

Pharmacognostic, Phytochemical, and Multi-analytical Profiling of the Leaves and Fruit of Terminalia Catappa Integrated With in-silico Docking Studies

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Abstract

Background: *Terminalia catappa* L (Indian almond), a tropical medicinal tree, has traditionally been used for its therapeutic properties. Despite its ethnopharmacological relevance, comprehensive validation of its bioactive constituents and pharmacological potential remains underexplored.

Objective: This study aimed to evaluate the pharmacological, physicochemical, phytochemical, microbial, and metabolomic attributes of *T. catappa* leaves and fruits, along with *in silico* docking, to assess its antiepileptic potential.

Methods: Pharmacobotanical studies included macroscopic, microscopic, and physicochemical analyses. Heavy metal and microbial loads were assessed using AAS and USP guidelines. Phytochemical profiling employed GC-MS, LC-MS, and HPTLC to identify bioactive compounds. *In silico* docking (Schrödinger) targeted epilepsy-related receptors: dopamine D2, serotonin 5-HT2A, NMDA, and GABA_A.

Results: Pharmacognostic evaluation revealed distinct anatomical features (trichomes, lignified cells). Phytochemical screening highlighted tannins, flavonoids, and cardiac glycosides. LC-MS identified key compounds, including gallic acid, ellagic acid, quercetin, and punicalagin. HPTLC confirmed their presence in leaves (ellagic acid: R_f 0.306; gallic acid: R_f 0.881; kaempferol: R_f 0.073; quercetin: R_f 1.050) and fruits (gallic acid: R_f 0.47; ellagic acid: R_f 0.81; rutin: R_f 0.10) with spectral correlations ($r = 0.824$) and methodological consistency (R_f deviations <10%). Derivatization with ANS reagent enhanced phenolic visualization. GC-MS detected 43 (leaf) and 50 (fruit) compounds, including phenolics and terpenoids. Heavy metals were within limits, except trace Pb in fruits. Microbial counts met safety standards. Docking highlighted rutin as a promising ligand, strongly binding to dopamine D2 (-8.215 kcal/mol) and NMDA receptors (-6.227 kcal/mol), suggesting antiepileptic potential.

Conclusion: Integrating GC-MS, LC-MS, and HPTLC data validated *T. catappa*'s rich phytochemical diversity. The prominence of gallic acid, ellagic acid, and rutin, corroborated by spectral and docking results, underscores its potential in managing epilepsy and oxidative stress. Further pharmacological studies are required to translate these findings into clinical applications.

1.0 INTRODUCTION

It is universally acknowledged that the importance of herbal medicines and phytonutrients is continually growing worldwide, with many individuals focusing on these naturally occurring substances to treat various health problems [1]. Approximately four billion individuals living in undeveloped nations still manage their medical ailments using herbal and traditional methods [2, 3]. Herbal remedies are made up of plant parts or plant extracts that include various phytochemicals thought to have medicinal benefits [4].

Worldwide, traditional medicine makes extensive use of *Terminalia*, a genus of plants in the Combretaceae family [5]. These geniuses, which number over 200 species, are known for their high tannin content, which might explain their many therapeutic qualities [6]. Fever, headaches, pneumonia, geriatric, flu, cancer, stomach pain, back pain, memory enhancement, cough and cold, diarrhoea, conjunctivitis, heart disease, sexually transmitted infections, leprosy, and urinary tract disorders are just a few of the maladies that the *Terminalia* genus may cure [7].

In tropical and subtropical areas, *Terminalia catappa* L. is widely distributed. Common names for it include red almond, wild badam, tropical almond, sea almond, and Indian almond [8]. Many important phytochemicals identified and isolated from *T. catappa*, i flavonoids like (isovitexin, vitexin, and rutin), triterpenoids (ursolic acid and asiatic acid), gallic acid, squalene, and hydrolysed tannins like punicalagin component, punicalin, tergalagin, tercatatin, chebulagic acid, corilagin, terflavin A and B [9]. The seed section is edible and includes fixed oil, mainly oleic and linoleic, olein, palmitin, protein, fat, carbohydrate, and the bark contains tannin, according to the literature [10, 11]. According to peer-reviewed articles, the nut portion has reported phytochemicals such as methyl laurate, methyl palmitate, syringol, palmitic acid, palmitoleic acid, glutamine, and methyl oleate [12]. Conversely, fatty acids identified from seed oil include 9,12-hexadecadienoic acid, hexadecanoic acid, octadecanoic acid, 9,12-octadecadienoic acid,, 9-octadecenoic acid, 10-octadecenoic acid, tetracosanoic acid and docosanoic acid [13]. Various plant parts have traditionally been used for antimicrobial, anthelmintic, antidiabetic, antifungal, and cytotoxicity purposes [14, 15]. *T. catappa* is one of the most often utilised herbs in Ayurveda, earning it the title of "King of Medicine" [16, 17]. *T. catappa* is effective in the treatment of inflammatory illnesses, wound healing, allergies, skin issues, asthma, ulcers, cardiovascular diseases, diarrhoea, and other ailments, according to Ayurveda and Siddha [18]. It is also claimed to aid in restoring the senses' strength. Various pharmacological properties have been reported from various parts of the plant, including antimicrobial, antioxidant, antifertility, larvicidal, immunostimulant, hypolipidemic, hypoglycaemic, anti-inflammatory, diuretic, antihypertensive, cardiac stimulant, antispasmodic, anti-anasarca, antipyretic, analgesic, anthelmintic, etc [19–25].

Although there is much research on *T. catappa*, there are still a lot of phytochemical constituents to identify and comprehend its therapeutic effect in diverse activities. The study's current goal is to look into the pharmacognostic, phytochemical, and analytical evaluation of *T. catappa* leaves and fruits. Furthermore, a comprehensive analysis of metabolites will be carried out using GC-MS analysis and in-silico docking studies of ethanolic extract of leaves and fruit. Additionally, as a quality control standard, a *Terminalia catappa* authenticity parameter could be created using all of this information.

2.0 MATERIALS AND METHODS

2.1 Authentication and collection of Plant material

Terminalia catappa leaves and fruit were gathered from a fully grown tree in Hyderabad, Telangana state, India, and authenticated by Botanica Survey of India, Deccan Regional Centre, Hyderabad

(BSI/DRC/2022-23/Identification/39, dated 18-04-2023). For future use, a voucher specimen has been stored in our research lab.

2.2 Plant materials grating and extract preparation

Prior to being ground into a fine powder for use in sensory, macroscopic, microscopic, physicochemical, fluorescence, heavy metal, and microbiological investigations, the collected plant materials were first gently cleaned in tap water to remove any dirt, and then they were shade-dried in the lab for two weeks at room temperature ($24\pm 2^{\circ}\text{C}$) to create a coarse powder. Various plant extracts were prepared for initial phytochemical analysis. The dried plant material was first ground in a mechanical grinder before being sieved through sieve number 40 to create a coarse powder. The coarsely crushed plant material was then gradually extracted using petroleum ether (60°C to 80°C), ethanol (99.99%), and deionised distilled water in a Soxhlet device. A rotary vacuum evaporator was used to extract the residual solvents, and the semisolid residues were dried and concentrated. Prior to GC-MS analysis, the coarsely ground plant material was extracted separately using ethanol (90% v/v) for 48 hours using a Soxhlet extractor. After the surplus solvents were removed using a rotary vacuum evaporator, the crude ethanol extract was dried and concentrated for analysis.

2.3 Pharmacobotanical enactment

The fresh leaves stems, fruits and powders of *Terminalia catappa* were investigated macroscopically and microscopically for pharmacobotanical features. The plant materials purity profile was investigated using several physicochemical parameters, as well as heavy metal and microbiological analyses.

2.3.1 Sensory and macroscopic anatomization

Various types of parameters evaluated for the fresh leaves were leaf colour (fresh and dried), shape, size, venation, margin, base, apex, texture, fracture, odour, and taste; similarly, the shape, size, gloss, and vibrant colour of the fruits were also closely examined; furthermore, only colour, odour, and taste were evaluated for the powder [26, 27].

2.3.2 Qualitative microscopic exploration

Using a sharp razor blade, the fresh plant parts (leaf and stem) were split into transverse sections. After softening the sectioned matrix in distilled water, it was stained with safranin (0.5%v/v), followed by glycerol. The observation was analysed using a compound microscope. Transverse sections were extensively contemplated to identify epidermal cells' structure and distribution, vascular bundles, stomata, and trichomes [28].

2.3.3 Assessment of Quantitative microscopy

Leaf constants like stomatal number, stomatal index, palisade ratio, vein termination number, and vein islet number were assessed using quantitative microscopy as per the Ayurvedic Pharmacopoeia of India.

[29].

2.3.4 Powder analysis

By treating the sample (dry fruit and leaf powder) independently with the various reagents and mounting it on glass slides using glycerol for powder microscopy, cell walls and other components in the powdered sample were identified histochemically. Phloroglucinol and concentrated hydrochloric acid (1:1, v/v) were utilized for lignified cell walls; calcium oxalate crystals were treated with 60% chloral hydrate and 25% concentrated sulfuric acid; starch and aleurone grains were treated with 2% iodine; tannins were treated with 5% alcoholic ferric chloride; and cellulose was treated with iodine solution and concentrated sulfuric acid. Each glass slide was observed under the microscope to identify different cell components [26,30].

2.3.5 Screening of preliminary phytochemical classes

In three solvent extracts of *T. catappa* leaves and dried fruits, standard procedures were used to determine and identify the presence of diverse natural compounds and chemical classes like proteins, amino acids, alkaloids, carbohydrates, tannins, sterols, triterpenoids, cardiac glycoside, saponins, flavonoids, phenols, and phenolic compounds [31,32].

2.3.6 Physicochemical panorama

A physiochemical evaluation of *Terminalia catappa*'s finely powdered leaves and fruit followed the [Ayurvedic Pharmacopoeia of India] [29] criteria. The criteria that were assessed were total ash, water-soluble ash, sulfated ash, acid-insoluble ash, foreign matter, drying loss, foaming index, swelling index, and pH. Cold maceration was used to determine the extractive values, which was done according to normal protocols with minor modifications [26,33]. To begin, 5 grams of coarse powder (plant material) was weighed and kept in each of the three conical flasks with glass stoppers. The plant material was then macerated for 6 hours with frequent shaking in 100 ml of each of the prescribed solvents (petroleum ether, ethanol, and deionized distilled water) and left to stand for 18 hours at room temperature ($24\pm 2^{\circ}\text{C}$). Filtered the extract, and removed the surplus solvents with a rotary vacuum evaporator before being concentrated and weighed.

Furthermore, the fluorescence behavior of the fine powders and extracts was investigated using standard techniques [34, 35]. Various extracts were made using the cold maceration procedure, using solvents such as petroleum ether, ethanol, and deionized distilled water. In an ultraviolet cabinet, extracts and finely ground plant material treated with different solvents and reagents were shown to glow under visible and ultraviolet light (long 365 nm and short 254 nm).

2.3.7 Heavy metals itemization by Atomic Absorption Spectroscopy

Atomic Absorption Spectroscopy was used as part of standard procedures to identify arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) in the fine powder [36,37]. A combination of 5 ml of strong

hydrochloric acid, 5 ml of nitric acid, and 0.5 g of powdered plant material was stirred until the vapors were completely dissolved and a clear solution formed. Following digestion, the material was transferred to a 50 ml volumetric flask and filled with deionised distilled water to the required level. The material was then set aside for future research. For calibration, a blank solution with a value of zero was used. The absorbance was then measured after loading the reference samples and the experimental samples.

2.3.8 Microbiome scanning

Plants cultivated in space are subjected to different environmental conditions than those on Earth. Emerging data suggests that plants and their microbiota interact closely, with unique and varied microbial populations being essential to plant viability. [38]. Microbiological analysis of the fine powder of *T. catappa* leaf and fruit was carried out to determine the total microbial load and the particular pathogen as mentioned in US pharmacopeia protocols. The parameters used to evaluate the overall microbial load were the total aerobic microbial count and the cumulative yeast and mould count. The test drug was analysed for pathogens such Salmonella species, E. coli, Pseudomonas aeruginosa, and Staphylococcus aureus. [39,40].

2.4 Gas Chromatography/Mass Spectrometry-Based Metabolomic Monitoring

The Gas Chromatography-Mass Spectrometry (GC-MS) analytical platform is one of the most popular for metabolomics, this is used to quantify metabolites linked to chromatographic peaks, compare the amount of a particular metabolite in various samples, and, when combined with pathway analysis, comprehend the biochemical interactions between multiple metabolites that vary in a coordinated or differential way. [41,42]. Thermo GC-Trace Ultra Ver: 5.0, Thermo MS DSQ II, was used to perform the GC-MS analysis of the ethanolic extract of *T. catappa* leaves and fruit. Compounds were separated using the DB 35-MS Capillary Standard Non-Polar Column (30 m × 0.25 mm inner diameters, film thickness: 0.25 µm). The temperature of the column oven was set to rise from 70°C (hold for 2 minutes) to 260°C at a rate of 6°C per minute, with a final hold time of 10 minutes. The temperature of the injector was kept at 250°C. The carrier gas was helium (He) at a 1.0 ml/min flow rate. GC was performed in a splitless mode. The electron ionisation mode, which has a mass range of 50–650 m/z and an ionisation energy of 70 eV, was employed for MS detection. The MS transfer line was kept at 280 degrees Celsius, while the mass spectrometer's ion source was run at 220 degrees. One microlitre of the sample was used as the injection volume. The percentages of phytoconstituents by peak area were reported for the ethanolic crude extract of the leaves and fruit components. GC retention time was used to identify and characterise the phytochemical components present in the ethanolic crude leaf and fruit extracts. The mass spectra were computer-matched with standards available in mass spectrum libraries such as Wiley 9.

2.5 LC-MS analysis

LC-MS analysis of the *Terminalia catappa* leaf and fruit extract was performed using a Waters Xevo TQD triple quadrupole mass spectrometer coupled with an ACQUITY UPLC system. An XBridge BEH Amide

C18 column (150 mm × 2.1 mm, 2.5 µm) kept at 35°C was used to achieve chromatographic separation. The mobile phase was composed of 5 mM ammonium acetate (Solvent A) and acetonitrile (Solvent B), which were delivered at a flow rate of 0.3 mL/min with a gradient elution: 0–6 min (95% B), 6–12 min (70% B), 12–16 min (40% B), and 16–20 min (20% B). Following this, the conditions were re-equilibrated to their initial state. The injection volume used was 2 µL. Electrospray ionization (ESI) was employed in both positive (ES+) and negative (ES-) modes, with capillary voltages set to 3.50 kV. The source parameters included a desolvation temperature of 350°C, source temperature of 120°C, cone gas flow rate of 50 L/h, and desolvation gas flow rate of 950 L/h. Full-scan mass spectra were acquired over 40–2040 Da with a resolution of 15.0, using a collision energy of 3.0 eV for MS/MS. Enhanced mass data were collected in the centroid mode. The calibration was performed using sodium formate (Naics) with polynomial coefficients to ensure mass accuracy. Data acquisition and processing were performed using MassLynx v4.2 software. The method included a photodiode array (PDA) detector scanning 210–500 nm, although the primary analysis focused on the MS data. The system pressure ranged from 1578 to 6762 psi, and the column temperature and sample stability were maintained at 35°C and 25°C, respectively [43,44].

2.6 HPTLC Fingerprinting

High-performance thin-layer chromatography (HPTLC) analysis of *T. catappa* extract was performed using Merck HPTLC Silica gel 60 F254 plates (100 × 100 mm). Samples, including the extract (TB), standards (ellagic acid, gallic acid, kaempferol, and quercetin), and a control (BP), were applied as 8.0 mm bands at a position Y = 8.0 mm using a Linomat 5 applicator. Each track received 2.0 µL of the sample, with a dosage speed of 150 nL/s and a pre-dosage volume of 0.20 µL. The mobile phase comprised chloroform, methanol, and water (70:30:4, v/v/v), with chamber saturation for 20 min using a TTC 10x10 tank. The solvent front migrated to 70 mm, which was followed by plate drying at room temperature for 5 min. To find phytoconstituents like alkaloids, carbohydrates, flavonoids, tannins, and other compounds, chemical tests were performed on these different extracts; the results are listed in A. TLC Scanner 4 with a slit size of 5 × 0.2 mm, a scanning speed of 20 mm/s, and a data resolution of 100 µm/step was used to do the detection at 254 nm (deuterium and tungsten lamps). Spectrum scans (254–450 nm) and ANS reagent derivatization (heated at 100°C for 3 min) were performed for better visibility. TLC Visualiser 2 captured images in white light at 254 and 366 nm. System suitability and correlation analysis were performed to verify the proposed method (Table 4) [45,46].

2.7. In-silico Docking studies

An in silico docking study was conducted using Schrodinger software to evaluate the binding affinity of *Terminalia catappa* phytoconstituents to epileptic targets. The three-dimensional structures of target proteins, including dopamine D2 receptor (PDB ID: 6VMS), serotonin 5-HT2A receptor (PDB ID: 6WHA), NMDA receptor (PDB ID: 7EOQ), GABA_A receptor (PDB IDs: 6HUO, 4COF), and carbonic anhydrase II (PDB ID: 3F8E), were retrieved from the Protein Data Bank. Ligands were optimized using LigPrep, and protein structures were pre-processed using the Protein Preparation Wizard. Glide docking was

performed to determine ligand-receptor interactions, and docking scores were analyzed to identify potential antiepileptic candidates [47,48,49].

3.0 RESULTS

3.1 *Sensory and macroscopic assessment, as well as morphological overview*

Terminalia catappa is a tall, erect deciduous tree that grows to 20-30 meters and has a main stem diameter of 1-2 meters. The trunk is vertically erect, stiff, and sturdy, with the branches holding itself upright. The branches are arranged in a lateral racemose sequence, giving the tree a conical or pyramidal shape. The leaves are unlobed and simple. Mature leaves are dark greenish in colour, oval in form, and are grouped whorled on branches with a length of 10-15 cm and a width of 5-9 cm. The venation is pinnately reticulate, and the leaf's surface is glabrous. The inflorescence is axillary, with a simple raceme, no branches, and an acropetal arrangement. Male and female parts are found in flowers. The seed is encased in a fleshy fruit. Both the fruit's outer covering and the seed are edible. Powder leaves have a dark green colour, a moderate odour, and a pleasant taste, according to the organoleptic investigation. Table 1 and Figure 1 delineated the macroscopic and morphological descriptions.

3.2 *Qualitative microscopic ramification*

Transverse sections of a young leaf at the midrib position (Figure 2a) reveal single-lined epidermal cells. It has a layer of cuticle on top of it. They have trichomes and are rectangular (Figure 2c). There are 3-5 layers of collenchymatous cells adjacent to the epidermal layer and 5–7 layers of parenchymatous cells next to it. The bundles of vascular tissue are aligned in a triangle pattern. The phloem surrounds the upper and bottom sides of the xylem. Near the vascular bundles, air cavities were recognised. The arrangement pattern of a fresh branch is nearly identical to the transverse section of a fresh branch (Figure 2b). Multiple layers of collenchymatous and parenchymatous cells lie underneath an epidermal layer. The appearance of stomata on the dorsoventral leaf is depicted in Figure 2d. In an anomocytic manner, each stomata is surrounded by 4-5 epidermal cells.

3.3 *Quantitative microscopic impression*

The quantitative investigation is carried out by assessing the leaf constant parameters illustrated in Figures 3a and 3b. The stomatal number was found to be 22.5 on average, with stomatal indexes of 23; however, the Vein-islet number was 22, and the Veinlet termination number was 20 on average. Table 2 highlights the detailed report.

3.4 *Powder Analysis*

A microscopic study of powdered *Terminalia catappa* leaf and fruit indicated the presence of various components (Figure 4a-d). Unicellular trichomes, phloem fibres, xylem vessels, and parenchymatous cells are also visible. The proper reagents listed in Table 3 may be used to detect them. The powder microscopic analysis also revealed the presence of tracheids and xylem channels. Following

histochemical analysis, the powdered samples included calcium oxalate crystals, cellulose, tannins, hydroxyanthraquinones, lignified cell walls, and starch grains.

3.5 Exposition of Preliminary Phytochemical Screening

Terminalia catappa leaves and fruit were pulverized and extracted separately using water, ethanol, and petroleum ether solvents. To ascertain the presence of phytoconstituents including alkaloids, carbohydrates, flavonoids, tannins, and other substances, chemical tests were performed on these different extracts; the test findings are listed in Table 4. The findings show that the leaves and berries are rich in tannins, flavonoids, carbs, and cardiac glycosides. The presence of alkaloids and steroids is modest. Compared to the other three solvents, the ethanolic fraction contains more phytoconstituents.

3.6 Physicochemical reflection

Both the powder leaf and the fruit were evaluated for extractive value, ash value, and other factors such as foreign matter, drying loss, and so on, with the assertions shown in Table 5. These parameters can provide knowledge about the presence of inorganic matter or any impurities by determining Ash values. Extractive values are used to identify contaminated compounds. Any alien organism or its pieces can be detected by knowing foreign matter. The swelling factor can be used to determine mucilage content. Tables 6 and 7 show the fluorescence behaviour of both powder and extract. The sample's colour signature was captured in the visible and ultraviolet ranges.

3.7 The consequence of the heavy metal analysis

The heavy metal analysis of the fine powder of both leaves and fruits was accomplished with atomic absorption spectroscopy, and the results are portrayed in Table 8. Heavy metal concentrations of arsenic (As), cadmium (Cd), and mercury (Hg) were less than 0.01 ppm in both leaf and fruit powder; however, lead (Pb) was slightly higher at 1.24 ppm and 2.02 ppm, respectively. All the elements indicated, however, are within the parts per million (ppm) limits of the Ayurvedic Pharmacopoeia of India.

3.8 Information regarding the microbiome assessment

Total aerobic microbial count (TAMC), total yeast and mould count (TYMC), and specific pathogen detection were used to evaluate the microbial presence in the leaf and fruit powders. The findings are presented in Table 9. It was observed that the TAMC value of both leaves and fruit is less than 1000 colony-forming per gram per milliliter (Cfu/gm/ml), while the TYMC value is less than 10 Cfu/gm/l, both of which are within the Ayurvedic Pharmacopoeia of India's limit. No other specific pathogen was identified from the samples.

3.9 Metabolomic portray using Gas Chromatography/Mass Spectrometry analysis

The metabolomic composition of the crude ethanolic extract of *T. catappa* fruit and leaf was examined separately using GC-MS analysis. There were 53 peaks in all, and out of the 49 notified peaks, the fruit

extract had 50 different phytoconstituents, and the leaf extract had 43 different phytoconstituents. Tables 10 and 11 list the phytochemical compounds that were discovered along with their names, molecular formula, molecular weight, compound retention time (R_t), and percentage area. Figures 5a and 5b show the chemical structures of such compounds. Alcohol, ketone, oxime, anisole, and damascones derivative chemicals, fatty aldehydes, hydrocarbons, fatty acid esters, saturated fatty acids, phthalate ester, terpenes, acyclic hydrogenated diterpene, monoterpene, pentacyclic triterpenoids, oxygenated sesquiterpene, pentose alcohol, carbocyclic, resorcylic acid lactone group, and various phenolic compounds were found from the ethanolic extracts for both leaves and fruits.

3.10. Analysis of LC-MS

LC-MS analysis of *Terminalia catappa* leaf extracted from methanol and fruit revealed a diverse array of phytochemicals, showing its complex chemical composition. The identified compounds include phenolic acids such as gallic acid (R_t : 32.55, Mol wt: 170), gentisic acid (R_t : 14.79, Mol wt: 154), and ellagic acid (R_t : 20.55, Mol wt: 302). Flavonoids like apigenin (R_t : 27.2, Mol wt: 270), quercetin (R_t : 6.16, Mol wt: 302), and kaempferol (R_t : 17.03, Mol wt: 286) were also detected, along with flavone glycosides such as vitexin (R_t : 14.61, Mol wt: 432) and isovitexin (R_t : 6.16, Mol wt: 432). Fatty acids, including palmitic acid (R_t : 22.12, Mol wt: 256) and palmitoleic acid (R_t : 25.68, Mol wt: 254), were present, as well as triterpenoids like ursolic acid (R_t : 14.61, Mol wt: 456). The extract also contained complex tannins and hydrolysable tannins, such as punicalagin (Mol wt: 1084), chebulagic acid (Mol wt: 954), and corilagin (R_t : 27.04, Mol wt: 634). Additionally, other notable compounds like ascorbic acid (R_t : 24.39, Mol wt: 176), caffeic acid (R_t : 6.16, Mol wt: 180), and β -carotene (R_t : 32.3, Mol wt: 536) were identified. These phytochemicals, including flavonoids, phenolic acids, tannins, and fatty acids, highlight the rich and varied chemical profiles of *T. catappa* (Table 12). This comprehensive LC-MS analysis provided valuable insights into the phytochemical composition of the extract, which can serve as a foundation for further chemical characterization and research (Table 13) (Figure 6a-d).

3.11. HPTLC Fingerprinting

HPTLC analysis revealed distinct bands for the phytocompounds in the leaf and fruit extract. For the leaf extract, the retention factor (R_f) values for standards were ellagic acid (0.275 ± 0.118), gallic acid (0.885 ± 0.054), kaempferol (0.060 ± 0.072), and quercetin (0.977 ± 0.093). Comparable R_f values were observed in the following sample tracks: ellagic acid (0.306 in Track 6), gallic acid (0.881 in Track 5), kaempferol (0.073 in Track 3), and quercetin (1.050 in Track 4). Spectrum correlation analysis indicated a strong agreement between the sample and standard peaks, with the highest correlation for kaempferol and quercetin ($r = 0.824$). Derivatization with ANS reagent under 366 nm illumination enhanced the bands' visibility, confirming the presence of phenolic acids and flavonoids. The chromatogram obtained at 254 nm showed well-separated bands, validating the resolution of the method. The R_f deviations ($<10\%$) and spectral matches confirmed the identity of ellagic acid, gallic acid, kaempferol, and quercetin.

in the extracts. These results demonstrate the efficacy of HPTLC in profiling key bioactive compounds in *Terminalia catappapaleaf*, aligning with its known phytochemical composition (Figure 7a&b).

The HPTLC analysis of *Terminalia catappa* fruit extract from ethanol was prepared using silica gel 60 F₂₅₄ plates. and Toluene, ethyl acetate, methanol, and formic acid (4.9:4.1:2:0.5) make up the mobile phase, revealed the presence of phenolic compounds, including gallic acid (R_f 0.47, 40.80% peak area), ellagic acid (R_f 0.81, 55.04% peak area), quercetin (R_f 0.58, 4.63%), and rutin (R_f 0.10, 34.94%), when compared to standard markers. Gallic acid, a hydrolyzable tannin precursor, and its dimeric derivative ellagic acid dominated the profile, aligning with the species' known accumulation of ellagitannins. Though present in lower quantities, quercetin and rutin indicated flavonoid contributions to antioxidant activity. Unidentified peaks (e.g., R_f 0.36, 0.53, 0.67) suggested additional secondary metabolites, potentially tannins or glycosides requiring LC-MS/NMR characterization. Detection at 254 nm confirmed aromatic systems in phenolic compounds, while post-derivatization with anisaldehyde-sulfuric acid improved visualization of non-UV-active components. Methodological reproducibility was ensured via duplicate tracks (e.g., gallic acid at 15.0 mm and 85.0 mm), with minimal R_f variance, underscoring analytical consistency (Figure 7a&b).

3.12. *In-silico* Docking

The docking results provide insight into the potential interactions of various ligands with epileptic targets, particularly receptors, and enzymes involved in neuronal excitability and seizure modulation. Among the tested targets, the dopamine D2 receptor (PDB ID: 6VMS) exhibited significant binding affinity with multiple ligands. Rutin demonstrated the strongest interaction with a Glide G-Score of -8.215, suggesting it may have a considerable effect on dopamine-mediated pathways related to epilepsy. Other ligands such as Peonidin-3,5-O-di-beta-glucopyranoside (-7.833), Narcissin (-7.595), and Isoquercetin (-7.334) also displayed moderate binding, while Chlorogenic Acid (-6.864), Vanillic Acid (-6.353), Cyclohexane carboxylic Acid (-6.17), and Retinol (-6.132) showed comparatively weaker interactions. Since dopamine plays a crucial role in seizure modulation, strong binding ligands like Rutin may hold promise as potential therapeutic candidates.

The serotonin 5-HT_{2A} receptor (PDB ID: 6WHA) did not show any binding interactions with the tested ligands. As serotonin pathways are linked to seizure susceptibility, the absence of binding suggests that these compounds may not exert a significant effect on serotonin-mediated mechanisms of epilepsy. Similarly, no interactions were observed with carbonic anhydrase II (CA2) (PDB ID: 3F8E), an enzyme known to influence neuronal excitability and often targeted by antiepileptic drugs like acetazolamide. This indicates that the tested compounds may not modulate epilepsy through carbonic anhydrase inhibition.

No ligands displayed notable binding affinity for the GABA_A receptor (PDB IDs: 4COF and 6HUO). Given that GABA_A receptor activation is essential for enhancing inhibitory neurotransmission and preventing seizures, the lack of interaction suggests that these ligands do not directly contribute to increasing

GABAergic activity. However, the human GluN1/GluN2A NMDA receptor (PDB ID: 7EOQ) showed moderate binding with Rutin, which recorded a GScore of -6.227. Since NMDA receptors are involved in excitatory neurotransmission, this interaction suggests a potential role in seizure control by modulating excessive neuronal excitation (Table 14) (Figure 8a-f). A 2D picture of docking interactions of individual ligand with different receptor was presented in the Figure 9a-d.

4.0 DISCUSSION

Terminalia catappa is established as a morphologically unique, anatomically rich, and chemically active plant species based on the evidence presented. Its large, leathery leaves, effective vascular and stomatal structure, and abundance of secondary metabolites all lend credence to its ethnobotanical uses. Particularly, the presence of tannins, lignin, and anthraquinones suggests medicinal promise, and its resilient architecture enables survival in a variety of climates. Given the presence of flavonoids, tannins, phenols, and carbohydrates, the combined phytochemical and physicochemical analysis of *T. catappa* leaves and fruits supports its traditional medicinal usage. For the extraction of important bioactive components, ethanol proved to be the most efficient solvent. A solid foundation for standardisation and formulation of herbal products is provided by physicochemical data, which attest to the stability, purity, and effectiveness of the plant components' extraction.

For the purpose of identifying and distinguishing between plant components and extraction solvents, fluorescence properties offer a fingerprint. Key bioactive chemicals may be present in ethanol and aqueous extracts, as they exhibited the strongest fluorescence responses. The fluorescence behaviour of *T. catappa* with different reagents supports its application in qualitative pharmacognostic investigation and demonstrates its chemical richness. These responses can be used to diagnose genuineness. For both medicinal and dietary purposes, *T. catappa* leaf and fruit powders are safe from heavy metal contamination. When proper manufacturing procedures are followed, *T. catappa* powder samples are microbiologically safe and appropriate for topical or oral treatments because to their low microbial count and lack of dangerous pathogens.

The different phytochemical profiles found in the ethanolic leaf and fruit extracts by GC-MS (Gas Chromatography-Mass Spectrometry) investigations suggest that their biological activities and uses may differ. The results are compared here. The leaf extract contains a wider variety of fatty acids, alcohols, esters, and aromatic acids (such as benzoic acid, salicylates, and phytol). The substantial amount of furans, aldehydes, phthalates, and sugar alcohols (such as arabinitol and DL-arabinitol) in fruit extract suggests that it may have uses as an antioxidant or antibacterial. The fruit extract tends to be more flavouring and antioxidant-supporting, whilst the leaf extract exhibits more potential as a source of bioactive and antioxidant-rich compounds.

The medicinal richness of *T. catappa* is highlighted by the LC-MS data, especially its presence of polyphenols and antioxidant flavonoids. Both its prospective utility in contemporary phytopharmaceuticals and its historical use in ethnomedicine are supported by the presence of both low

and high molecular weight phytoconstituents. Because fruit and leaf extracts include strong tannins and antioxidants, they can be used in medicinal formulations, cosmetics, and functional meals. In particular, GC-MS, LC-MS, and HPTLC were among the sophisticated analytical methods used to identify substances such as gallic acid, ellagic acid, quercetin, and rutin. Known for their strong anti-inflammatory and antioxidant qualities, these chemicals probably help the plant treat conditions like oxidative stress, inflammation, and epilepsy.

According to the docking tests, rutin, a flavonoid that is widely present in leaf and fruit extracts, had high binding affinities with both the NMDA and dopamine D2 receptors. This shows that rutin is a promising option for treating epilepsy since it may be important in regulating neuronal excitability. The therapeutic effects of *T. catappa* may not be mediated through serotonin or GABA_A receptors, which are frequently targeted in the treatment of epilepsy. It is noteworthy that no significant interactions with these receptors were found. This might point to a brand-new way that the bioactive substances in the plant regulate seizures.

However, while interpreting these findings, a number of limitations must be taken into account. To completely comprehend the pharmacokinetics and pharmacodynamics of the active substances, *in vivo* investigations are necessary to supplement the encouraging initial insights provided by the *in-silico* results. All things considered According to the findings of the pharmacognostic, phytochemical, and *in silico* investigations, *Terminalia catappa* (*T. catappa*) has therapeutic potential, especially for its bioactive components that are relevant to illnesses associated to oxidative stress and neurodegeneration.

5.0 DISCUSSION:

Based on the evidence presented, *Terminalia catappa* is recognized as a morphologically distinct, anatomically complex, and chemically active plant species. Its big, thick leaves, effective water and gas exchange systems, and many useful chemicals back up its traditional uses. The presence of tannins, lignin, and anthraquinones highlights its medicinal potential, while its robust structural features allow it to thrive in diverse climates. Tests on *T. catappa* leaves and fruits show that they contain flavonoids, tannins, phenols, and carbohydrates, supporting their use in traditional medicine. Ethanol proved the most effective for extracting key bioactive compounds among the solvents tested.

Physicochemical data provide a solid foundation for the standardization and formulation of herbal products, confirming the stability, purity, and efficiency of extractions from *T. catappa* components. The plant's fluorescence properties, especially in ethanol and aqueous extracts, offer a distinctive fingerprint for identifying plant constituents and solvents. The way *T. catappa* glows under certain conditions shows it has a lot of different chemicals, which helps in checking its quality and confirming its identity. Furthermore, the absence of heavy metal contamination and the low microbial load in properly processed leaf and fruit powders ensure safety for medicinal and dietary applications.

GC-MS analysis shows that the ethanolic extracts from leaves and fruits have different chemical makeups, indicating they may have different biological functions. Leaf extracts have a wider variety of fatty acids, alcohols, esters, and aromatic acids (like benzoic acid, salicylates, and phytol). Leaf extracts contain a broader range of fatty acids, alcohols, esters, and aromatic acids (e.g., benzoic acid, salicylates, and phytol). In contrast, fruit extracts are rich in furans, aldehydes, phthalates, and sugar alcohols like arabinitol and DL-arabinitol, indicating antioxidant and antibacterial potential. While fruit extracts promise flavor enhancement and antioxidant support, leaf extracts appear more suitable for sourcing bioactive, antioxidant-rich compounds.

LC-MS analysis highlights the healing possibilities of *T. catappa*, especially because it contains polyphenols and antioxidant flavonoids. The existence of both small and large plant compounds supports their traditional use in natural medicine and their potential in today's herbal medicine development. Strong antioxidants and tannins in leaf and fruit extracts render them valuable in medicinal, cosmetic, and functional food formulations. Advanced testing methods like GC-MS, LC-MS, and HPTLC found important compounds such as gallic acid, ellagic acid, quercetin, and rutin, which are known to help reduce inflammation and fight free radicals, indicating they could be useful in treating oxidative stress, inflammation, and epilepsy.

In computer studies, it was found that rutin, a flavonoid found in leaf and fruit extracts, strongly binds to NMDA and dopamine D2 receptors, which suggests it could help treat epilepsy by affecting how neurons behave. However, it showed little interaction with serotonin and GABA_A receptors, which are usually targeted in epilepsy treatments, indicating a new way that *T. catappa*'s active compounds might work. Interestingly, there were few interactions with serotonin and GABA_A receptors, which are often focused on in epilepsy treatments, indicating a new way that *T. catappa*'s active compounds might work.

However, these findings should be interpreted cautiously. Further, in vivo studies are essential to understand the pharmacokinetics and pharmacodynamics of these active constituents fully. Overall, studies on the plant's properties, chemical makeup, and computer simulations together show that *Terminalia catappa* has potential benefits for conditions related to oxidative stress and neurodegeneration.

5.0 CONCLUSION

The current study investigated the pharmacobotanical, phytochemical, and metabolomic attributes of *Terminalia catappa* leaves and fruits, revealing its comprehensive medicinal potential. Morphological and microscopic examinations confirmed distinct pharmacological features, such as dorsiventral leaves with characteristic trichomes, vascular bundles, stomata, and key powder characteristics, including lignified cell walls, tannins, and starch grains. Physicochemical analyses, including ash content and extractive values, ensured the purity and quality of the plant material, while heavy metal analysis verified safety within permissible limits.

Both leaf and fruit extracts had high levels of flavonoids, tannins, and cardiac glycoside polysaccharides, according to preliminary phytochemical screening, with ethanol serving as the most efficient solvent. GC-MS metabolomic profiling identified diverse bioactive compounds, including phenolics, fatty acids, and terpenoids. LC-MS analysis expanded this profile, revealing specific phytochemicals such as gallic acid, ellagic acid, quercetin, kaempferol, rutin (R_f 0.10), and complex tannins (e.g., punicalagin and chebulagic acid), corroborating the plant's antioxidant and anti-inflammatory potential. HPTLC validation confirmed the presence of ellagic acid (leaf: R_f 0.306; fruit: R_f 0.81), gallic acid (leaf: R_f 0.881; fruit: R_f 0.47), kaempferol (R_f 0.073), quercetin (leaf: R_f 1.050; fruit: R_f 0.58), and rutin (R_f 0.10) through distinct bands, spectral correlations ($r = 0.824$ for kaempferol-quercetin), and methodological consistency (R_f deviations < 10%). Derivatization with ANS reagent under 366 nm illumination enhanced phenolic acid and flavonoid visualization, while chromatograms at 254 nm confirmed aromatic systems and resolution efficacy. Duplicate tracks (e.g., gallic acid at 15.0 mm and 85.0 mm) ensured reproducibility, reinforcing analytical reliability.

Microbiological assessments affirmed the samples' safety, with low microbial counts and no pathogens detected. Molecular docking highlighted rutin as the most promising ligand for epileptic targets, showing strong binding to the dopamine D2 receptor (-8.215 kcal/mol) and moderate affinity for NMDA receptors (-6.227 kcal/mol). The LC-MS and HPTLC identification of rutin and related flavonoids supports its potential role in modulating dopamine-mediated seizure pathways. However, the absence of interactions with GABA_A or serotonin receptors suggests a focused mechanism on excitatory neurotransmission.

The integration of LC-MS and HPTLC data with GC-MS and docking results collectively underscores *T. catappa*'s multifaceted bioactive repertoire, aligning with its traditional uses. These findings position the plant as a viable candidate for nutraceutical and pharmaceutical development, particularly for neurodegenerative and oxidative stress-related disorders. Further *in vivo* studies and clinical trials are essential to validate these *in silico* and *in vitro* insights and elucidate mechanistic pathways for therapeutic application.

Abbreviations

T. catappa-Terminalia Catappa

GC-MS-Gas chromatography -mass spectroscopy

R_f -Retention Factor

Pb -Lead

Cd-Cadmium

Hg-Mercury

He- Helium

PDB-Protein Data Bank

HPTLC-High Performance Thin Layer Chromatography

USP-United State Pharmacopoeia

AAS-Atomic Absorption Spectroscopy

Declarations

Ethical Approval and Consent-Not Applicable

Funding-Not Applicable.

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Tables

Tables 1 to 14 are available in the Supplementary Files section

Figures



Figure 1

Plant morphology of *Terminalia catappa* L., (A) General appearance; (B) Leaf (upper side and lower side); (C) Mature ripe fruit ripe; (D) Dried fruit; (E) inflorescence; (F) Flower with male and female parts

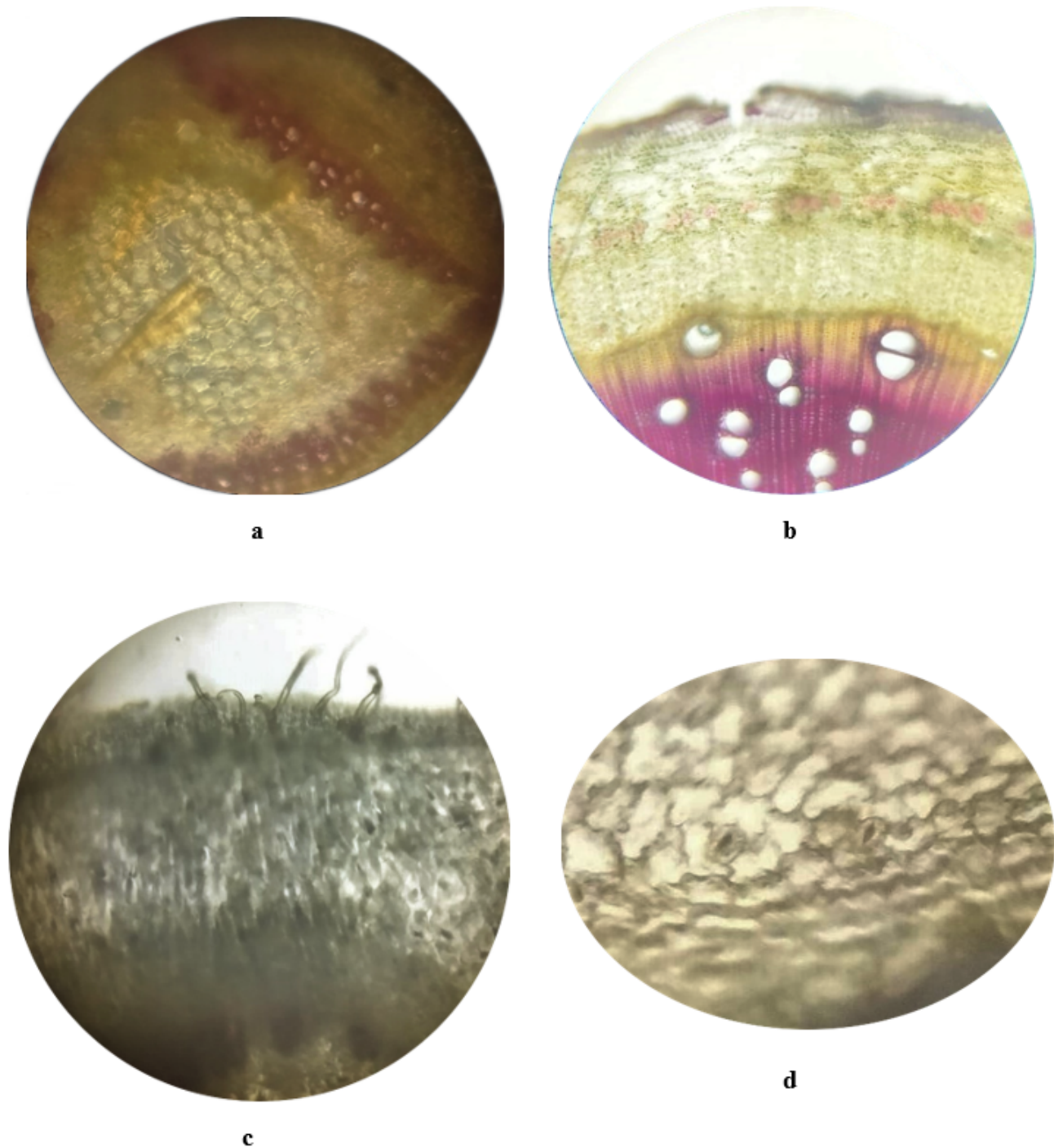


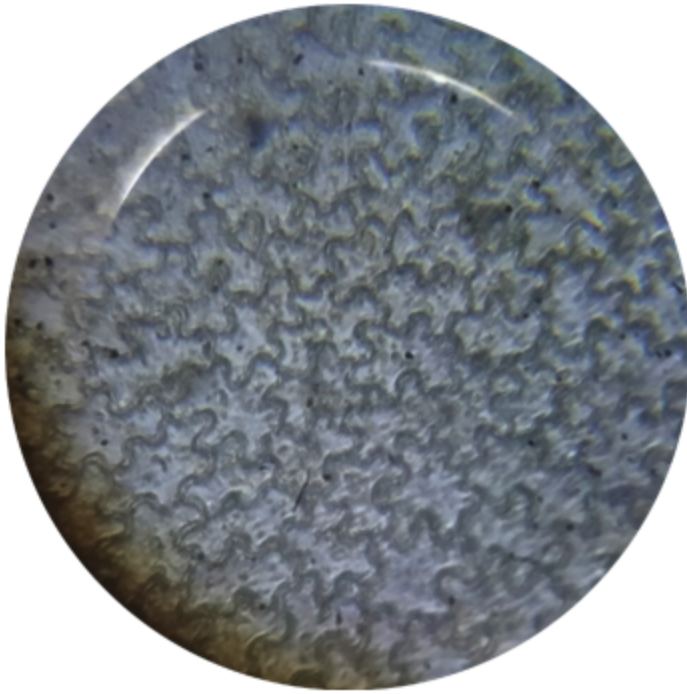
Figure 2

(a) Transverse sections of a *T. catappa* young leaf

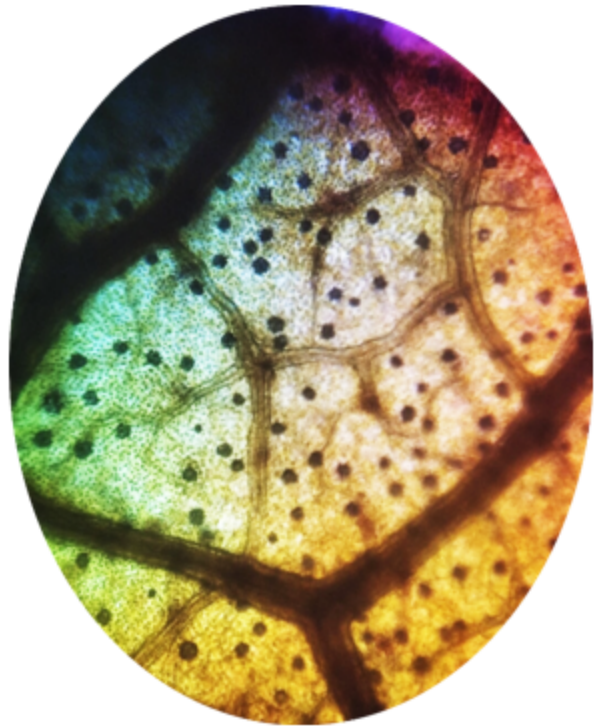
(b) Transverse sections of fresh stem

(c) Trichomes present on leaf

(d) Stomata on dorsoventral leaf



a



b

Figure 3

(a) Determining the stomatal number and stomatal Index.

(b) Appearance of vein islets and veinlet termination.

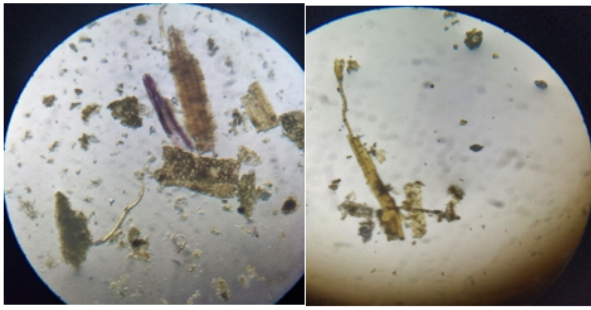


Figure 4: (a)

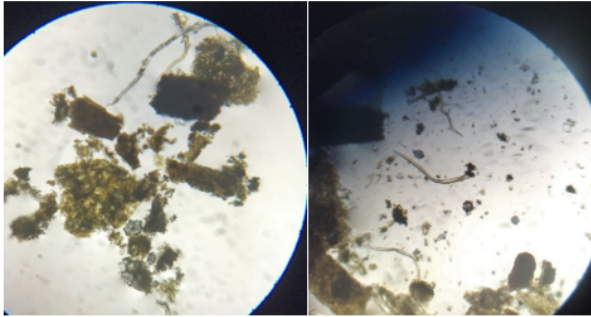


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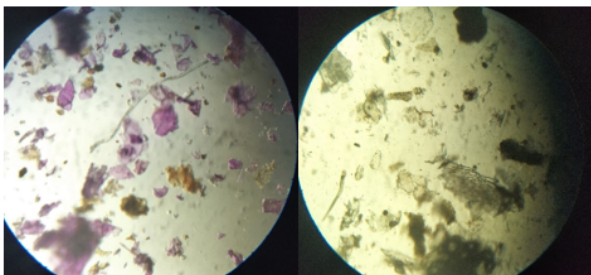


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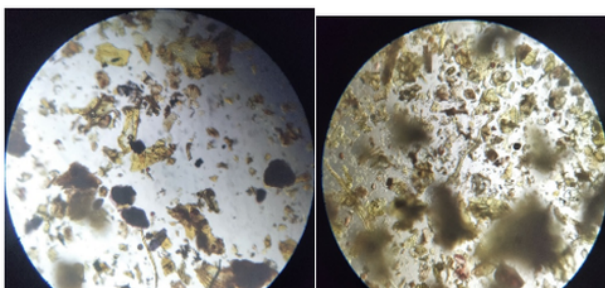


Figure 4: (d)

Figure 4

- (a) Lignified cells and cellulose present in leaf powder.
- (b) Tannin containing cells and starch present in leaf powder.
- (c) Lignified cells and cellulose present in fruit powder.

(d) Tannin containing cells and starch present in fruit powder.

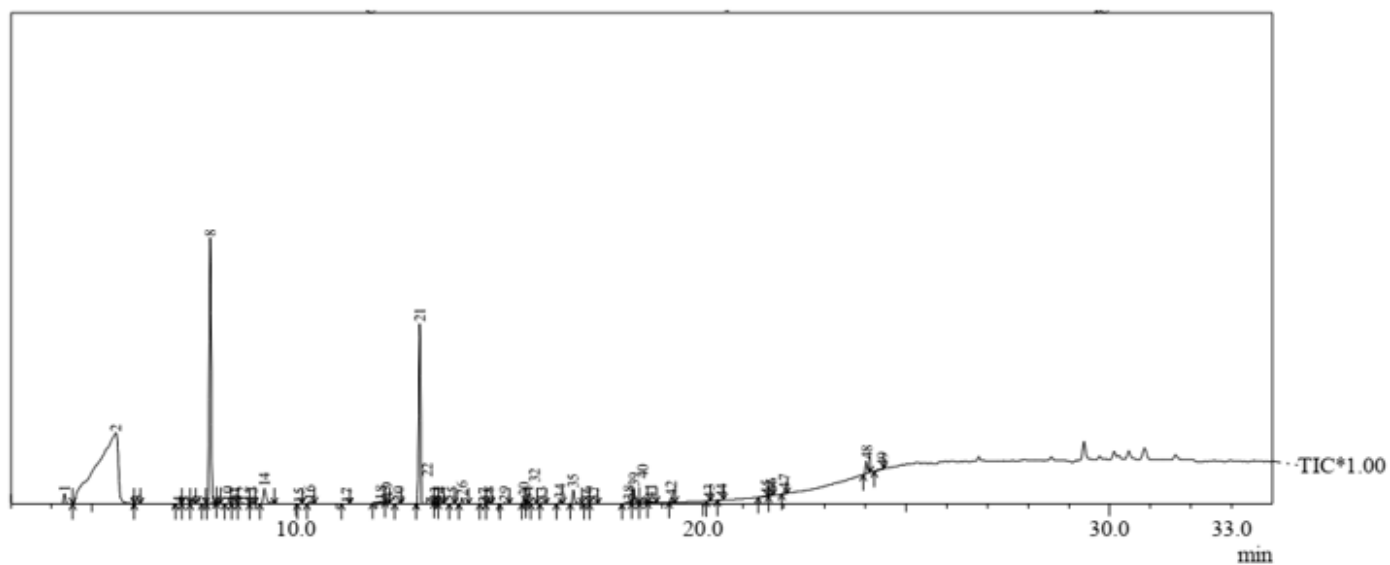


Figure 5(a)

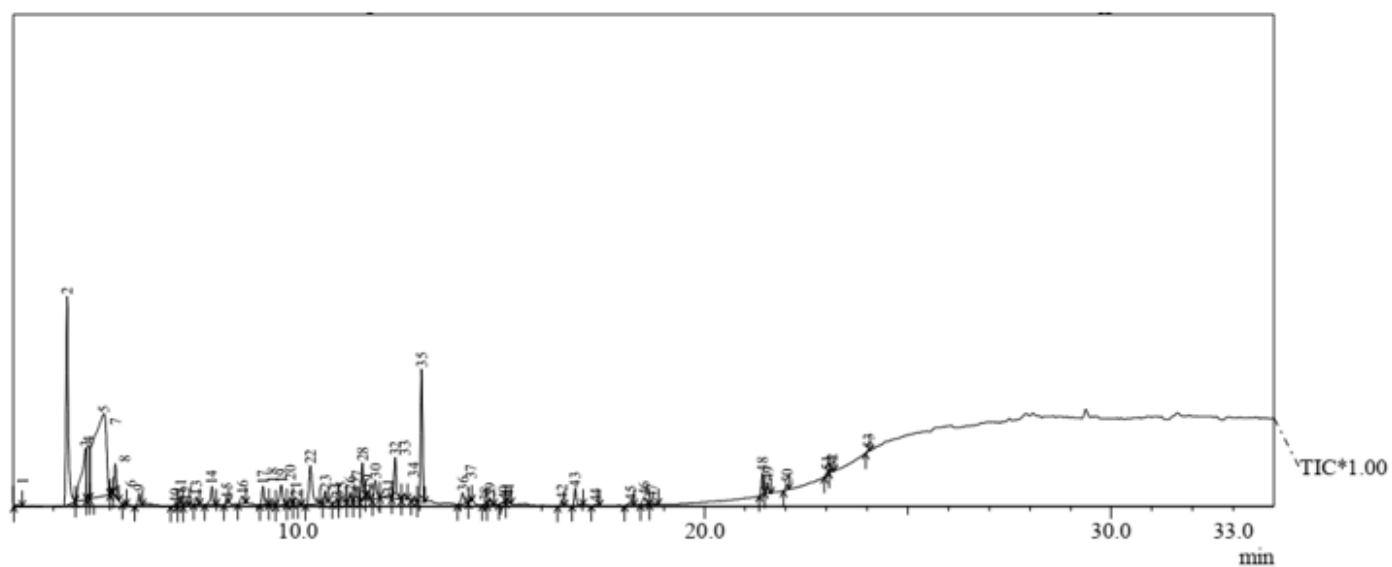


Figure 5(b)

Figure 5

(a) Peaks obtained from the GC MS analysis of ethanolic leaf extract.

(b) Peaks obtained from the GC MS analysis of ethanolic fruit extract.

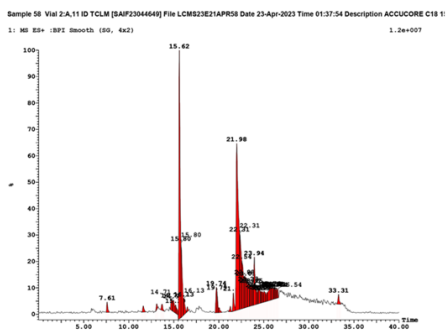


Figure 6(a)

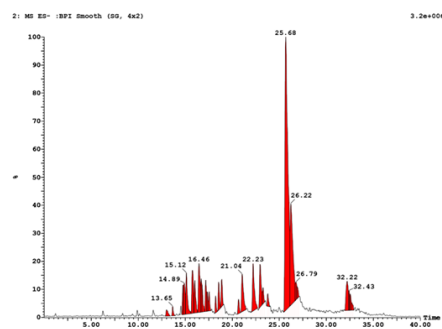


Figure 6(b)

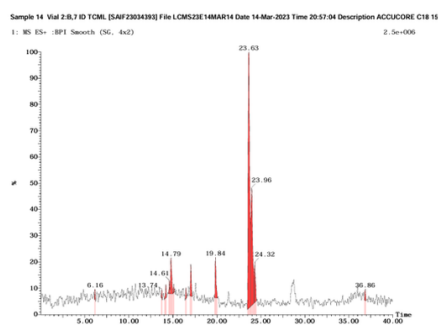


Figure 6(c)

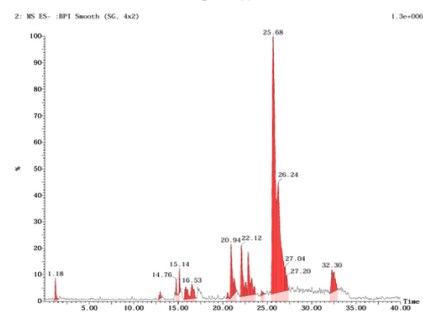


Figure 6(d)

Figure 6

(a): LC-MS of Chromatogram in positive mode for leaf extract.

(b): LC-MS of Chromatogram in Negative mode for leaf extract.

(c): LC-MS of Chromatogram in positive mode for fruit extract.

(d): LC-MS of Chromatogram in Negative mode for fruit extract.

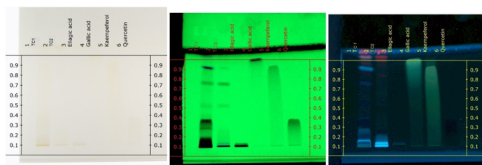


Figure 7(a)

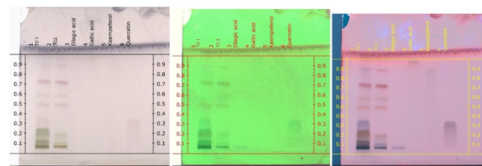


Figure (7b)

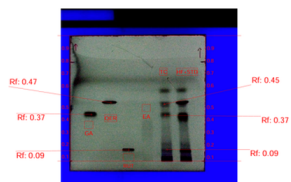
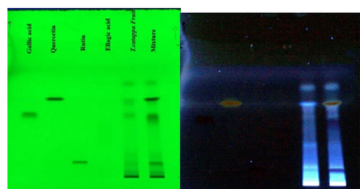
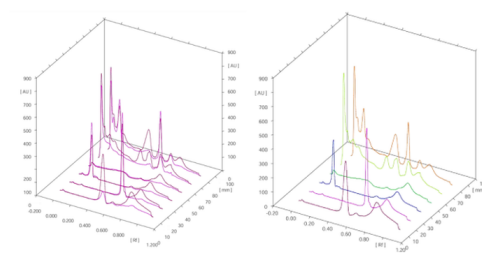


Figure (7c)

Figure 7

(a). HPTLC Chromatogram &Densitogram before derivatization of *T.catappa* leaf extract.

(b): HPTLC Chromatogram &Densitogram after derivatization *T.catappa* leaf extract.

(c): HPTLC Chromatogram & Densitogram before and after derivatization of *T.catappa*fruit extract.

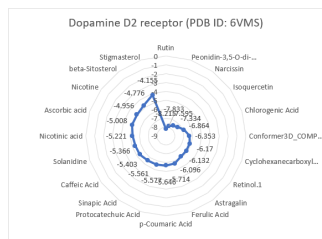


Figure (8a)

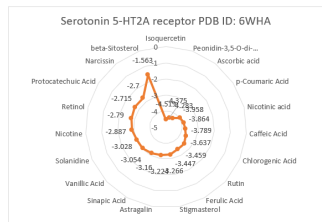


Figure (8b)

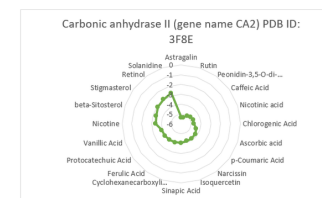


Figure (8c)

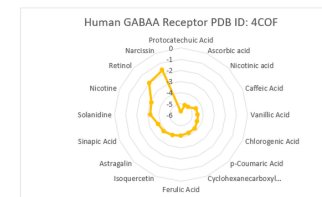


Figure (8d)

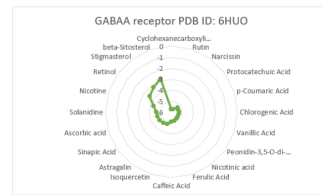


Figure (8e)

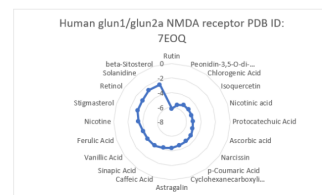


Figure (8f)

Figure 8

(a): Docking interactions of serotonin 5-HT2A receptor (PDB ID: 6WHA) with various ligands

(b): Docking interactions of serotonin 5-HT2A receptor (PDB ID: 6WHA) with various ligands

- (c): Docking interactions of serotonin carbonic anhydrase II (CA2) (PDB ID: 3F8E) with various ligands
- (d): Docking interactions of GABAA receptor (PDB IDs: 4COF) with various ligands
- (e): Docking interactions of GABAA receptor (PDB IDs: 6HU0) with various ligands
- (f): Docking interactions of Human Glun1/Glun2a NMDA receptor (PDB ID: 7EOQ)with various ligands

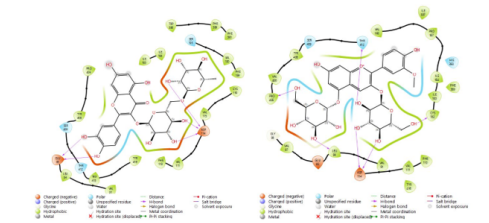


Figure (9a)

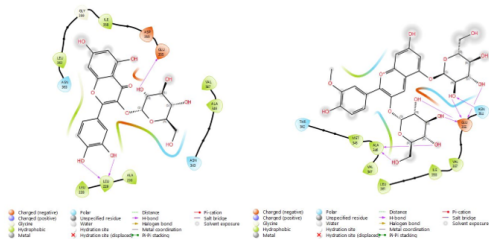


Figure (9b)

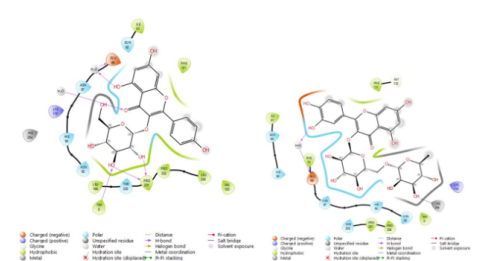


Figure (9c)



Figure (9d)

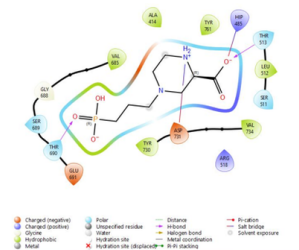


Figure (9e)

(a):Docking interactions of Dopamine D2 receptor (PDB ID: 6VMS)with ligandRutin and Peonidin-3,5-O-di-beta-glucopyranoside.

(b):Docking interactions of Serotonin 5-HT2A receptor PDB ID: 6WHA with ligandIsoquercetin and Peonidin.

(c):Docking interactions of Carbonic anhydrase II (gene name CA2) PDB ID: 3F8E with ligandAstragalin and Rutin.

(d):Docking interactions of **Human GABAA Receptor PDB ID: 4COF**and **6HUO**with ligandAscorbic acidCyclohexanecarboxylic acid respectively.

(e): Docking interactions of Human glun1/glun2a NMDA receptor PDB ID: 7EOQ with ligand Rutin.

Supplementary Files

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