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Phytochemical Screening of Lantana Camara

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Abstract

Phytochemicals are biologically active compounds derived from plants that contribute significantly to human health and disease prevention. The present study focuses on the phytochemical, physicochemical, and pharmacological evaluation of Lantana camara leaves, along with an overview of supercritical fluid extraction as an advanced extraction technique. Qualitative and quantitative analyses revealed the presence of important bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, terpenoids, saponins, steroids, phenolics, and anthraquinones. The total phenolic content (318.63 mg/g GAE) and total flavonoid content (284.49 mg/g RE) were higher in the 50% ethanolic extract compared to the aqueous extract, indicating superior extraction efficiency with hydroalcoholic solvents. Proximate analysis demonstrated that the leaves contain appreciable amounts of crude protein (24.84%), crude fibre (16.41%), ash (10.77%), and moisture (10.15%), with low crude fat content (2.99%). Mineral analysis confirmed the presence of essential elements such as potassium, calcium, iron, magnesium, manganese, copper, phosphorus, and sulphur, while zinc was not detected. Anti-nutritional factors including phytate, tannins, and oxalate were also identified. Physicochemical parameters such as total ash, acid-insoluble ash, extractive values, and loss on drying complied with standard quality control measures. Pharmacological investigations reported significant antiulcerogenic, antioxidant, anti-inflammatory, antibacterial, antifungal, anticancer, antimotility, antifilarial, antiurolithiatic, cardiovascular, antitubercular, and mosquito larvicidal activities of various extracts and essential oils of Lantana camara. These biological effects are attributed to the presence of diverse secondary metabolites, including lantadenes and pentacyclic triterpen.

Keywords: *Phytochemicals, Lantana camara, medicinal plants, phytochemical screening, pharmacological properties, secondary metabolites.*

1. INTRODUCTION

The term “phyto” in phytochemicals comes from the Greek word “phyto” meaning plant. therefore, phytochemicals are chemicals derived from plants. [1] Phytochemicals are biologically active, naturally occurring chemical compounds present in all plants that offer health benefits to humans by acting as medicinal substances and nutrients. Phytochemicals are classified into primary and secondary metabolites based on their function in plant metabolism. Primary metabolites include common sugars, amino acids, proteins, purines and pyrimidines found in nucleic acids, chlorophyll, and similar essential compounds. Secondary metabolites consist of other plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, phenolics, and glucosides. Literature surveys show that phenolic compounds are the most abundant and structurally diverse phytochemicals found in plants [2]. Today's world phytochemicals are more popular due to their many medicinal uses. Phytochemical plays a important role against number of disease. Eg.- asthma, arthritis, cancer, etc. It does not have any side effect [3]. Medicinal plants are a natural gift that offer numerous health benefits to humans. In our country, these plants have been used for centuries because of their medicinal properties and continue to be used even today. India possesses a wide range of traditional medical systems such as Ayurveda, Siddha, Unani, and a vast body of ethnomedicine [4]. Various parts of plants such as bark, flowers, fruits, leaves, resins, rhizomes, roots, seeds, and stems are used in traditional medicine [5]. Plants produce secondary metabolites such as essential oils, phenolic compounds, terpenoids, alkaloids, steroidal compounds, glycosides, terpenes, and tannins, which are responsible for a wide range of therapeutic effects.

These plant constituents exhibit several beneficial physiological activities, including antioxidant, anti-inflammatory, and anti-atherosclerotic properties [6]. Numerous studies have shown that organic good products possess a wide range of biological activities, including stimulation of the immune system, as well as antibacterial, antiviral, anti-hepatotoxic, anti-inflammatory, antioxidant, antimutagenic, and anticancer effects [7].

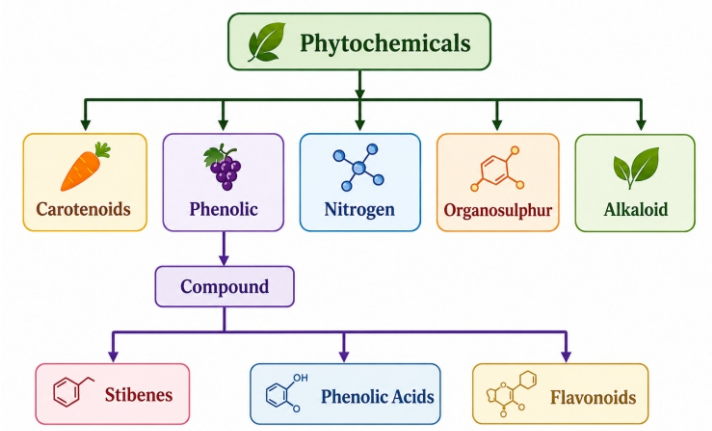

Figure 1: Classification Of Phytochemicals

A supercritical fluid (SCF) is a substance maintained at a temperature and pressure exceeding its critical point, under which conditions separate liquid and gaseous phases no longer exist [8]. Supercritical fluid extraction has received growing attention compared to conventional methods such as steam distillation and liquid solvent extraction due to its use of non-toxic and volatile solvents like carbon dioxide [9]. The primary limitation of the SCF process is its high production cost, which is not only attributed to the requirement of high-pressure equipment [10]. A broad range of solvents can be used as supercritical fluids (SFs), including carbon dioxide, nitrous oxide, ethane, propane, n-pentane, ammonia, fluoroform, sulfur hexafluoride, and water. Among these, carbon dioxide is most commonly preferred because it readily attains supercritical conditions and offers significant advantages over other fluids, such as low toxicity, non-flammability, low cost, and high purity [11].

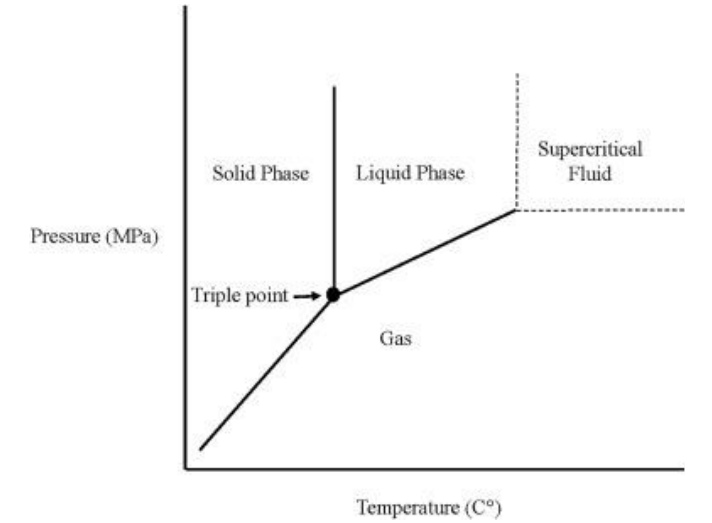

Figure 2: A schematic diagram showing the scf

The application of supercritical fluids (SCFs) in the analysis of fat-soluble vitamins represents an effective alternative to traditional organic solvents. The key benefits of employing supercritical fluids over conventional solvents include reduced use of organic solvents, elimination of oxygen exposure, and lower thermal stress. Modern supercritical fluid extraction (SFE) techniques provide shorter extraction durations, potentially improved selectivity, and greater sample processing capacity (owing to automated systems) when compared with conventional solvent extraction methods [12].

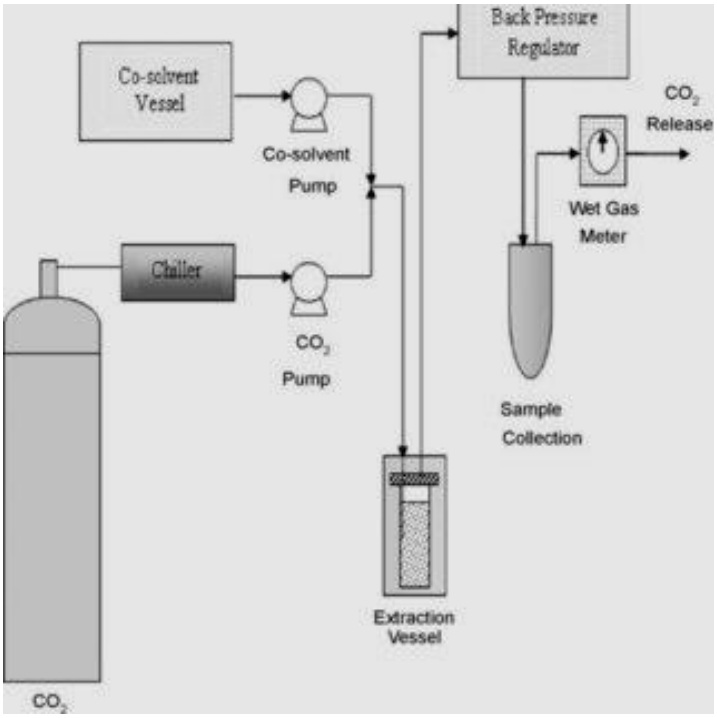

Figure 3: flow diagram of the Supercritical Fluid Extraction

Lantana camara is a well known ornamental garden plant that grows to a height of about 2–4 meters and bears flowers in various colors such as yellow, red, pink, and white (Figure 1). It also occurs naturally along roadsides and riverbanks at elevations up to 2,000 meters in tropical and subtropical regions [13]. Flowers occur in many different colours, such as Red, Yellow, White, Pink, and Orange, and these variations depend on their position in the inflorescence, as well as their age and stage of maturity. The flower emits a tutti-frutti aroma with a subtle peppery undertone. Phytochemical investigations of Lantana camara have revealed the presence of flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, isocatechins, alkaloids, tannins, saponins, and triterpenoids [14].

Taxonomy [15]
Kingdom Plantae
Subkingdom Tracheobionta
Superdivision Spermotophyta
Division Magnoliophyta
Class Magnoliopsida
Subclass Asteridac

Order Lamiales
Family Verbenaceae
Genus Lantana
Species Lantana Camara

The chemical constituents of Lantana camara include caryophyllene, 1-α-phellandrene, lantadene A, lantadene B, lancamarone, quinine, and lantanine [16]. The leaves possess medicinal properties and provide therapeutic effects in the treatment of cuts, ulcers, catarrhal infections, tetanus, rheumatism, malaria, cancer, chickenpox, asthma, swelling, eczema, tumors, high blood pressure, sores, measles, and fever [17].

Physicochemical Analysis of L.C. Leaf

2. Preliminary Phytochemical Analysis

2.1 Quantitative Analysis Of Phytochemicals

The total phenol content was determined using the method described by Edeoga et al. (2005). Tannin content was estimated following the procedure of Van-Burden and Robinson (1981). Saponin content was determined according to the method of Obdoni and Ochuko (2001). Alkaloid content was analyzed using the method described by Harborne (1973). Flavonoid content was determined following the procedure of Bohm and Kocipai-Abyazan (1994). Qualitative analysis of vitamins was carried out using the method described by Pearson (1976) [18].

2.2 Qualitative Analysis Of Phytochemicals

Using standard procedures, phytochemicals such as flavonoids, alkaloids, steroids, terpenoids, glycosides, saponins, catechol tannins, anthocyanins, carbohydrates, and amino acids were detected in the methanolic extract of Lantana camara leaves [19].

2.2.1 Test For Alkaloids

A 2 mL sample of the plant extract was acidified with a few drops of dilute hydrochloric acid. To this acidic solution, 1 mL of Dragendorff's reagent (potassium bismuth iodide) was added to test for the presence of alkaloids. The formation of an orange or reddish-brown precipitate indicates the presence of alkaloids [20].

2.2.2 Test For Saponins

One gram of each powdered, dried sample was boiled separately with 10 mL of distilled water in a water bath for 10 minutes. The hot mixture was filtered and then allowed to cool. The following tests were subsequently performed :

Test for frothing : A 2.5 mL portion of the filtrate was diluted to 10 mL with distilled water and shaken vigorously for 2 minutes. The formation of persistent froth indicated the presence of saponins in the filtrate.

Test for emulsifying properties : Two drops of olive oil were added to the solution prepared by diluting 2.5 mL of the filtrate to 10 mL with distilled water (as described above). The mixture was shaken vigorously for a few minutes. The formation of a relatively stable emulsion confirmed the presence of saponins [21].

2.2.3 Test For Flavonoids

1ml of the extract was treated with 1 mL of 10% lead acetate solution ($Pb(OAc)_4$). The development of an intense yellow coloration in the plant extract indicated the presence of flavonoids [22].

2.2.4 Test For Tannins

A few drops of 10% lead acetate solution were added to 2 mL of each extract. The formation of a white precipitate indicated the presence of tannins [23].

2.2.5 Test For Terpenoids

2 ml of the extract were combined with 2 mL of acetic anhydride, after which 2–3 drops of concentrated H_2SO_4 were carefully added. The formation of a reddish-brown coloration at the interface indicated the presence of terpenoids [24].

2.2.6 Test For Molisch's

A small quantity of the extract was treated with an alcoholic solution of α-naphthol and shaken thoroughly. Concentrated H_2SO_4 was then carefully added along the side of the test tube. The formation of a violet ring at the interface of the two liquids was observed and indicated a positive result [25].

2.2.7 Test For Steroids

1 Mg of the crude plant extract was placed in a test tube and dissolved in 10 mL of chloroform. An equal volume of concentrated sulphuric acid was then carefully added along the sides of the test tube. The upper layer turned red, while the sulphuric acid layer appeared yellow with green fluorescence, indicating the presence of steroids [26].

2.2.8 Test For Xanthoproteic

The extracts were treated with a few drops of concentrated nitric acid. The appearance of a yellow coloration indicated the presence of proteins [27].

2.2.9 Test For Carbohydrate

100 mg of the plant extract were dissolved in 5 mL of water and then filtered. To 0.5 mL of the filtrate, 0.5 mL of Benedict's reagent was added, and the mixture was heated in a boiling water bath for 3 minutes. The development of a characteristic color indicated the presence of carbohydrates [28].

2.2.10 Test For Ninhydrine

A 0.25% ninhydrin reagent was added to the extract and the mixture was boiled for a few minutes. The development of a blue color confirmed the presence of amino acids [29].

2.2.11 Test For Coumarin

Precisely 1 mL of 10% sodium hydroxide was added to 0.2 g of each plant extract. The development of a yellow coloration indicated the presence of coumarin [30].

2.2.12 Test For Cardiac Glycosides

5 ml of each plant extract were treated with 2 mL of glacial acetic acid containing a drop of $FeCl_3$ solution. Concentrated H_2SO_4 (1 mL) was then carefully added beneath the mixture. The formation of a brown ring at the interface indicated the presence of deoxy-sugars characteristic of cardenolides, thereby confirming the presence of cardiac glycosides (Akinyemi et al., 2005; Naz & Bano, 2013) [31].

2.2.13 Test For Resins

10 ml of 1% copper acetate solution were added to 10 mL of the diluted extract, and the mixture was shaken vigorously. The formation of a distinct green color indicated the presence of resin (Sofowara, 1993) [32].

2.2.14 Test For Anthraquinines

A 0.5 g portion of the extract was shaken with 10 mL of benzene. The mixture was then filtered, and 5 mL of 10% ammonia solution was added to the filtrate. After shaking the final mixture, the appearance of a pink, red, or violet color in the ammoniac (lower) layer indicated the presence of anthraquinones (Brain and Turner, 1975) [33].

3. Medicinal / Pharmacological Properties Of Lantana Camara

3.1 Antiulcerogenic Activity

The methanol extract of Lantana camara leaves exhibited antiulcerogenic effects against gastric lesions induced by aspirin, ethanol, and cold restraint stress in rats. Pretreatment of the rats with the extract at doses of 200 and 400 mg/kg body weight produced a significant protective effect in all three ulcer models. The extract demonstrated a dose-dependent antiulcerogenic activity across all tested models [34].

3.2 Antioxidant Activity

The ethanolic extract of L. camara demonstrated significant antioxidant activity in in vivo studies. Treatment with the extract reduced lipid peroxidation in the kidneys of urolithic rats. In vitro studies were conducted using the DPPH radical scavenging assay and the nitric oxide free radical scavenging assay, where the extract showed strong antioxidant activity in both assays. The antioxidant potential of Lantana Camara leaves was also reported through reducing power activity and the DPPH radical scavenging assay. The leaf extracts exhibited notable antioxidant effects, with younger leaves showing stronger antioxidant activity compared to older or mature leaves [35].

3.3 Antifungal Activity

The antifungal activity of the essential oil was assessed using the Disc Diffusion Method, following the guidelines of CLSI (Clinical and Laboratory Standards) M44-A2 (2009). Candida krusei ATCC 6258, kindly provided by the Adolfo Lutz Institute of São Paulo, along with a clinical isolate of Candida albicans obtained from patients with onychomycosis in Teresina, Piauí, were used in the study. Approximately 100 mg of the essential oil was dissolved in distilled water with 20% acetone to prepare concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL. Antifungal activity was determined by measuring the zones of inhibition around the oil. All analyses were conducted under strict aseptic conditions. The diameters of the inhibition zones were recorded using an antibiotic zone scale (Cecon-Brasil), and each experiment was performed in triplicate. Standard antifungal agents (fluconazole 25 µg, itraconazole 10 µg, amphotericin B 2 µg, and terbinafine 2 µg – Cecon-Brasil) served as positive controls, while 20% water/acetone was used as the negative control [36].

3.4 Anti-Inflammatory Activity

The carrageenan-induced paw oedema model was employed to evaluate the anti-inflammatory activity of Lantana extract. Inflammation in the hind paw of rats was measured in millimeters, and the changes were recorded as variations. The findings demonstrated that Lantana possessed significant anti-inflammatory properties.In another study, the aqueous leaf extract of Lantana was also assessed for anti-inflammatory effects in albino rats. Various doses were administered, and the 500 mg/kg body weight dose notably decreased paw volume in the carrageenan-induced paw oedema test. Overall, Lantana leaf extracts exhibited promising analgesic and anti-inflammatory potential [37].

3.5 Antibacterial Activity

Different varieties of Lantana camara leaves and flowers have been investigated for their antibacterial properties. Three solvent extracts prepared from the leaves and flowers of four distinct varieties of Lantana camara demonstrated notable antibacterial activity against Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa, while showing comparatively weak activity against Staphylococcus aureus.

Ethanolic extracts obtained from the leaves and roots of Lantana camara were also evaluated for antibacterial effects. The in vitro antibacterial assessment was carried out using the microdilution technique. These extracts exhibited antimicrobial activity against Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio cholerae, Escherichia coli, including two multidrug-resistant strains of Escherichia coli and Staphylococcus aureus.

Furthermore, methanolic extracts from various parts of Lantana camara were tested for antimicrobial activity against ten bacterial and five fungal species using both the disk diffusion and broth microdilution methods. Among the tested parts, the leaf extract exhibited the strongest activity against the Gram-positive Bacillus cereus and the Gram-negative Salmonella typhi [38].

3.6 Anticancer Activity

Badakhsan et al. (2011) evaluated the in vitro anticancer activity of L. camara using different solvent extracts,

including petroleum ether, chloroform, ethanol, and aqueous extracts, and found that the ethanolic extract exhibited the most significant effect. The anticancer potential of root and leaf extracts was assessed against Jurkat leukemia cells using the MTT assay. The results suggest that the root and leaf extracts may be further investigated for the identification and fractionation of novel anticancer compounds [39].

3.7 Mosquito Controlling Activity

The methanol and ethanol extracts of leaves and flowers of L. camara have been reported to show mosquito larvicidal activity against the 3rd and 4th instar larvae of Ae. aegypti and Cx. quinquefasciatus. Both extracts demonstrated significant larvicidal effects on both mosquito species; however, at lower concentrations (1 mg/ml), the extracts were more effective against Ae. aegypti compared to Cx. quinquefasciatus.The essential oil obtained from the leaves of L. camara was also found to exhibit adulticidal activity against Aedes aegypti, Culex quinquefasciatus, Anopheles culicifacies, An. fluvialitis,and An. stephensi. The LD_{50} values were recorded as 0.06, 0.05, 0.05, 0.05, and 0.06 mg/cm², while the LD_{90} values were 0.10, 0.10, 0.09, 0.09, and 0.10 mg/cm² against Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluvialitis,and An. stephensi respectively [40].

3.8 Antifilarial Activity

The stem extract of lantana was evaluated for its anti-filarial properties. When administered at a dose of 1 g/kg for five days, the crude stem extract resulted in the death of approximately 43.05% of adult Brugia malayi parasites. Remarkable anti-filarial activity was also demonstrated by the essential oil derived from the lantana stem against adult Brugia malayi. At the same dosage (1 g/kg), nearly 80% of the adult worms were eliminated in the gerbil model. The anti-filarial effects may be attributed to the presence of oleanonic acid and oleanolic acid [41].

3.9 Wound Healing Activity

Wounds are among the earliest medical challenges encountered by humankind. Despite advancements in medicine, knowledge regarding wounds and their proper management remains relatively underdeveloped. A wound refers to the disruption of normal tissue structure caused by physical, chemical, microbial, or immunological injury, and it heals either through regeneration or through fibroplasia. The alcoholic extract of the stem and bark of Annona muricata demonstrated significant wound healing activity, as evidenced by a marked reduction in wound area in albino rats, indicating its potential usefulness in wound management [42].

3.10 Cardiovascular Activity

The cardiovascular effects of the ethanolic extract of Lantana camara leaves were assessed using various experimental models. The extract exhibited both negative inotropic and negative chronotropic effects. It was found to decrease the workload of the heart, regulate inotropic activity through its negative chronotropic action, and promote relaxation of smooth muscles [43].

3.11 Antimicrobial Activity

Nutrient agar was prepared by dissolving 14 g of the medium in 500 ml of distilled water, followed by autoclaving for 30 minutes. The sterilized medium was then poured into Petri plates and allowed to solidify. After solidification, a selected microorganism was evenly spread over the surface using an L-rod. Wells were then made in the agar, ensuring that no more than three wells were created per plate and that a minimum distance of 1 cm was maintained between each well using a borer. AgNP samples were added into the wells with the help of a micropipette, and the plates were incubated at 37°C for 24 hours. Subsequently, the zones of inhibition were measured in millimeters. Antibacterial activity was evaluated based on the clear inhibition zones formed around the discs containing plant extract and synthesized AgNPs [44].

4. Result & Discussion

4.1 Biochemical Characterization–screening of Phytochemicals, TPC & TFC :

Qualitative phytochemical screening was carried out to detect phenolic constituents, specifically alkaloids, flavonoids, tannins, saponins, and terpenoids. The analysis revealed that alkaloids, flavonoids, and tannins were present in both extracts of Lantana camara, while saponins and terpenoids were absent. Additionally, the total phenolic content (TPC) and total flavonoid content (TFC) were higher in the 50% ethanol extract than in the aqueous leaf extract of L. Camara [45].

Table No. 1:

Solvent Extract	TPC (mg/g GAE)	TFC (mg/g RE)
Distilled Water	166.56	107.15
50% ethyl Alcohol	318.63	284.49

TPC & TFC Of L.Camara Leaf extract

All Values are mean of triplicate.
GAE : Gallic acid Equavalent
RE : Rutin Equivalent

4.2 Proximate Analysis Of L.Camara Leaf

The findings of the proximate analysis of Lantana camara leaves are presented in Table 2. The leaf was found to contain high levels of crude protein (24.84%), crude fibre (16.41%), ash (10.77%), and moisture (10.15%), whereas crude fat (2.99%) was present in a relatively low amount.

Table 3 presents the mineral composition of Lantana camara leaves. The analysis indicated the presence of phosphorus (0.07 ± 0.01 ppm), calcium (0.5 ± 0.01 ppm), manganese (0.99 ± 0.02 ppm), sulphur (0.73 ± 0.03 ppm), potassium (1.05 ± 0.02 ppm), iron (0.84 ± 0.01 ppm), magnesium (0.43 ± 0.03 ppm), and copper (0.53 ± 0.01 ppm), while zinc was not detected.

The anti-nutritional constituents of Lantana camara are shown in Table 3. The results revealed that the leaf contains phytate (41.06 mg/100 g), tannins (3.35 mg/100 g), and oxalate (280.75 mg/100 g) as anti-nutritional factors [46].

Table No. 2:

Proximate Indices	Composition
Moisture	10.15 ± 0.57
Crude Protein	24.84 ± 0.51
Crude Fibre	16.41 ± 0.58
Crude Fats	2.99 ± 0.01
Ash	10.77 ± 0.43

Values are expressed as Mean ± SEM (n=3)

Table No. 3:

Minerals	Composition (ppm)
Phosphorous	0.07 ± 0.01
Calcium	0.54 ± 0.01
Sulphur	0.73 ± 0.03
Potassium	1.05 ± 0.02
Iron	0.84 ± 0.01
Zinc	Nd
Magnesium	0.43 ± 0.03
Copper	0.53 ± 0.01
Manganese	0.99 ± 0.02

Values are expressed as mean ± SEM (n=3)
Nd : Not Detected

Table No. 4:

Antinutrients	Composition (mg/100g)
Phytate	41.06 ± 0.15
Tannins	3.35 ± 0.05
Oxalate	280.75 ± 1.06

Values are expressed as Mean ± SEM (n=3)

4.3 Physicochemical analysis of L. Camara

In physicochemical analysis, parameters such as moisture content, loss on drying, total ash, acid-insoluble ash, alcohol-soluble extractive value, and water-soluble extractive value are evaluated. The ash value is used to determine the quality and purity of a crude drug, as it reflects the presence of impurities like carbonates, oxalates, and silicates. Water-soluble ash helps estimate the amount of inorganic compounds present in the drug, whereas acid-insoluble ash mainly represents silica and indicates contamination with earthy materials. Maintaining low moisture content is essential to prevent the growth of bacteria, yeast, and fungi during storage.

The estimation of extractive values determines the quantity of active constituents present in a specific amount of plant material when extracted with a particular solvent. Extraction of a crude drug with a suitable solvent yields a solution containing various phytoconstituents. The composition of these phytoconstituents, which depends on the nature of the drug and the solvent used, indicates whether the crude drug has been completely exhausted (Tatiya et al., 2012).

The results of the physicochemical parameters, including description, loss on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash, alcohol-soluble extractive, and water-soluble extractive, are presented [47].

4.4 Qualitative Analysis Of Vitamins

Vitamins are organic compounds required in very small amounts for the body's growth and proper functioning. They are naturally obtained from both plant and animal sources. In this context, “organic” refers to their chemical structure, meaning that their molecules contain carbon. It also implies that vitamins can be damaged and lose their effectiveness in the body. Excessive heat, certain types of light, and exposure to oxygen can destroy some vitamins. The quantities of vitamins consumed through food are measured in micrograms or milligrams.

Vitamin C also known as ascorbic acid, is a vitamin that humans cannot produce on their own and must obtain from food. It helps maintain cell structure, promotes wound healing, and supports the development of bones and teeth. Rich sources of vitamin C include citrus fruits, strawberries, melons, and leafy green vegetables. In addition, vitamin C aids in the absorption and utilization of iron. To prevent the loss of vitamins in fruits and vegetables, simple preservation methods such as refrigeration, washing before cutting, storing in airtight containers, and avoiding prolonged exposure to high temperatures during cooking should be practiced (Okwa, 2003). The analysis of the vitamins present in Lantana camara leaves, as summarized in Table 8, revealed the presence of vitamins A, C, and E, while vitamin D was not detected [48].

Table 5. Anti-nutrient Content of Lantana Camara leaf [49]

Anti-Nutrients	Composition (mg/100g)
Phylate	41.06 ± 0.15
Tannins	3.35 ± 0.05
Oxalate	280.75 ± 1.06

Values are expressed as mean ± SEM (n=3)

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