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RESEARCH ARTICLE

EVALUATION OF THE BIOLOGICAL ACTIVITY OF THE METHANOLIC EXTRACT OF LANTANA CAMARA

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ABSTRACT

Herbs contain a wide range of bioactive compounds, including alkaloids, flavonoids, terpenoids, and essential oils, which contribute to their medicinal properties. These compounds can exhibit anti-inflammatory, antioxidant, antimicrobial, and analgesic effects, among others. The efficacy of herbal remedies is often attributed to the synergistic action of multiple constituents present in the plants, which may enhance therapeutic outcomes while minimizing side effects. Recent interest in herbal medicine has surged, driven by a growing demand for natural and alternative therapies. Research studies continue to explore the pharmacological properties of various herbs, aiming to validate traditional uses and uncover new applications in modern healthcare.The study evaluated the anti-cancer, anti-inflammatory and anti-bacterial properties of the methanolic extract of Lantana camara. The cytotoxicity of HepG2 cell line was examined using MTT Assay and protein denaturation test to study anti-inflammatory while the effect of the extract on E. coli and B. subtilis for antibacterial evaluation

INTRODUCTION

Lantana camara, commonly known as wild sage, tickberry, or Spanish flag, is a flowering plant belonging to the family Verbenaceae. Native to the tropical regions of Central and South America, **L. camara** has spread globally and has become naturalized in many tropical and subtropical regions, including Africa, Asia, and Australia. It thrives in a variety of habitats, ranging from disturbed areas to forests, and is often considered an invasive species due to its aggressive growth and ability to dominate native vegetation(Bhakta et al. (2007))(Chougale et al. (2012). Despite its notoriety as an invasive plant, *L. camara* has garnered attention for its wide range of pharmacological properties. The plant has been traditionally used in folk medicine for the treatment of various ailments, including skin diseases, respiratory infections, and gastrointestinal disorders(Kirtikar, et al. (2005)(Sharma, et al. (2017).. In recent decades, the plant has become a subject of intense biological and pharmacological studies aimed at isolating and characterizing its bioactive compounds. These studies have shown that *L. camara* contains several classes of secondary metabolites with potential therapeutic applications, including anti-inflammatory, antimicrobial, antioxidant, and anticancer properties(Shalini, et al. (2010). (Sujatha, et al. (2005)*Lantana camara* is a perennial shrub that can grow up to 2 meters in height. It features a woody stem, rough-textured leaves, and clusters of small, tubular flowers that change color as they age, typically shifting from yellow or orange to pink, red, or purple. This color variation in flowers is one of the plant's most distinctive characteristics. The leaves of *L. camara* are opposite, simple, and serrated, with a strong odor when crushed, a feature that is believed to deter herbivores (Chougale et al. (2012).. The fruits of the plant are small, black, and fleshy drupes that are attractive to birds, which help in seed dispersal(Bhakta et al. (2007)) (Sharma, et al. (1989)..In traditional medicine, different parts of *L. camara*—including the leaves, flowers, stems, and roots—have been used for various treatments. Leaf extracts are applied topically to wounds and ulcers due to their purported antibacterial properties, while decoctions of the leaves or roots are consumed to treat respiratory ailments, fevers, and digestive disorders(Kirtikar, et al. (2005) (Day,et al. (2003). In certain cultures, *L. camara* is also used to treat skin conditions such as eczema and psoriasis(Kirtikar, et al. (2005). Its use as an insect repellent has also been documented, as the strong odor of the leaves is thought to deter mosquitoes and other pests(Bhakta et al. (2007)) (Shalini, et al. (2010).

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Phytochemical studies have revealed that *L. camara* contains a wide variety of bioactive compounds, including triterpenoids, flavonoids, phenolics, and essential oils(Sharma, et al. (2017). (Sharma, *et al.* (1989). Among the most notable compounds are the pentacyclic triterpenoids, such as lantadene A and lantadene B, which are believed to be responsible for many of the plant's pharmacological activities. These triterpenoids have been shown to possess significant anticancer and anti-inflammatory properties, making them subjects of interest for drug discovery and development(Sharma, *et al.* (2017).. (Sujatha, *et al.* (2005). In addition to triterpenoids, the plant is rich in flavonoids, which are known for their antioxidant properties. These compounds can scavenge free radicals and protect cells from oxidative stress, thereby offering potential benefits in the treatment of chronic diseases such as cancer and cardiovascular diseases(Shalini, *et al*. (2010). Essential oils extracted from *L. camara* have also demonstrated antimicrobial activities against a variety of bacterial and fungal pathogens, further supporting its use in traditional medicine as a treatment for infections(Sharma, *et al.* (2017).. (Sharma, et al. (1989).

MATERIALS AND METHOD

Plant Material

Lantana camara was collected from Aurangabad -India,the leaves were thoroughly washed and dried in the shade until completely dry, then ground to obtain a fine powder. Conventional extract: 30 g of Lantana camara powder was extracted in 300 ml of methanol using Soxhlet. The extraction process continued until the color of the leaves totally disappeared. Then the solvent was removed through a rotary evaporator, and the sample was left until it dried completely for use in analysis.

INVESTIGATION OF BIOLOGICAL ACTIVITIES

ANTICANCER

MTT Assay

Cytotoxicity of the provided samples on HepG2 cell line was determined by MTT Assay. The cells (5000-8000 cells/well) were cultured in 96 well plates for 24 h in DMEM medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO2. Next day cells were treated from 0-1000 µg of the formulations (different concentrations were prepared in incomplete medium). After incubation for 24 hours, MTT Solution (a final concentration of 250µg/ml) was added to cell culture and further incubated for 2 h. At the end of the experiment, culture supernatant was removed and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO) and read in an Elisa plate reader (iMark, Biorad, USA) at 595 nm and 660 nm.

(Morgan (1998)(van Meerloo et al. (2011)(Fotakis& Timbrell (2006) (Tihauan et al. (2020)

ANTI-INFLAMMATORY

Anti-inflammatory: anti-inflammatory activities were investigated by two examinations, i.e., a hemolysis assay and a protein denaturation assay. preparation of erythrocyte suspension and heat-induced hemolysis. The previously described protocol was followed to make the erythrocyte suspension, with a few minor modifications (Okoli et al., 2008.). Perfectly healthy human blood was taken. For five minutes, blood was poured into centrifuge tubes coated with EDTA while spinning at 3,000 rpm. The erythrocytes (blood cells) were washed three times with an equivalent amount of natural saline solution (0.9% sodium chloride) to ensure there was no colour in the floating material. To restore blood cells as 10% suspension (v / v), after centrifugation, the volume of blood cells was determined and mixed with an isotonic dielectric solution (10 mm sodium phosphate buffer pH 7.4 containing 0.9% sodium chloride). The heat-induced hemolysis process was created by mixing 2.90 ml of phosphate solution (pH 7.4) with 0.05 ml of blood cell suspension. The plant extracts were introduced in a 0.05-millilitre amount. An additional 0.05 ml of phosphate buffer was used for the control pipes. The mixture was incubated in a vibrating water bath at 54 °C for twenty minutes. Following the incubation period, the 540 nm absorbance of the flotant was measured after the mixture was centrifuged for three minutes at 2500 rpm. Impact on protein denaturation: The previously established standard procedure (Gambhire et al. 2009.) was followed in the protein denaturation analysis. The examination tubes with 4.4 ml of phosphate-stored brine (PBS, pH 6.4) were filled with 0.1 ml of extract and 0.5 ml of 0.4% bovine serum albumin. The pipes were placed in a sporadic vibration water bath and incubated at 37 ° C for 15 minutes, then at 70 ° C for 5 minutes. At 660 nm, the turbidity of the tubes was measured after they had melted to ambient temperature. As a control, phosphate buffer was employed. (Tesfaye at al 2021) (okoli et al 2008)

Antibacterial:

Antibacterial-Zone Inhibition Test

The Antibacterial activity was checked by following Zone Inhibition Method (Kirby-Bauer method). The MHA plates were inoculated by spreading with 100 µl of Bacterial culture, S. aureus (adjusted to 0.5 McFarland Unit - Approx cell density (1.5 X 108 CFU/mL) and followed by placing the discs containing 10 µl of different concentration (0 to 100 mg/ml). 10 % of the sample was taken and serially diluted to achieve the required amount to be loaded on the disc. One disc in each

plate was loaded with solvent alone which served as vehicle control and Ciprofloxacin disc (10µg) was taken as positive control. The plates of Bactria were incubated (Basil Scientific Corp. India) at 37 °C for 24 hrs. A clear zone created around the disc were measured and recorded.

RESULTS AND DISCUSSION

The Percentage Yield of the Crude Extract

The percentage of the extract were found to be 36.63% forMethanol extract,The extract was investigated for their phytochemical content and their biological activities.

Fourier transform infrared spectroscopy (FTIR):

The Fourier Transform Infrared (FTIR) spectroscopy peaks for the methanolic extract of Lantana camara are as follows:

Interpretation of FTIR Peaks:

3319.74 cm□¹: This peak corresponds to the O–H stretching vibration, which is typically associated with hydroxyl groups (–OH) in alcohols, phenols, or carboxylic acids. The broadness of the peak often indicates hydrogen bonding.

2943.05 cm□¹ and 2831.16 cm□¹: These peaks are typically attributed to C–H stretching vibrations of aliphatic compounds, indicating the presence of –CH□ or –CH□ groups.

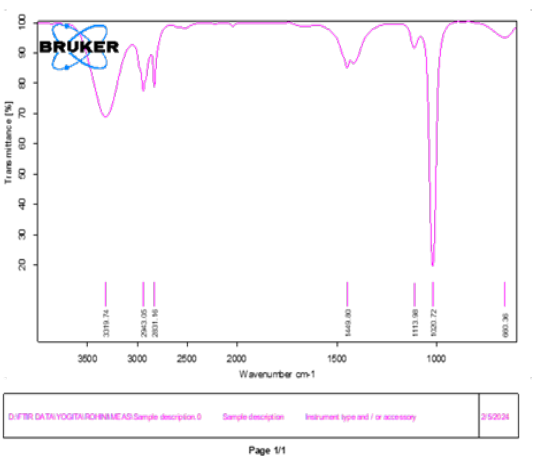
1449.80 cm□¹: This is characteristic of C–H bending vibrations (scissoring) in alkanes. It further confirms the presence of aliphatic hydrocarbons in the extract.

1113.98 cm□¹: This peak is usually related to C–O stretching vibrations, commonly found in alcohols, ethers, or esters.

1020.72 cm□¹: This peak suggests the presence of C–O stretching, which is characteristic of polysaccharides or carbohydrate-like compounds.

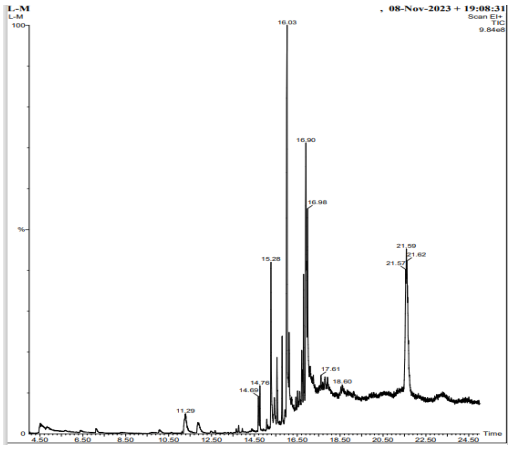
660.36 cm□¹: This low wavenumber peak is often related to C–H out-of-plane bending vibrations, potentially indicating aromatic compounds or alkyl halides.

The FTIR spectra suggest that the methanolic extract of Lantana camara contains hydroxyl groups (–OH), aliphatic hydrocarbons (–CH□, –CH□), alcohols or ethers (C–O), and possibly some aromatic compounds or carbohydrate derivatives. These functional groups are indicative of various bioactive compounds such as flavonoids, phenolic acids, terpenoids, and polysaccharides, which could contribute to the plant's known medicinal properties, including anti-inflammatory and anticancer activity.(Moein et al. (2015)



GC-MS

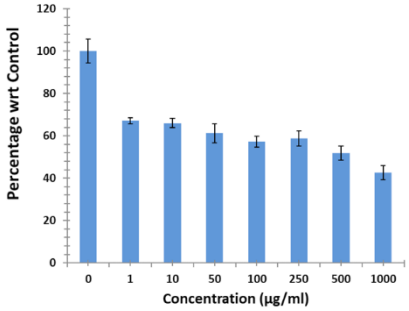
The GC-MS of the methanolic extract of Lantana camara indicates a complex mixture of phytochemicals, including terpenoids, fatty acids, phenolic compounds, and possibly steroids. These compounds are known for their pharmacological properties, such as antimicrobial, antioxidant, and anti-inflammatory activities.



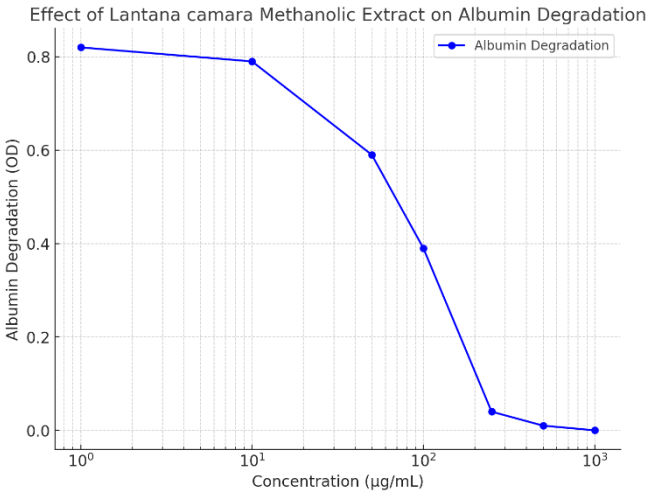
BIOLOGICAL ACTIVITIES

Anti-cancer

Control (0 µg/mL): At zero concentration (control), the cell viability is 100%, representing the baseline viability of untreated HepG2 cells.Low concentrations (1–50 µg/mL): At 1, 10, and 50 µg/mL, cell viability decreases slightly from 87.89% to 84.78%. This shows a minor cytotoxic effect of the methanolic extract at these lower doses, suggesting minimal interference with the cell's proliferation.Intermediate concentrations (100–250 µg/mL): A more pronounced reduction in viability is observed at 100 µg/mL (75.99%) and 250 µg/mL (73.70%). This indicates moderate cytotoxicity, suggesting that the extract begins to exert significant effects on the HepG2 cells.High concentrations (500–1000 µg/mL): The cell viability dramatically drops to 53.15% at 500 µg/mL and to 48.03% at 1000 µg/mL. These higher concentrations demonstrate a strong cytotoxic effect, reducing the viability of HepG2 cells by nearly half at 1000 µg/mL, highlighting the potential anticancer activity of the Lantana camara extract.The methanolic extract of Lantana camara shows a concentration-dependent cytotoxic effect on HepG2 cells. While the impact at low concentrations (1–50 µg/mL) is relatively mild, higher concentrations (500–1000 µg/mL) lead to a significant reduction in cell viability, indicating potent anticancer properties. This suggests that the bioactive compounds in Lantana camara have potential as therapeutic agents against liver cancer.



Anti-inflammatory



Analysis of the Effect of Lantana camara Methanolic Extract on Albumin Degradation:

Low concentrations (1–10 µg/mL): At the lower concentrations of 1 and 10 µg/mL, the optical density (OD) values of 0.82 and 0.79, respectively, indicate limited degradation of albumin. This suggests that the methanolic extract has a minimal inhibitory effect on albumin degradation at these low doses.Moderate concentrations (50–100 µg/mL): With a concentration of 50 µg/mL, the OD drops to 0.59, showing a moderate inhibition of albumin degradation. At 100 µg/mL, the OD value decreases further to 0.39, suggesting that the extract exerts a significant effect at this concentration, inhibiting albumin breakdown more effectively.High concentrations (250–1000 µg/mL): At higher concentrations, particularly 250 µg/mL and above, the OD values approach zero (0.04 at 250 µg/mL, 0.01 at 500 µg/mL, and 0.00 at 1000 µg/mL). This demonstrates almost complete inhibition of albumin degradation, indicating that the methanolic extract of Lantana camara is highly effective at preventing albumin breakdown at these higher doses.The methanolic extract of Lantana camara shows a concentration-dependent inhibition of albumin degradation. While low concentrations have a minimal effect, moderate concentrations (50–100 µg/mL) significantly reduce albumin degradation, and higher concentrations (250–1000 µg/mL) nearly eliminate it. These findings suggest that the extract contains compounds with potent protease inhibitory activity, which may be beneficial in conditions where the inhibition of protein degradation is desired.

Antibacterial activity

Results from the Antibacterial Zone Inhibition Test of E. coli

The antibacterial activity of methanol at various concentrations was evaluated using an inhibition zone test against E. coli. The results obtained from the different concentrations of methanol (µg/disk) are as follows:

0 µg/disk: No inhibition zone observed (0 mm)

50 µg/disk: Small inhibition zone (approximately 5 mm)

125 µg/disk: Moderate inhibition zone (approximately 6.33 mm)

250 µg/disk: Noticeable inhibition zone (approximately 7.67 mm)

500 µg/disk: Significant inhibition zone (approximately 9 mm)

1000 µg/disk: Maximum inhibition zone observed (approximately 10 mm)

The results indicate a clear correlation between the concentration of methanol and the antibacterial activity against E. coli. As the concentration of methanol increased, the size of the inhibition zone also increased, suggesting that methanol exhibits dose-dependent antibacterial properties. Further studies could explore the mechanism of action and potential applications of methanol in antibacterial formulations.

Results from the Antibacterial Zone Inhibition Test of B. subtilis

The antibacterial activity of methanol against B. subtilis was assessed through an inhibition zone test. The results from various concentrations of methanol (µg/disk) are summarized below:

0 µg/disk: No inhibition zone observed (0 mm)
50 µg/disk: Inhibition zone measured at 7 mm
125 µg/disk: Inhibition zone measured at 8 mm
250 µg/disk: Inhibition zone measured at 9 mm
500 µg/disk: Inhibition zone measured at 10 mm
1000 µg/disk: Inhibition zone measured at 11 mm

The results demonstrated a significant dose-dependent relationship between the concentration of methanol and its antibacterial effectiveness against B. subtilis. As the concentration of methanol increased. The size of the inhibition zone also increased. indicated that higher concentrations of methanol are more effective in inhibiting bacterial growth. Further investigations should provide insights into the mechanisms of action and potential therapeutic applications of methanol as an antibacterial agent

CONCLUSION

The methanolic extract of Lantana camara exhibits promising biological activities, including anti-inflammatory, anti-cancer, and anti-bacterial properties, which suggest its potential as a therapeutic agent.

Anti-inflammatory Activity: The extract was demonstrated a concentration-dependent inhibition of albumin degradation, with higher concentrations (250–1000 µg/mL) almost completely preventing albumin breakdown. These findings revealed indicating its potential as a protease inhibitory compound in the extract, which could be beneficial in conditions where the regulation of protein degradation is crucial, such as in various inflammatory diseases.

Anti-cancer Activity: The methanolic extract was showed a dose-dependent cytotoxic effect on HepG2 liver cancer cells, with higher concentrations (500–1000 µg/mL) significantly reducing cell viability. These findings highlighted the anticancer potential of Lantana camara, suggesting that its bioactive compounds could be explored further for the development of therapeutic should be further investigated for the development of medicinal agent s against liver cancer.

Anti-bacterial Activity: The extract was exhibited strong antibacterial activity against both Escherichia coli and Bacillus subtilis. The antibacterial effect was also dose-dependent, with

larger inhibition zones observed at higher concentrations. These findings emphasised the potential of Lantana camara as a natural antibacterial agent, particularly in combating infections caused by both Gram-negative and Gram-positive bacteria.

To summarise, the methanolic extract of Lantana camara exhibits a wide range of bioactivity, indicating its prospective utility in the development of therapeutic interventions for cancer, inflammation, and bacterial infections. Future research should concentrate on isolating and characterising the specific bioactive chemicals responsible for these effects, as well as looking into their mechanisms of action and therapeutic application.

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