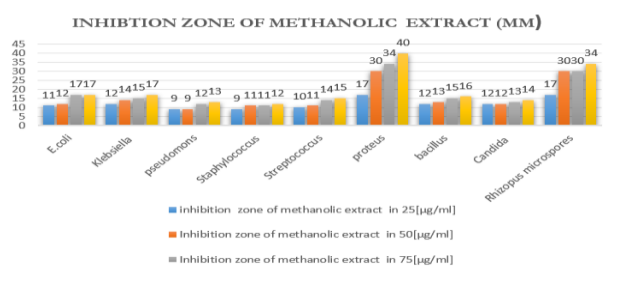

Figure (6): Agar well diffusion method of methanolic extract.

4. Discussion

The fact that Lantana camara grows in so many different places shows how well it can adapt to different environments. The species lives in a wide range of places, from open areas with no shade, like wastelands, to the edges of rainforests, beachfronts, and forests that have been damaged by things like fire pits or logging (36, 37). Along roads, railroad tracks, and canals, for example, where there is a lot of activity, the species thrives. Due to human action, the invasion worsens and has a chance of spreading (38).The GC-MS of lantana camara extract showed different compounds have different biological activities such as phytol, squalene, neophytadiene, DL-alpha-tocopherol, Hexadecanoic acid and beta-amyrin. The Agar well diffusion method was used to investigate biological activity. Lantana camara n-Hexane extract showed higher activity in six types of bacteria (Klebsiella pneumonia, Bacillus subtilis, Proteus vulgaris, E. coli, Pseudomonas, and Streptococcus mutans) and in two types of fungi (Candida albicans and Rhizopus microspores).

Lantana camara ethanolic extract was more effective against four types of bacteria (Klebsiella pneumonia, staphylococcus aureus, bacillus subtilis, and Proteus vulgaris) and two types of fungi (Candida albicans and Rhizopus microspores), while the methanolic extract was more effective against seven types of bacteria (Klebsiella pneumonia, staphylococcus

aureus, bacillus subtilis, Proteus vulgaris, E.coli, Pseudomonas aeruginosa , and Streptococcus mutans) and two types of fungi (Candida albicans and Rhizopus microspores).The biological activity of the each extract against bacteria and fungi increased with increasing concentration (a concentration of 100 [µg/ml] was more effective than a concentration lower than 75[µg/ml] and the latter had a higher effectiveness than 50 [µg/ml] and 25 [µg/ml]). In Klebsiella pneumonia, the n-Hexane extract showed greater activity. In Bacillus subtilis, the ethanol extract showed greater activity. Proteus vulgaris showed greater activity upon exposure to the methanolic extract. The two different types of fungi are responsive to all extracts, but the methanolic extract outperformed ethanol and n-Hexane in terms of efficacy.

5. Conclusion

The aerial part of Lantana camara was used in the current study for a variety of phytochemical analyses and biological activity purposes. n-Hexane, ethanol, and methanol were used as solvents to extract the material from the dried and powdered aerial part. The extract's phytoconstituents, antibacterial, and antifungal properties were examined. The presence of phytoconstituents like phytol, squalene, neophytadiene, beta-amyrin, Hexadecanoic acid and tocopherol was determined by GC-MS analysis of the extract. Antibacterial activity and antifungal activity of the extract were determined by using the agar well diffusion method against seven pathogenic bacteria and two fungi. n-Hexane extract showed higher activity in Klebsiella pneumonia. Ethanolic extract showed higher activity in Bacillus subtillis. Methanolic extract showed higher activity in Proteus vulgaris. All the extracts showed efficacy against the two types of fungi, but the methanolic extract was more effective than n-Hexane and ethanol.

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