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GCMS-based Phytochemical Profiling of *L. camara* Induces Apoptosis and Cell Cycle Arrest in Lung Cancer

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ABSTRACT

In this paper, the focus is on the phytochemical study of *Lantana camara L.* leaf extract, specifically its antioxidant and anti-cancer effects. The study shows that all solvent extracts have significant antioxidant and anticancer activity. However, the methanol liquid extract of *L. camara* leaf contains higher levels of metabolites than other solvents. The methanolic extracts of the leaves of four distinct species of *Lantana camara* also have high levels of terpenoid chemicals, which demonstrate strong antioxidant and free radical elimination properties. With further research, it is possible that useful medications can be developed for the treatment of various human diseases, especially lung cancer.

Keywords: Phytochemicals, Plant extract, Antioxidant, Metabolites, *Lantana camara*, GCMS.

INTRODUCTION

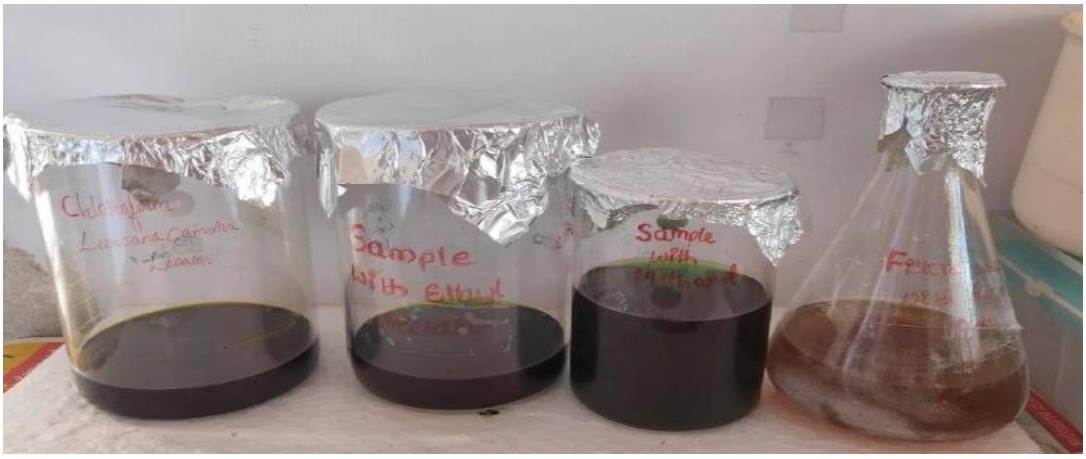
Nature and human illness treatment coexist, with natural plant products aiding ecosystem balance and providing food through photosynthesis. Phytochemicals and biologically plant-based reactive substances are beneficial for an individual's well-being by enhancing plant colour, flavour, and perfume while shielding them from environmental threats. Over 4,500 phytonutrients have been identified, with 350 currently under investigation. Common foods with phytochemicals include fruits, vegetables, and whole foods. The phytochemical analysis of the Ocimum plant extract, used in traditional remedies for swelling, diarrhoea, and persistent diarrhoea, revealed increased polyphenols as well as antioxidant function. Gillela S et. al. found the potential application of *Lantana camara* (LC) stem for lignocellulosic fibre in bio-composites to prevent its spread into forested and agricultural areas [1]. Khan A et. al. studied the phytochemicals and herbs with pharmaceutical potential for medicinal purposes in the treatment of cancer, the biggest cause of mortality globally [2]. Dawood AS et. Al. investigated the mechanism of action of LA inhibition on prostate cancer cells, finding that an increase in LA content reduced cell viability, leading to mitochondrial membrane breakage and nucleus collapse [3]. Yadav K et. Al. discovered strong antibacterial and antifungal activity in LC leaves [4]. Negi and colleagues investigated the ecology of the invasion and extermination of the lantana plant in India, highlighting the possible adverse effects on biodiversity and ecosystem services [5]. Cui Y et al. assessed the anti-cancer, antioxidants, and cell cycle arrest capabilities of cyclic triterpenoids pentacyclic LC leaf in opposition to MCF-7 cells [6]. Herbal medications can cure a variety of ailments, and research on the chemical makeup and biological properties of plants has been conducted in great detail. In 2013, Rabia Naz and Asghari Bano discovered that the plant has the strongest antibacterial strains [7]. In 2010, Ghosh S et. al. investigated cancer-fighting as well as anti-inflammation compounds from LC [8]. Sharma and Sarita Sharma examined the hepatotoxic consequences of lantana leaf on female rats, guinea pigs, and rabbits, as well as the possible restorative properties of Chinese medicinal tea and the molecular makeup of lanatadenes [9].

MATERIALS AND METHODS

Phytochemicals Analysis and Extraction from *L. Camara*

The LC plants were collected in Hubli in March 2023, cleaned, dried, and broken into little pieces. It was then kept in storage at room temperature until needed again. Crushing leaves, applying organic solvents, and stirring the concoction were all part of the investigation. After that, the mixture was cold extracted, filtered, and constantly stirred. After being yield-weighed, the finished extracts were kept for later research at 37°C. Following the recommended technique, the phytochemical analysis of *L. camara* leaflets revealed several different chemicals, including the saponins alkaloid compounds, protein molecules, flavonoids that are glycosides, phytosterols, tannins, terpenoids which are oils, and lipids.

(a) (b) (c)



(d)

Figure 1: (a) *L. camara* leaves **(**b)Extraction of *L. camara* leaves (c) Extraction of Photochemical from *L. camara* leaves with different solvent systems. (d) Filtrate of extracted Photochemical from *L. camara* leaves.

Figure 2: Summaries of various tests involved in the research work towards phytochemical analysis

Terpenoid Level Complete Identification with the Fcr Approach

600 microliters of distilled water were thoroughly mixed with one millilitre of *L. camara* extract (200 µg to 1 mg of methanol extract), and 0.2 millilitres of Folin-Ciocalteu reagent were added. After five minutes of incubation, 1ml in sodium carbonate (Na_2CO_3) was added, stirred, and diluted with distilled water to make 1ml. After that, let it sit for 40 min at ambient temperatures. In a spectrophotometer, the intensity of absorption was measured at 765 nm about a blank. An ordinary curve of gallic acid (first test tube blank, subsequently 200, 400, 600, 800, 1000, µg/ml) was used to quantify the total phenols.

DPPH Test Antioxidant Action

The standard solution (20, 40, 60, 80, 100 µg/ml) and 1.0 ml of DPPH functioning solution (0.2 mm) were combined with 0.5 ml of multiple quantities (50, 100, 150, 200, and 250µl) straight from the provided stock of test samples in a 2 ml centrifuge tube. The proportions were then cultivated for thirty minutes in the gloom at ambient temperatures with the help of a Labman UV Spectrophotometer transparent, the transmittance had been established at 517nm.

MTT Assesses Anticipatory Conduct

Using the modified MTT assay technique, the lung malignant cell line (A549) was assessed for viability. After the living cells were taken out onto a centrifuge tube, 96-well plates were filled with the cell suspension. Different drug test concentrations were put on the plates after 24 hours. Once the growth media had been removed, DMSO was used to solubilize the formazan. To get rid of the crystals, the dish then underwent three-hour incubation. The absorbance was measured at 570nm and 630nm, and the percentage of propagation retardation had been computed. The cell line's dose-effect relationship pattern was used to calculate the IC_{50}.

Apoptosis Assay by Flow-Cytometery

Seeds of tissues were planted. in a microplate and incubated overnight at 37^0C. The IC50 concentration was treated for 24 hours. After washing, centrifuging, and resuspending in 1X binding buffer, AbFlour 488 Annexin V, and PI were added. the proliferation of cells was analyzed by flow cytometry within 30 minutes.

RESULTS AND DISCUSSION

Red sage, or *Lantana camara L*, is a popular spice and significant medicinal herb. Pentacyclic triterpenes and lantadenes, which have a variety of biological actions such as antifungal, antiproliferative, and antibacterial qualities, are present in them. Nevertheless, the anti-cancer potential of the phytochemicals in *Lantana camara* has not received much attention, which is why this study was conducted for our dissertation.

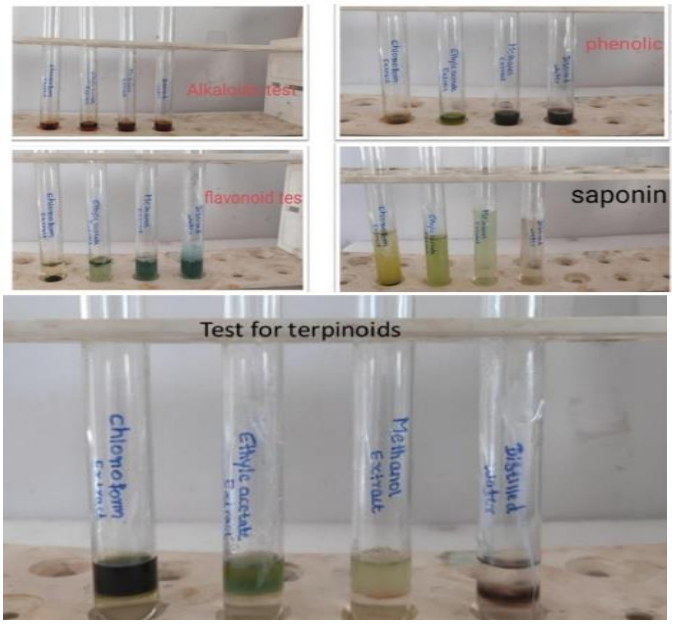


Figure 1: Phytochemical analysis showing negative results from *Lantana camara* leaves extract and Phytochemical analysis showing positive results from *Lantana camara* leaves extract in methanol (Brown colouring formation) and distilled water sample.

Principal Groups

The principal groups present in the phytochemicals are shown in the figure 2. referring to figure 2 Phenolics occupied the highest contribution with a value of 45% followed by Terpinoids and Steroids with a value of 27%, Alkaloids with a value of 18%. Chemicals are contributed with a value of 10%.

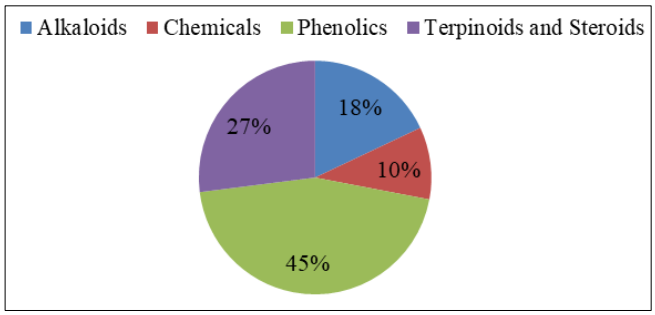
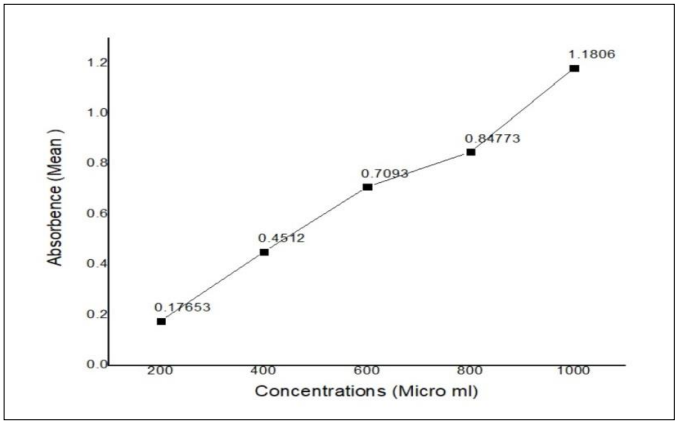


Figure 2: Contributions of Extracted *L. camara* Leaves

Terpenoid Level Complete Identification with The Fcr Approach

600 microliters of distilled water were thoroughly mixed with one millilitre of *L. camara* extract (200 µg to 1 mg of methanol extract), and 0.2 millilitres of Folin-Ciocalteu reagent were added. After five minutes of incubation, 1ml in sodium carbonate (Na_2CO_3) was added, stirred, and diluted with distilled water to make 1ml. After that, let it sit for 40 min at ambient temperatures. In a spectrophotometer, the intensity of absorption was measured at 765 nm about a blank. An ordinary curve of gallic acid (first test tube blank, subsequently 200, 400, 600, 800, 1000, µg/ml) was used to quantify the total phenols.

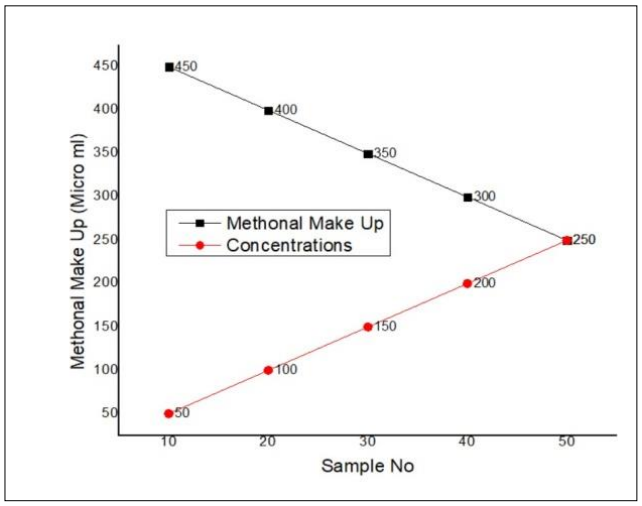


Graph 1: Standard Calibration Curve Preparation

Graph 3.1 observed that whenever the concentration values of standard gallic acid increased from the range of 200 microgram/ml to 1000 microgram/ml, the mean absorbance values also increased. The highest mean absorbency of standard gallic acid is found against the concentration value 1000 as 1.806, and the lowest mean absorbency of standard gallic acid is found against the concentration value 200 as 0.1763 respectively.

Antioxidant Assay

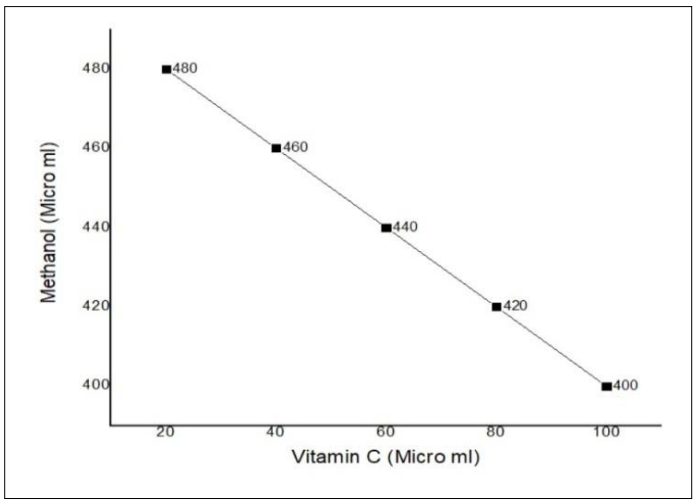
Graph 3.2 observed that whenever the sample size values increased from the range of 10 to 50, both the methanol makeup as well as concentration values were also increased. The highest Methanol makeup was found against the sample size of 10 with a value of 450 microlitres. The lowest methanol makeup was found against the sample size of 50 with a value of 250 microlitres. The highest concentration value was found against the sample size of 50 with a value of 250, whereas the lowest concentration value was found against the sample size of 10 as 50. Both quantifications of terpenoids and antioxidant assay for methanol extraction were evaluated with the DPPH value 1, After incubating for thirty minutes at location temperatures in a light-free environment, the sample was read at 577 nm respectively.



Graph 2: Antioxidant Assay for Methanol Extraction

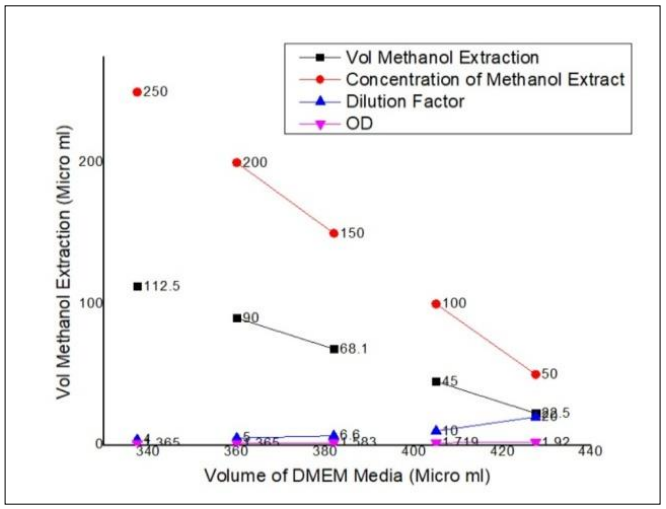
Calibration Curve of Ascorbic Acid for Antioxidant Assay

Referring to Graph 3.3 the methanol absorbance the vitamin C availability is nonlinear. Whenever the vitamin C availability values are increased the methanol absorbance values are decreased. The highest methanol absorbance was found as 480 µl against the Vitamin C availability with a value of 20 µl followed by methanol absorbance as 460 micro ml against the Vitamin C availability with a value of 40 µl, methanol absorbance was found as 440 µl against the Vitamin C availability with a value of 60 µl, methanol absorbance was found as 420 µl against the Vitamin C availability with a value of 80 µl. The lowest methanol absorbance was found as 400 µl against the Vitamin C availability with a value of 100 µl respectively.



Graphs 3: Calibration Curve of Ascorbic Acid for Antioxidant Assay at DPPH

MTT Assay towards Viability of Lung Cancer Cell Lines (A549 Cell Lines)



Graphs 4: MTT assay to check the viability of lung cancer cell lines in the presence of Methanol extract (A549 Cell Lines).

Vol. of Methanol Extraction: Referring to Graph 3 whenever the volume of DMEM media values was decreased, the volume of methanol extraction, and extracted methanol concentration values were also increased, and the dilution factor values and OD values were decreased respectively. The highest volume methanol extraction was found with a value of 112.5 µl against the volume of DMEM media value of 337.5 µl whereas the lowest value was 22.5 µl against the DMEM Media value of 427.5 µl.

Concentration of Methanol Extract: Referring to Graph 3 the highest concentration of methanol extract was found as 250 against the volume of DMEM media value of 337.5 µl, and the lowest concentration of methanol extract was found to be 50 against the DMEM Media value of 427.5 µl.

Dilution Factor: Referring to Graph 3 the highest dilution factor value is noticed as 20 against the DMEM media value of 427.5 µl, and the lowest dilution factor value is noticed as 4 against the volume of DMEM media value of 337.5 µl.

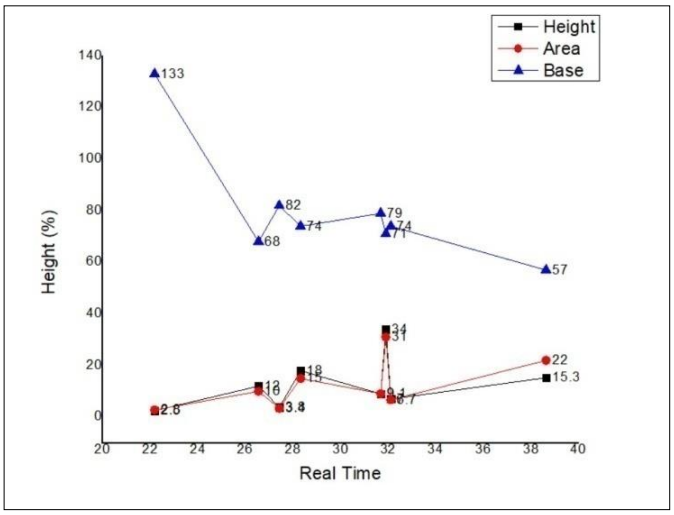
OD: Referring to Graph 3 the highest OD value is recorded as 1.92 against the DMEM media value of 427.5 µl, and the lowest OD value is recorded as 1.365 against the volume of DMEM media value of 337.5 µl.

GC-MS ANALYSIS

GC-MS is a method employed in calculating the molecule weight and elemental composition of compounds. It uses high-resolution Electron Impact Mass Spectroscopy and a Shimadzu ELITE 5MS column. The analysis uses helium gas as the carrier gas and a 43-minute runtime. The software used for handling mass spectra and chromatography is GCMS solution version 2.53. Eight compounds were identified in the LC leaf extract.

Test	Chloroform	Ethyl acetate	Methanol	Distilled water
Alkaloids	Negative	Negative	Negative	Negative
Phenols	Negative	Negative	Negative	Negative
Flavanoids	Negative	Negative	Negative	Negative
Terpenoids	Negative	Negative	Positive	Positive
Saponins	Negative	Negative	Positive	Positive
Tanins	Negative	Negative	Negative	Negative
Anthroquinone glycoside	Negative	Negative	Negative	Negative
Steroids	Negative	Negative	Positive	Positive

Figure 3: Scientific Examination *L. Camara*



Graph 5: GS-MS Fragmentation

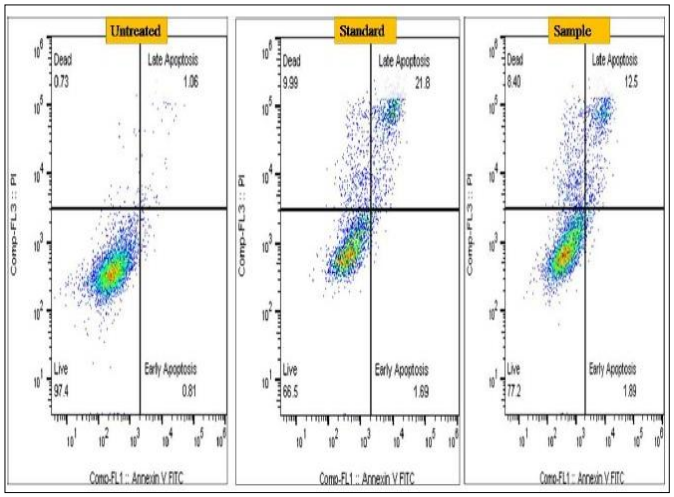


Figure 4: Assay of Apoptosis in A549 Cell Line

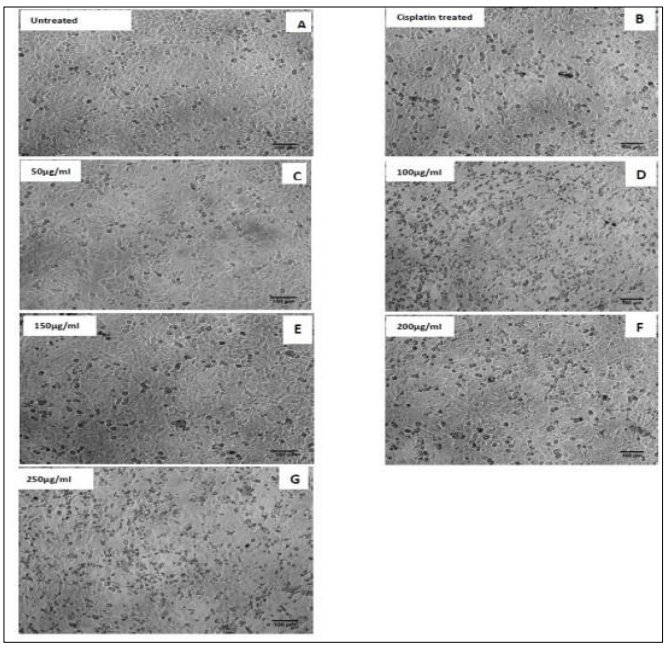
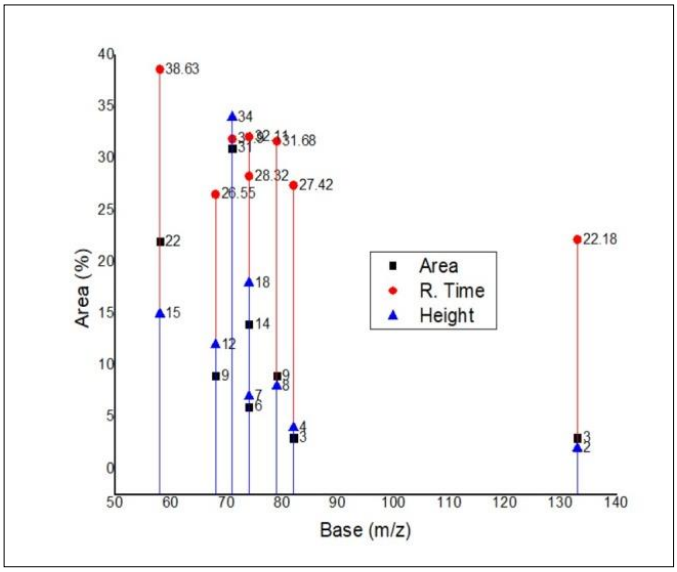
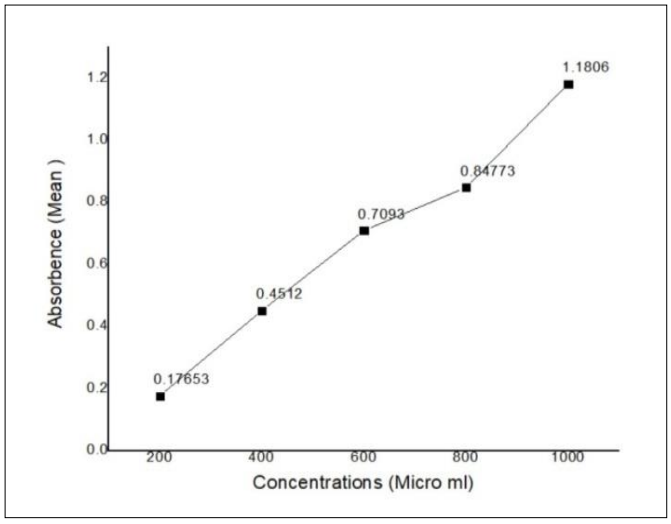


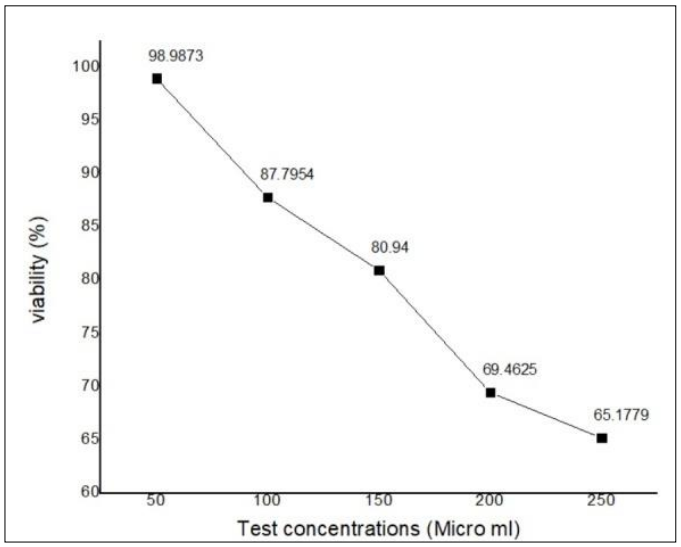
Figure 5: Lung Cancer Cell line A549 treated with Methanol extract of Latan



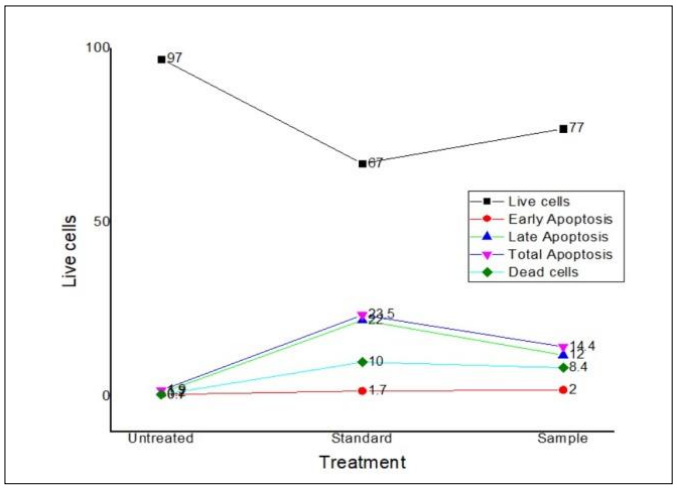
Graph 6: GS-MS Data of Methanol Extract



Graph 7: Standard Calibration Curve Preparationmby using the concentration of Gallic acid Vs OD at 570nm.Cammaral Leaves



Graph 8: Concentrations vs viability %



Graph 9: Cell Distribution (%)

Phytochemical Analysis

The study's main focus is on the phytochemicals that were separated from powdered *Lantana camara* leaves using distilled water, methanol, ethyl acetate, and chloroform. Terpenoids and steroids are the primary secondary metabolites; they have antibacterial, free radical-scavenging, and antioxidant properties. These substances slow the onset and spread of illness. Fruits of the *Lantana camara* plant include phenolic chemicals, flavonoids, phytosterols, glycosides, and saponins, all of which have different biological effects. Nevertheless, nothing is known about *Lantana camara*'s ability to fight cancer. Because leaves contain phytochemicals, this investigation is pertinent to the project dissertation.

Anti-Cancer Activity

Lung cancer is a prevalent human disease, with 43% of annual incidents in Iraq. The plant *Lantana camara* contains active compounds like pentacyclic, saponins, flavonoids, alkaloids, and phenols. Studies have shown that Lantadene A and B have potent antitumor properties against the mammary tumour cell line MCF-7. Lantadene B significantly decreased cell viability, leading to 75% cell death at 400µg/ml. Lantadene C and iatrogenic also showed antiproliferative effects. These findings suggest the phytochemical role of *Lantana camara* leaves in cancer prevention.

Antioxidant Activity

The study uses a DPPH combating free radicals test to assess the antioxidant qualities of *Lantana camara* extracts. We tested the extracts of distilled water, methanol, ethyl acetate, and chloroform with ascorbic acid, a conventional antioxidant. According to the findings, methanol extracts exhibited strong scavenging action, shielding organisms from aggressive oxygen radicals. The potential of organic anti-oxidants to protect and promote health has led to a high demand for them. The DPPH experiment demonstrated the potent antioxidant and DPPH radical inhibition that *Lantana* leaf extracts exhibited. The greatest antioxidant was shown to be the ethanol fermentation preparation of Lantana trifolia. LC premature leaves may also have antioxidant properties.

Apoptosis Assay by Flow-Cyotometery

The presence of Annexin on the plasma membrane, especially in A549 cells subjected to *L. camara*, is measured in this work using flow cytometry. The findings demonstrate that the proportion of cells that have died relative to all treated cells is shown by Annexin V/PI staining.

GC-MS Analysis of *L. camara* Leaves Methanol Extract

Similar to the 32 phytochemicals reported in Mallappa et al.'s 2015 study, this study reveals 8 phytochemicals in methanol extracts from *Lantana camara* leaves. Findings of six different phytochemicals point to the necessity for additional research on the extraction of pure phytochemicals and the assessment of their biological activity to comprehend their potential applications in medicine. Dawood et al. found that LNCaP cell viability decreased with increased LA concentration, leading to cellular disintegration, mitochondrial membrane rupture, and cytochrome C release [10]. High LA concentrations also caused dose-dependent increases in caspase activity and cell cycle arrest. This research highlights the importance of controlling LA levels in biological processes. Cabrido et al. found alkaloids, saponins, flavonoids, steroids, tannins, and fifteen phytocompounds with antioxidant and antibacterial properties in their phytochemical screening [11]. These results suggest ethnomedicinal benefits in the plant. Bhakta D et al. found that proanthocyanidin levels were higher than phenolics and flavonoids in different leaf positions of a twig [12]. Additionally, they observed that leaf extracts from positions I to III exhibited higher antioxidant activities. The study by Ganjewala D et. al analyzed the biochemical composition and antibacterial activities of four *Lantana camara* plants with different coloured flowers [13]. The plants showed similar carbohydrate and lipid compositions, but the levels varied among different plant parts. The ethyl acetate extracts from the leaves and flowers exhibited significant antibacterial activities against bacteria, although the activities varied between plants and tissue types. Hiremath et al. purified *Lantana camara* (LCL) leaves using chromatography and found that they showed strong binding to human colon adenocarcinoma HT29 cells and exhibited potent antibacterial and anti-fungal activity [14]. These findings suggest that LCL has potential for clinical applications.

Lantana camara plant contains active compounds that have shown anti-cancer activity on MCF-7 and lung cancer cell lines in various studies. These active compounds include pentacyclic, saponins, flavonoids, alkaloids, and phenols. Lantadene B is a promising candidate for caspase 9 induction and cell cycle arrest experiments. Essential oils from *Lantana camara* Linn and *L. motevidensis* Briq have scavenging effects on DPPH radicals. Lantana leaf extracts have strong antioxidant effects, with IC50 values of 7.57 µg/ml for *L. camara* and 2.55 µg/ml for *L. montevidensis. L. camara* premature leaves show 62% DPPH scavenging activity.

Swamy MK et. al. identified eight phytochemicals in *Lantana camara* leaves methanol extracts via GCMS analysis [15]. Six distinct phytochemicals were found, indicating a need for further research on

isolation and biological activity evaluation to understand their medicinal role. *Lantana camara L* is a medicinal plant and spice containing lantadenes and pentacyclic triterpenes with antifungal, antiproliferative, and antimicrobial effects. However, limited research has been done on its anti-cancer properties.

CONCLUSION

More extractable metabolites were found in the *L. camara* leaf solvent extract than in any other solvent. Furthermore, due to variations in their phytochemical makeup, all solvent extracts of *L. camara* exhibited notable antioxidant and anticancer activities. Based on the findings of the current investigation, it is determined that the methanol as the extracts of leaves from four distinct *L. camara* varieties, which are rich in terpenoid compounds, have strong antioxidant, free radical scavenging, as well as *in-vitro* peroxidation of lipids inhibition properties. Therefore, our research indicates that the methanol-based extract of the leaves of *L. camara*, which contains a variety of bioactive chemicals, may be used as a medicinal source to help produce medications that may help treat a range of individual diseases and disorders. Our investigation leads us to the conclusion that the plant L. camara exhibits a wide range of activities. We can isolate pure medication using this *L. camara* extract. A medication that is employed to treat tumours or cancer can also be separated from this medication. Metabolites and anti-cancer medications may be found in good amounts in *L. camara*.

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Conflict of interest

The authors declared no conflict of interest.

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