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Research Article

Evaluation of Phytochemicals in crude extracts derived from the Aerial parts of *Lantana camara*

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ABSTRACT

Natural products plays a crucial role in the ongoing quest for new pharmaceutical discoveries and developments. They serve as valuable sources for clinically effective drugs, act as essential precursors for synthesizing pharmaceuticals, and offer foundational compounds for designing entirely synthetic drugs. Among the vast array of natural sources, the genus Lantana stands out with approximately 2500 species distributed worldwide, renowned for their bioactive secondary metabolites and essential oils. Lantana camara, a species within this genus, has garnered attention for its rich reservoir of chemical compounds with potential pharmacological significance. The samples obtained from aqueous, petroleum ether, benzene, chloroform and ethanol underwent both qualitative and quantitative analyses to determine their phytochemical composition. Various compounds such as alkaloids, phenolic compounds, flavonoids, saponins, tannins, and cardiac glycosides were assessed in extracts from different solvents. Notably, positive results were observed for six phytochemical tests in the aqueous, ethanol, chloroform, and petroleum ether extracts of L. camara, while the benzene extract yielded positive results for five tests. Additionally, concentrations of key secondary metabolites were quantified in all extracts, with the ethanol extract showing the highest levels of phytochemicals. The primary aim of this study was to identify and evaluate the bioactive compounds present in the plant, which could potentially contribute to its therapeutic properties. The findings provide valuable insights into the chemical makeup of the plant, serving as a foundation for future

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investigations into its pharmacological effects.

INTRODUCTION

Plants serve as vital sources of medicine, offering a plethora of biological benefits such as antioxidant, antibacterial, and antifungal properties. Approximately 25% of conventional drugs and a significant portion of global healthcare rely on plants for treatment.[1] Natural antioxidants play a crucial role in combating oxidative stress caused by free radicals, offering a safe and effective means of regulation. While modern medicine predominantly utilizes synthetic or semi-synthetic antibiotics, the emergence of antibiotic-resistant microbes and the high cost of these drugs have led to an increased interest in plant extracts and their derivatives as alternative therapies.[2,3] Several research has demonstrated the therapeutic potential of various botanicals in treating chronic ailments like cancer, diabetes, inflammation, stroke, and aging, indicating plants as valuable sources for discovering novel drugs and therapeutic compounds.[4] In contrast to plant cells, human cells sometimes lack sufficient antioxidants, necessitating the use of synthetic antioxidants.[5] However, these synthetic alternatives often come with toxicity concerns, prompting the search for safer natural alternatives such as vitamins, phenolics, flavonoids, tannins, and carotenes found in plants.[6] High dietary intake of natural antioxidants has shown positive effects on chronic heart diseases and cancer, contributing to the growing demand for natural antioxidants in pharmaceuticals, nutraceuticals, and food additives.[7,8] Similar challenges exist in agriculture, where fungal and bacterial infections pose significant threats to crop yields. Throughout history, humans have relied on plants for energy and shelter, with approximately 80% of all natural products originating from plants. [9, 10] Lantana camara (Raimuniya) play a vital role in discovering and manufacturing new pharmaceuticals, either as direct ingredients or as inspiration for synthetic drugs. Belonging to the Verbenaceae family, which comprises over 100 genera and around 2600 species, Lantana camara is commonly cultivated for decorative purposes or as a boundary plant in gardens, lawns, or fields. Its various parts have been utilized in traditional medicine to address a range of ailments including itching, ulcers, inflammation, eczema, malaria, tumors, and rheumatism. [11, 12] Numerous compounds have been isolated from different parts of L. camara, including 3,24-dioxo-urs-12-en-28-oic acid, camaryolic acid, methylcamaralate, pentacyclic triterpenoids, and others such as octadecanoic acid, camaric acid, beta-sitosterol 3-O-beta-D-glucopyranoside, among others.[13] Chemical structures of these compounds were identified through various analytical techniques including spectroscopy and 2D NMR. Oleanolic acid, extracted from L. camara roots, has been found to exhibit hepatoprotective activity and can be converted into 28 → 13ß lactone through photooxidation.[14] Additionally, compounds inhibiting testosterone-5a reductase, useful in skin cosmetics and hair preparation, were isolated from L. camara roots. Flavonoids, triterpenoids, and a mixture of stigma sterol, campesterol, and 13-sitosterol were isolated from the stem of L. camara's pink-flowering taxa. Hispidulin, a flavonoid, was isolated from the genus Lantana in 1998. Moreover, the production of lactones closely related to those found in L. camara extracts has led to the development of new inhibitors for human thrombin, chymotrypsin, trypsin, and human leucocyte elastase. [15]

Fig.1: Aerial parts of Lantana camara

EXPERIMENTAL SECTION

The experiments took place in the Medicinal chemistry laboratory of the Faulty of Medical Science and Research at Sai Nath University, Ranchi, conducted between 4:00 P.M. and 5:00 P.M. daily for 2 months.

Plant materials and reagents

Fresh leaves and flowers of L. camara were collected from the campus of Sai Nath University, which is located in Ranchi, Jharkhand, India (Geographical coordinates 23° 29′ 18.47″ N, 85° 24′ 28.73″ E). The botanical identification and authentication were conducted at Shibpur Botanical Garden in West Bengal, India. To remove any unwanted dirt particles, the leaves and flowers were gently washed with tap water and then air-dried in the shade. Various chemicals including Ethanol, Petroleum ether, chloroform, benzene (for the process of extraction) and Sodium Hydroxide, concentrated Hydrochloric acid, Magnesium powder, Ferric chloride solution, Distilled water, Folin-ciocalteu reagent, Sodium bi carbonate, Potassium Iodide, Mercuric chloride and Baljet's reagent (for qualitative analysis) were supplied by Sai Nath University, all of which were of analytical grade. Working standards and samples were prepared by diluting the stock solution (1 mg/ml) in ethanol and double-distilled water, adjusting concentrations as needed for the experiment. The solvents used in the study were also of analytical grade.

Extraction procedure [16]

Freshly collected aerial parts of L. camara were underwent a meticulous washing to eliminate impurities, followed by drying in a shaded location. Once fully dehydrated, the leaves were pulverized into a fine powder using a blender. 50 grams of this powder underwent a series of extraction procedures using various solvents in a Soxhlet extractor over a 72-hour period. The solvents employed for extraction comprised petroleum ether, benzene, chloroform, ethanol, and distilled water. Post-extraction, all the acquired extracts were concentrated and meticulously preserved in airtight containers for subsequent utilization.

PHYTOCHEMICAL ANALYSIS

Qualitative Analysis

The phytochemical screening of the aerial parts of L. camara conducted following a standard method to identify the presence of flavonoids, tannins, saponins, cardiac glycosides, phenolic compounds and alkaloids.

Quantitative Analysis

To identify and quantify the phytochemicals present in the various extract of L. camara, standard methodologies were followed.

Determination of total Flavonoids [17]

The method relies on forming a complex between flavonoids and Aluminum, with its maximum absorption observed at 415nm. For the analysis, 100µl of plant extracts dissolved in methanol (10 mg/ml) were mixed with 100µl of 20% Aluminum trichloride in methanol and a drop of acetic acid. This mixture was diluted with methanol to a final volume of 5ml. After 40 minutes, the absorbance at 415nm was recorded. Blank samples were prepared using 100µl of plant extracts, a drop of acetic acid, and methanol, then diluted to 5ml. Additionally the absorbance of a standard rutin solution (0.5 mg/ml) in methanol was measured using the same procedure. All measurements were conducted in triplicate.

Determination of total Tannins [18]

To initiate the experiment, a 500 mg aliquot of the sample was meticulously weighed and introduced into a plastic bottle with a capacity of 50 ml. Following this, 50 ml of distilled water was added to the bottle, and the contents were vigorously agitated for one hour using a mechanical shaker. Subsequently, the mixture was filtered into a 50 ml volumetric flask, and the flask was topped up to the mark to ensure precise volume measurement. Following filtration, 5 ml of the filtered solution was transferred using a pipette into a test tube. Within the test tube, the solution was combined with 2 ml of 0.1 M FeCl3 solution in a mixture containing 0.1 N HCl and 0.008 M potassium ferrocyanide. The resulting solution's absorbance was then measured at a wavelength of 120 nm over a duration of 10 minutes to assess its characteristics.

Determination of total Saponins [19]

Ground samples, each weighing 20 grams, were placed into a conical flask. To this, 100 cubic centimeters of a 20% aqueous ethanol solution were added. The flask was then heated on a hot water bath, with continuous stirring at approximately 55°C, for a duration of 4 hours. Following the heating process, the mixture underwent filtration, and the residue underwent another extraction using 200 milliliters of 20% ethanol. The extracts from both processes were combined and concentrated to 40 milliliters using a water bath at approximately 90°C. The concentrated solution was then transferred to a 250 milliliter separatory funnel. To this, 20 milliliters of diethyl ether were added and vigorously shaken. The aqueous layer was separated and retained, while the ether layer was discarded. This purification step was repeated, followed by the introduction of 60 milliliters of n-butanol. The combined n-butanol extracts were washed twice with 10 milliliters of a 5% aqueous sodium chloride solution. Subsequently, the remaining solution was heated in a water bath. After evaporation, the samples were dried in an oven until a constant weight was achieved. The saponin content was then calculated based on these dried samples.

Determination of total Cardiac glycosides [20]

To determine the presence of cardiac glycosides, a 10% extract from each generation and the total extract of seeds were mixed with 10 mL of freshly prepared Baljet's reagent, composed of 95 mL of 1% picric acid and 5 mL of 10% NaOH. After one hour, the mixture was diluted with 20 mL of distilled water, and the absorbance was measured at 495 nm using a Shimadzu UV/VIS spectrophotometer model 160A (Kyoto, Japan). For the preparation of the standard curve, solutions with different concentrations (ranging from 12.5 to 100 mg/L). The total glycosides obtained from triple replicates were quantified and expressed as milligrams of L.camara per gram of dried extracts.

Determination of total Phenolic compounds [21]

A precisely measured sample extract weighing 100 milligrams was dissolved in 100 milliliters of triple distilled water (TDW). Following this, 1 milliliter of this solution was transferred to a test tube. Then, 0.5 milliliters of 2N Folin-Ciocalteu reagent and 1.5 milliliters of 20% Na2CO3 solution were added. The volume was adjusted to 8 milliliters with TDW, followed by vigorous shaking. The mixture was left to stand for 2 hours, after which the absorbance was measured at 765 nanometers. These absorbance readings were utilized to calculate the total phenolic content by referring to a standard calibration curve established using various diluted concentrations of gallic acid.

Determination of total Alkaloids [22]

5-gram portion of the sample was accurately measured and placed into a 250-milliliter beaker. Then, 200 milliliters of a 10% acetic acid solution in ethanol were added to the beaker, which was covered and left undisturbed for 4 hours.

Following this, the mixture underwent filtration, and the resulting extract was concentrated on a water bath until it reached one-quarter of its original volume. To ensure complete precipitation, concentrated ammonium hydroxide was slowly added drop by drop to the concentrated extract. The entire solution was then allowed to settle to facilitate the formation of precipitate. The formed precipitate was carefully collected and subjected to washing with dilute ammonium hydroxide. Once washed, the precipitate was filtered, leaving behind a residue containing the alkaloid. Finally, the alkaloid residue was dried and weighed for further analysis.

RESULT

Qualitative phytochemical analysis

In a qualitative examination of L. camara extracts using four different solvents (ethanol, chloroform, petroleum ether, and benzene), various phytochemical properties were assessed (Table 1). The findings revealed that each solvent produced positive outcomes in at least one of the six phytochemical tests. The ethanol, petroleum ether, and chloroform extracts of L.camara demonstrated positive results across all six phytochemical tests, indicating a broad spectrum of chemical compounds present. Conversely, the benzene extract exhibited positive results in five tests, suggesting a slightly lesser diversity of phytochemicals compared to ethanol. In summary, the ethanol extract showed the highest number of positive outcomes, followed by benzene, chloroform, and petroleum ether extracts. The investigation primarily concentrated on screening phytochemical compounds within the four solvent extracts. The compounds under scrutiny included alkaloids, phenolic compounds, flavonoids, saponins, tannins, and cardiac glycosides. These compounds are recognized as significant secondary metabolites, celebrated for their medicinal properties within the plant. Additionally, researchers conducted supplementary analytical tests to quantify the phytochemical compounds present in the extracts.

Table.1 Qualitative phytochemical analysis of Lantana camara

Compounds	Aqueous	Ethanol	Pet. Ether	Chloroform	Benzene	Corresponding Test	Results
Alkaloids	+	+	+	+	+	Mayer's Test [23]	Cream precipitate arises
Cardiac Glycosides	+	+	+	+	+	Kedde reagent Test [24]	Distinctive reddish brown Colour arises
Flavonoid	+	+	+	+	+	Shinoda Test [25]	Yellow precipitate arises
Tannins	+	+	+	+	+	Ferric chloride Test [26]	Dark greenish black Colour arises
Phenolic compounds	+	+	+	+	+	Legal's reagent Test [27]	Greenish Colour arises

Saponins	+	+	+	+	_	Foam Test [28]	Foam arises

Fig. 2
Standard extract of L.camara

Fig. 3
Test for Alkaloids

Fig. 4
Test for Cardiac glycosides

Fig. 5
Test for Flavonoids

Fig. 6
Test for Tannins

Fig. 6
Test for Phenolic compounds

Fig. 7
Test for Saponins

Quantitative phytochemical analysis

Quantitative analysis was performed on the phytochemicals present in the aerial parts extract of the plant. The results revealed differing levels of various phytochemicals within different extracts of L.camara. Notably, flavonoids, tannins and cardiac glycosides were found to be the predominant constituents in the specific aerial parts analyzed. Following them were alkaloids and phenolic compounds, as detailed in Table 2.

Conversely, the presence of saponins in this extract was observed to be minimal.

Table 2 Quantitative phytochemical analysis of Lantana camara

Phytochemicals	Aqueous extract (mg.)	Ethanolic extract (mg.)	Pet. ether extract (mg.)	Chloroform extract (mg.)	Benzene extract (mg.)
Alkaloids	11.92 ± 0.25	17.12± 0.13	18.20 ± 0.10	14.29 ± 0.30	12.22 ± 0.29
Cardiac glycosides	12.32 ± 0.14	18.40 ± 0.25	17.60 ± 0.15	15.90 ± 0.50	11.60 ± 0.35
Flavonoids	11.20 ± 0.13	16.63 ± 0.24	15.50 ± 0.12	13.90 ± 0.55	14.01 ± 0.70
Tannins	12.50 ± 0.1	13.60 ± 0.29	15.90 ± 0.1	12.77 ± 0.90	13.07 ± 0.2
Phenolic compounds	8.50 ± 0.15	9.11 ± 0.30	12.01 ± 0.1	12.88 ± 0.90	12.77 ± 0.7
Saponins	6.2 ± 0.10	7.1 ± 0.5	5.01 ± 0.21	2.88 ± 0.90	-

The aqueous extract was analyzed and found to contain 11.92 mg of alkaloids, 12.32 mg of cardiac glycosides, 11.20 mg of flavonoids, 12.50 mg of tannins, 8.50 mg of phenolic compounds, and 6.2 mg of saponins. Similarly, the ethanol extract showed 17.12 mg of alkaloids, 18.40 mg of cardiac glycosides, 16.63 mg of flavonoids, 13.60 mg of tannins, 9.11 mg of phenolic compounds, and 7.1 mg of saponins. The petroleum ether extract exhibited 18.20 mg of alkaloids, 17.20 mg of cardiac glycosides, 15.50 mg of flavonoids, 15.90 mg of tannins, 12.01 mg of phenolic compounds, and 5.01 mg of saponins. Furthermore, the chloroform extract contained 14.29 mg of alkaloids, 15.90 mg of cardiac glycosides, 13.90 mg of flavonoids, 12.77 mg of tannins, 12.88 mg of phenolic compounds, and 2.88 mg of saponins. Lastly, the benzene extract was found to contain 12.22 mg of alkaloids, 11.60 mg of cardiac glycosides, 14.01 mg of flavonoids, 13.07 mg of tannins, and 12.77 mg of phenolic compounds.

All the above data are combined and shown in Chart number 1.

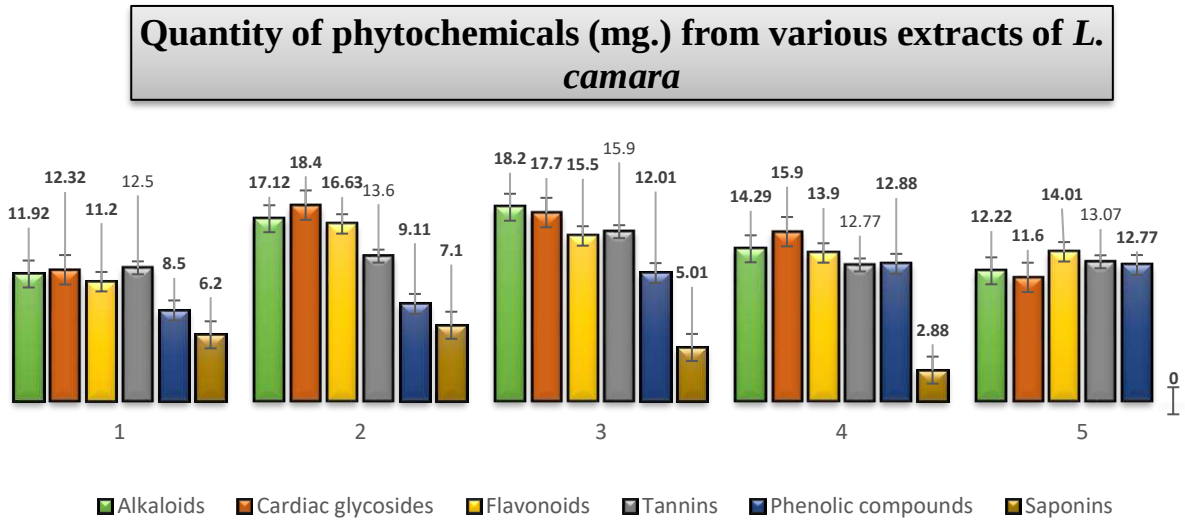

Chart.1: Quantitative phytochemical analysis of L. camara

DISCUSSION

The bioactive compounds discovered in Lantana camara show promising medicinal properties. Alkaloids exhibit various biological effects, including antimicrobial and antioxidant activities, as well as providing pain relief and antimalarial effects. They also influence the central nervous system and hold potential in combating inflammation, oxidation, and cancer. Additionally, cardiac glycosides primarily improve cardiac contractility by inhibiting the sodium-potassium ATPase pump, leading to increased intracellular sodium levels. This results in enhanced myocardial function and greater cardiac output. On the contrary, flavonoids possess anti-inflammatory properties and may hinder cancer cell growth. Phenolic compounds and tannins are associated with antioxidant properties and liver protection. Moreover, the presence of saponins in Lantana camara suggests potential anti-tumor effects. These findings highlight the importance of further exploring the pharmacological activities within Lantana camara. [29, 30, 31, 32, 33, 34]

CONCLUSION

The research of the chemical constituents present in the aerial parts of Raimuniya (Lantana camara) has revealed a rich variety of bioactive compounds with potential therapeutic benefits. Among these are alkaloids, cardiac glycosides, flavonoids, phenolic compounds, tannins, and saponins, each playing a role in the plant's medicinal properties. These findings provide a foundation for further exploration into the pharmacological effects and potential applications of Lantana camara in natural medicine. However, further research is necessary to understand the specific mechanisms through which these bioactive components function and to assess their safety and efficacy.

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