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Qualitative and quantitative estimation phytoconstituents and antioxidant activity of *Picrorhiza kurroa* and *terminalia arjuna*

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Abstract
Herbal medicines have long been a cornerstone of traditional healthcare systems, and contemporary research increasingly supports their therapeutic potential. The present study aimed to perform a comprehensive pharmacognostical, physicochemical, and phytochemical evaluation of *Picrorhiza kurroa* rhizomes and *Terminalia arjuna* bark, two widely recognized medicinal plants in Ayurveda. Morphological, microscopic, and organoleptic analyses established key diagnostic characteristics for plant identification and quality control. Physicochemical parameters including ash values, extractive values, moisture content, and microbial load were determined, confirming the purity and compliance of the crude drugs with pharmacopoeial standards. Elemental analysis using atomic absorption spectrophotometry revealed essential elements within permissible limits. Qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, tannins, and saponins in both plants. Quantitative estimations indicated that methanolic extracts consistently exhibited higher total phenolic and flavonoid content compared to other solvent extracts. Antioxidant potential assessed by DPPH and hydrogen peroxide scavenging assays demonstrated notable free radical scavenging activity, especially in methanolic extracts of *Terminalia arjuna* and *Picrorhiza kurroa*. The findings scientifically substantiate the therapeutic potential of these botanicals and provide validated baseline data for their safe incorporation into herbal and pharmaceutical formulations.

Keywords: *Picrorhiza kurroa*, *Terminalia arjuna*, DPPH, Antioxidant, Phytochemical

1. Introduction
Despite the extensive historical and ethnopharmacological use of herbal medicines, one of the major challenges in their wider clinical acceptance lies in the lack of systematic standardization and scientific validation. Herbal formulations often suffer from issues of batch-to-batch variability, adulteration, and inconsistent therapeutic efficacy due to differences in geographical origin, harvesting conditions, post-harvest processing, and extraction methods. This necessitates comprehensive pharmacognostical, physicochemical, and phytochemical evaluations to establish the identity, purity, and potency of medicinal plant materials. Additionally, qualitative and quantitative estimation of bioactive constituents, along with chromatographic profiling, is crucial to ensure the reproducibility, safety, and efficacy of herbal formulations. Scientific validation through preclinical and *in vitro* pharmacological screening further provides evidence-based support for traditional claims, facilitating the integration of plant-based therapies into modern healthcare systems. Therefore, establishing standardized protocols for the authentication and evaluation of medicinal plants like *Terminalia arjuna* and *Picrorhiza kurroa* is essential for promoting their safe, consistent, and therapeutically effective usage in contemporary phytomedicine. Herbal medicines have been deeply rooted in traditional healthcare systems, with *Terminalia arjuna* (Arjuna) and *Picrorhiza kurroa* (Kutki) holding significant positions in Ayurveda and other indigenous medicinal practices. Both plants have been revered for centuries for their therapeutic efficacy in managing various chronic and acute conditions. *Terminalia arjuna*, belonging to the family Combretaceae, has been historically documented in ancient Ayurvedic texts such as the Charaka Samhita and Sushruta Samhita as a potent cardiotonic, haemostatic and anti-inflammatory agent [12, 15].

Traditionally, its bark decoction was used for treating angina, hypertension, heart failure, and hemorrhagic disorders. Modern pharmacological research has validated its cardioprotective, antioxidant, anti-inflammatory, antimicrobial, and hypolipidemic activities, primarily attributed to its rich content of polyphenols, flavonoids (arjunolone, arjunic acid, arjunolic acid), tannins, and saponins. *Picrorhiza kurroa*, a small herbaceous plant from the family Plantaginaceae, has been extensively used in Ayurveda, Unani, and Siddha medicine systems for its hepatoprotective, anti-inflammatory, antipyretic, and immunomodulatory properties [9]. Traditionally prescribed for liver ailments such as hepatitis, jaundice, and dyspepsia, Kutki's therapeutic properties have been linked to its iridoid glycosides (picroside I, picroside II, kutkoside) and apocynin. Recent studies affirm its hepatoprotective, antioxidant, anti-ulcer, antimicrobial, and anti-asthmatic potentials [15, 14].

The primary objective of the present study was to perform comprehensive pharmacognostical, physicochemical, and phytochemical evaluations of two traditionally significant medicinal plants, *Terminalia arjuna* and *Picrorhiza kurroa*. The study aimed to establish the macroscopic, microscopic, and organoleptic characteristics of these plants for their botanical authentication. Additionally, physicochemical parameters such as ash values, extractive values, moisture content, and bitterness value were determined to assess their quality and purity [15]. Qualitative and quantitative phytochemical screening was conducted to detect and estimate the total phenolic and flavonoid content in various extracts. Furthermore, chromatographic profiling using Thin Layer Chromatography (TLC) was undertaken to support the phytochemical findings. The study intends to generate baseline standardization data for these botanicals, thereby scientifically validating their therapeutic potential and supporting their safe and effective incorporation into herbal and pharmaceutical formulations.

2. Material and Methods

2.1 Material

The rhizomes of *Picorhiza kurroa* (kutki) and bark of *Terminalia arjuna* were obtained from local vendors of Rohtak. All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

2.2 Methods

The collected plant parts (rhizomes and bark) of *P. kurroa* and *T.arjuna* were subjected to shed drying and further crushed to powder and then the powder was passed through the mesh 40 and stored in airtight container. [4].

2.2.1 Pharmacognostic Studies

Morphological Studies

The rhizomes of *Picrorhiza kurroa* (Kutki) and the bark of *Terminalia arjuna* were subjected to macroscopic, morphological, and organoleptic evaluation to aid in their preliminary identification and authentication. Visual inspection assessed key features such as color, texture, shape, and overall appearance, while organoleptic analysis involved evaluating odor and taste by slight crushing and smelling. These assessments help confirm the identity, purity, and selection of the correct plant parts for further extraction and formulation processes. [4] [7].

Microscopic Studies

Thin transverse sections of fresh *Picrorhiza kurroa* rhizomes and *Terminalia arjuna* bark were prepared, cleared with chloral hydrate, and stained with phloroglucinol-HCl to visualize lignified tissues. The sections were mounted in glycerin and examined microscopically to observe characteristic anatomical features for authentication and standardization. Photomicrographs were captured for documentation. [7].

Powder Microscopy

Powder microscopy was performed by mounting finely powdered plant material on glass slides with suitable stains to enhance cellular details. Diagnostic features like fibers, vessels, starch grains, calcium oxalate crystals, and trichomes were identified under a compound microscope, aiding in authentication and quality assessment of the crude drugs [1, ,7].

Determination of Physicochemical Properties

1. Ash value

Ash content represents the inorganic residue remaining after the incineration of a crude drug sample, providing valuable information regarding its identity, purity, and cleanliness.

A. Total Ash value: A precisely weighed 3 g of air-dried powdered crude drug was incinerated in a pre-weighed silica crucible by gradually increasing the temperature to dull red heat for complete combustion. After cooling in a desiccator, the crucible was weighed, and the process repeated until a constant weight was obtained [11]. The total ash content was calculated as a percentage relative to the weight of the air-dried crude drug using the following formula:

Total ash %=[Total ash formed in the crucible/ weight of sample] X 100

B. Acid Insoluble Ash Value: The Total ash obtained was boiled with 25ml of 2 N hydrochloric acid (HCl) for 5 minutes. The insoluble matter was collected on an ashless filter paper, then washed with hot water. The dried the filter paper, ignited and weighed it. Calculate the percentage of acid insoluble ash with reference to the air -dried crude drug [10, 11].

Acid soluble ash (%)=[Total ash formed in the crucible with ashless filter paper/weight of sample] X 100

C. Water-Soluble Ash Value: The total ash obtained was boiled with 25ml of water for 5 minutes. The insoluble matter was collected on an ashless filter paper. After that washed insoluble matter with hot water and ignited for 15 minutes at a temperature not exceeding 450 ℃. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried crude drug.

D. Sulphated Ash Value: Accurately weighed 3 g of air-dried powdered crude drug was placed in a pre-weighed

silica crucible, gently ignited, and the residue moistened with 1 ml of concentrated sulphuric acid. It was then heated until fumes ceased, followed by ignition at 750 ±50°C until carbon was fully removed. After cooling, a few more drops of sulphuric acid were added and the ignition repeated. The final weight was noted, and the percentage of sulphated ash was calculated with reference to the air-dried drug.

2. Extractive Values

Extractive values are important for determining the nature of the chemical components in a raw medicine. Two types of extractive values were calculated i.e., alcohol soluble extractives and water-soluble extractives [4, 11].

E. Alcohol Soluble Extractives: About 5 g of crude drug was macerated with 100 ml of 90% alcohol in a conical flask, sealed, and intermittently shaken for 24 hours. After filtration, 25 ml of the filtrate was evaporated to dryness in a porcelain dish, dried at 100 °C, cooled in a desiccator, and weighed. The extractive value was calculated in mg per g of air-dried material, with all tests performed in triplicate.

The crude drug percentage w / w of the extractive was measured using the following formula:

$$\% \text{ of soluble extractive value of the sample} = \frac{\text{Weight of extract residue}}{\text{Weight of crude drug}} \times 100$$

A. Water Soluble Extractives: About 5 g of air-dried crude drug was macerated with 100 ml of water containing a small amount of chloroform as a preservative in a 250 ml conical flask. The mixture was shaken intermittently and left to stand for 24 hours. After filtration, 25 ml of the filtrate was evaporated to dryness on a copper bath, dried at 100 °C in a hot air oven, cooled in a desiccator, and weighed. The water-soluble extractive values were calculated in mg/g of air-dried material. All experiments were conducted in triplicate.

3. Foreign Matter

The foreign matter present in the crude drug sample was assessed through visual inspection and magnification. A 200 g sample was evenly spread and examined with the naked eye and under a 5-10× magnifying lens. Any extraneous materials were carefully separated, weighed, and the percentage of foreign matter was calculated relative to the initial sample weight to ensure the purity and quality of the herbal material.

$$\text{Percentage of foreign matter} = \frac{\text{Weight of separated foreign matter}}{\text{Weight of the original sample}} \times 100$$

4. Moisture Content

This test estimates the amount of moisture and volatile matter present in a sample, which is essential for standardizing herbal drugs and preventing microbial contamination and degradation. In a silica crucible, add 1.5 g weight of powdered crude drug sample. The crude drug was then subjected to dryness at 105°C in hot air oven. After that crucible was then transferred to a desiccator and dried. [11]

$$\% \text{ Loss on Drying} = \frac{\text{Initial weight of sample} - \text{Weight after drying}}{\text{Weight of the Initial weight of sample}} \times 100$$

5. Swelling Index

Taken individually 1gm of *Terminalia arjuna* bark and *Picrorhiza kurroa* rhizomes and transferred into a 25 ml measuring cylinder having glass stopper. Add 20 ml of water into the above mixture. Then agitate rigorously at 10-minute intervals throughout the course of 1 hour. Subsequently, kept the sample undisturbed for duration of 24 hours at room temperature. After 24 hours, check the volume (ml) encompassed by the plant material, inclusive of any adhesive mucilage. The mean value of three measurements was computed. [11]

6. Foaming index

The foaming index of *Terminalia arjuna* bark and *Picrorhiza kurroa* rhizomes was determined based on their saponin content, which produces stable foam upon shaking. 1 g of each plant material was boiled in 100 ml of water for 30 minutes, filtered, and serially diluted in 10 test tubes. After shaking for 15 seconds and standing for 15 minutes, foam height was measured. If foam height reached 1 cm in a test tube, the foaming index was calculated using the formula:

Foaming index=1000/a, where *a* is the volume (in ml) of decoction in the test tube showing 1 cm foam. [16] [10]

7. Crude Fibre Content

The powdered plant materials were passed through a stainless-steel mesh sieve (No. 20). Firstly, fat was removed from 3gm of dried ground plant materials using Soxhlet extraction with petroleum ether. The defatted sample was digested with 1.25% sulphuric acid (H_2SO_4) and 1.25% sodium hydroxide (NaOH) solutions. After digestion, the plant materials were dried in an oven at 130°C for 2 hours. Then residue was ignited at 600°C for 30 minutes. The loss in plant materials weight on ignition and the weight of the ground plant materials before defatting was utilized to determine the percentage crude fibre content. [2]

$$\% \text{ Crude fibre in Ground Plant Material} = \frac{\text{Loss in weight on ignition}}{\text{Weight of ground plant material}} \times 100$$

8. Bitterness Value

The bitterness value was determined by comparing the threshold concentration of plant extract to that of quinine hydrochloride. Serial dilutions of both quinine (0.01 mg/ml) and plant extracts (10 mg/ml) were prepared. Volunteers tasted successive dilutions, noting the lowest concentration eliciting a bitter sensation after 30 seconds. The bitterness value was calculated using the formula: [2] [1]

Bitterness value=2000 × c / (a × b)

where *a* is the stock extract concentration (mg/ml), *b* is the extract volume at threshold, and *c* is quinine content (mg) at its threshold.

2.2.2 Determination of Microbial contamination of crude drug parts

Microbial contamination testing was conducted on Ilex paraguariensis and Bertholletia excelsa following standard pharmacopeial protocols. Plant materials were pre-treated based on their nature to neutralize inherent antimicrobial properties. Plate count methods for bacteria and fungi were performed using casein-soybean digest agar and Sabouraud glucose agar, respectively. Specific pathogen detection tests targeted *E. coli, Salmonella spp., Pseudomonas aeruginosa, Staphylococcus aureus*, and *Clostridium spp*. using selective enrichment, differential media, and confirmatory biochemical reactions. The materials passed all tests if no characteristic colonies appeared, ensuring microbial safety and compliance for pharmaceutical use. [7] [16]

2.2.3 Elemental analysis

Picrorhiza kurroa rhizomes and *Terminalia arjuna* bark was performed using Atomic Absorption Spectrophotometry (AAS) to detect both heavy metals and essential elements. Dried plant samples were digested with a nitric acid-perchloric acid mixture (2:1), and blanks were prepared similarly. Elements such as Na, K, Cu, Mg, Fe, Zn, Ca, and Mn were quantified using a hollow cathode lamp and calibrated against standard solutions. Results were compared against recommended daily allowance (RDA) limits to ensure elemental safety and quality compliance. [2] [6].

2.2.4 Extraction and Percentage Yield

The extraction will be done through Soxhlation process. The collected plant materials of *P.kurroa* rhizomes and *T. arjuna roots* dried in the shade for a specified period. Finally, selected plant materials were subjected to size reduction to get coarse powder and passed through sieve no. 40 to get uniform powder. The dried powder 200 g was subjected to continuous hot Soxhlet extraction with various solvents i.e., ethanol, hydroalcoholic (1:1), methanol and aqueous in desired ratio at a temperature of 50-75 ℃ during 32 cycles. After complete extraction, each solvent extract was concentrated under reduced pressure using a rotary evaporator and subsequently dried. The final weight of the dried extract was recorded.

The percentage yield was then calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extracts obtained}}{\text{Weight of material taken}} \times 100$$

2.2.5 Phytochemical Screening

Qualitative Analysis

A preliminary phytochemical investigation was conducted to identify the presence of various bioactive constituents in *Picrorhiza kurroa* rhizomes and *Terminalia arjuna* root extracts using solvents of increasing polarity i.e., ethanol, hydroalcoholic (1:1), methanol and aqueous. [11] [11].

1. Test for Alkaloids

- **Dragendorff's Reagent test:** Add 1ml of Dragendorff's reagent (potassium bismuth iodide solution) in one ml of extract solution. Presence of prominent buff colored precipitate showed the presence of alkaloids.
- **Mayer's test:** Add one ml of Mayer's reagent (potassium mercuric iodide solution) to the test tubes containing one ml of test extracts. Presence of white or cream-colored precipitate showed the presence of alkaloids.
- **Hager's test:** Add one ml of Hager's reagent (saturated aqueous solution of picric acid) in the test tubes containing one ml of test extracts. Presence of yellow colored precipitate indicated the presence of alkaloids.
- **Wagner's test:** Add 2ml of Wagner's reagent (iodine in potassium iodide) in one ml of test extract. Presence of reddish-brown precipitates indicated the presence of alkaloids.

2. Test for Glycosides

- **Legal test:** In few ml of extract, added pyridine and made alkaline by adding freshly prepared sodium nitroprusside solution. Formation of pink to red color indicates the presence of steroidal glycoside.
- **Baljet test:** In one ml of the test extract added with one ml of sodium picrate solution. Formation of yellow to orange color showed the presence of steroidal glycoside.
- **Borntrager's test:** In one ml of the test extract added few ml of dilute sulphuric acid, then boiled, filtered the mixture. Add few ml of chloroform to the filtrate. Separate the Chloroform layer and treated with one ml of ammonia. Presence of red color indicates of anthraquinone glycosides.

3. Tests for Carbohydrates

- **Molisch's Test:** In 2ml of the extract, add two drops of Molisch's reagent (alcoholic solution of α-napthol). Shake the mixture well in test tube. Slowly add 1 ml of concentrated sulphuric acid along the sides of the test tube. Appearance of purple or reddish violet color at the junction of the two liquids showed the presence of carbohydrates.
- **Fehling's test:** Add 1 ml of each of Fehling solutions A and B. Added 1 ml of the extracts to above solution and heat it. Appearance of red precipitate indicated the presence of sugars.
- **Barfoed's test:** In 5 ml of Barfoed's solution, add 1ml of extract solution. Heat the above mixture till boiling. Formation of ed precipitate of copper oxide confirmed the presence of carbohydrates.

4. Test for Steroids and Sterols

- **Liebermann Burchard's test**: In few ml of the extract, add 2 ml of chloroform. Add few drops of acetic anhydride and also add two drops of concentrated sulphuric acid. The above mixture showed red, then blue and finally bluish green color which indicated the presence of steroids.
- **Salkowski test:** In few ml of extract, added 2 ml of chloroform and equal volume of concentrated sulphuric acid. The appearance of bluish red to cherry red colour in Chloroform layer and acid layer assuming marked green fluorescence represents the steroid and sterol.

5. Test for Saponins: In one ml of extract added 20 ml of distilled water. Shake the above mixture in a graduated cylinder for 15 minutes. Appearance of foam about one cm indicated the presence of saponins.

6. Test for Proteins and Amino acids

- **Biuret test:** In 1 ml of the extract added 1ml of 40% sodium hydroxide solution and add 2 drops of 1% copper sulphate solution. The formation of violet color indicated the presence of protein.
- **Ninhydrin test:** In 2 drops of freshly prepared 0.2% Ninhydrin reagent, add the prepared test extract solution and heat it. The formation of purple color during cooling suggests the presence of amino acids.

7. Tests for Flavonoids

- **Shinoda test:** In 1ml of the extract added magnesium turnings and add 1-2 drops of concentrated hydrochloric acid. Formation pink or red color showed the presence of flavonoids.
- **Ferric chloride test:** In 1 ml of extract solution, added 3-4 drops of 10% FeCl3 solution in a test tube. Appearance of a greenish-blue to a violet color indicated the presence of flavonoids.
- **Sodium hydroxide test:** In 1 ml of the extract solution, added 2 ml of 10% NaOH solution into a test tube. The yellow color of the solution disappeared upon when dilute Hydrochloric acid was added, indicated the presence of flavonoids.

8. Test for Tannins and Phenolic Compounds

Ferric chloride test: To the extract, added ferric chloride solution. Appearance of dark blue or greenish black color showed the presence of tannins.

9. Test for Acidic Compounds: The extract solution was treated with sodium bicarbonate and produced effervescence. The above preparation then treated with warm water and filtered. To check the acidic compounds filtrate was tested with litmus paper.

10. Test for Mucilage: The test extract solution was treated with ruthenium red solution and appearance of pink color indicated the presence of mucilage.

11. Test of fixed oils and volatile oil

(a) Spot test: The small quantity of extract was pressed between two filter papers. Presence of oily stain indicates presence of fixed oil and no appearance of stain indicates the presence of volatile oil.

(b) Sudan Red IV Test: Dark red Sudan IV (a common dye) is added to a solution. Extract is dyed as red color in presence of fixed oils.

Quantitative Analysis

1). Determination of Total Phenolic Content

The total phenolic content of *Picrorhiza kurroa* rhizomes and *Terminalia arjuna* bark extracts was estimated by the Folin-Ciocalteu colorimetric method using gallic acid as the standard. Plant extracts (1 mg/mL) and gallic acid standards (100-500 µg/mL) were reacted with Folin-Ciocalteu reagent and sodium carbonate, incubated for 2 hours, and absorbance was measured at 750 nm. A calibration curve was prepared, and total phenolic content was expressed as mg gallic acid equivalents per gram of crude drug (mg GAE/g). All tests were performed in triplicate and results reported as mean± SD. [8]

2). Determination of Total Flavanoid Content

The total flavonoid content of *Picrorhiza kurroa* rhizomes and *Terminalia arjuna* bark extracts was determined using the Aluminium Chloride colorimetric method, with quercetin as the reference standard. Serial dilutions of quercetin (100-500 µg/mL) were prepared to construct a calibration curve. Plant extracts or standards were mixed with sodium nitrite, aluminium chloride, and sodium hydroxide in sequence, and the final volume was made up with distilled water. After incubation, the absorbance was measured at 510 nm using a UV-Vis spectrophotometer. The total flavonoid content was calculated from the quercetin calibration curve and expressed as mg quercetin equivalent (QE) per gram of crude drug, with all tests performed in triplicate. [8]

$$\text{TFC mg QE/g} = \frac{C \times V}{W}$$

Where:
C=concentration of quercetin obtained from the calibration curve (mg/mL)
V=volume of the extract (mL)
W=weight of the plant extract (g)

3). Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was performed to separate and identify phytoconstituents in the rhizome extracts of *Picrorhiza kurroa* and root extracts of *Terminalia arjuna*. Silica gel 60 F254 was used as the stationary phase, and plates were prepared, air-dried, and activated at 100°C. Samples and quercetin (standard) were dissolved in methanol and ethyl acetate, respectively, and applied to the plates using glass capillaries. Plates were developed in a pre-saturated chamber containing an appropriate organic solvent system. After development, spots were visualized using vanillin-sulfuric acid and 5% ethanolic $FeCl_3$, followed by heating. Rf values were calculated and compared with the standard for phytoconstituent identification. The Retention factor (Rf) values for each spot were calculated using the following formula:

$$Rf = \text{Distance travelled by solute (spot)} / \text{Distance travelled by solvent front}$$

3. Antioxidant Activity

I. DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging assay: The antioxidant activity of the plant extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. A reaction mixture was prepared by adding 1 mL of freshly prepared DPPH solution (0.135 mM in ethanol) and 450 µL of 50 mM Tris-HCl buffer (pH 7.4) to various concentrations (20, 40, 60, 80, 100 and 120) of the plant extracts. Ascorbic acid was used as the reference standard at the same concentrations. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to allow interaction between the antioxidants and DPPH radicals. After

incubation, the decrease in absorbance was measured at 517 nm using a UV-visible spectrophotometer. The percentage inhibition of the DPPH radical was calculated to assess the free radical scavenging ability of the test extracts and compare them with the standard. The percent inhibition was calculated from the following equation.

$$\text{Percentage Inhibition}=\left(1-\frac{\text{Absorbance of test sample}}{\text{Absorbance of control}}\right)\times 100$$

All the tests and analysis were run in triplicates. Standard and extracts concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration.

II. Hydrogen peroxide scavenging activity: A 20 mM solution of hydrogen peroxide was freshly prepared using phosphate buffer saline (PBS) with a pH of 7.4. To 2 mL of this hydrogen peroxide solution, 1 mL of plant extract samples at varying concentrations (20, 40, 60, 80, 100 and 120 µg/mL in ethanol) was added. A standard solution of ascorbic acid at the same concentrations served as the positive control. The reaction mixtures were incubated at room temperature for 10 minutes. Following incubation, the absorbance of each sample was measured at 230 nm using a UV-visible spectrophotometer against a blank containing PBS without hydrogen peroxide. The percentage scavenging of hydrogen peroxide was calculated to evaluate the antioxidant potential of the extracts and compare them with the standard.

$$\text{Percentage Inhibition}=\left(1-\frac{\text{Absorbance of test sample}}{\text{Absorbance of control}}\right)\times 100.$$

All the tests and analysis were run in triplicates and averaged. Standard and extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration.

4. Results

The collected plant parts (rhizomes and bark) of *P. kurroa* and *T. arjuna* were subjected to shed drying and further crushed to powder and then the powder was passed through the mesh 40 and stored in airtight container and were used for further evaluation.

4.1 Pharmacognostic Studies

4.1.1 Morphological Studies: *P. kurroa* (Kutki) is a small, hairy, creeping and herbaceous alpine species. It grows in rock crevices and moist, sandy soils with long creeping rootstocks that are bitter in taste. The leaves are sub radical, flat, oval, and sharply serrated.

Terminalia arjuna (T. arjuna) is a deciduous large sized fluted tree to 30 m tall and 2-2.5 m diameter at breast height, with an often-buttressed trunk. *T. arjuna* (family Combretaceae), a large tree, is found throughout the South Asian region. This tree is usually an evergreen tree with new leaves appearing in the hot season (February to April) before leaf fall. This tree is an exotic tree in India. It is one of the most versatile medicinal plants having a wide spectrum of biological activity. The outer surface of the bark appeared smooth, pale greenish yellow while the inner surface was finely longitudinally striated and pinkish in color. Bark has pieces that were flat, curved and recurved in shape. On the inner part fracture was short while laminated on the outer part. Sample (Arjuna bark) size 8.5 cm in length and 6.3 cm in width was also observed.

4.1.2 Microscopic Characteristics

Terminalia arjuna

In the microscopic examination of mature *Terminalia arjuna* bark, the outer cork layer comprised 4-8 rows of thick-walled, suberized cells, beneath which a narrow cork cambium and several layers of parenchymatous secondary cortex were present, containing starch grains, pigment granules, and calcium oxalate crystals. A broad secondary phloem region featured sieve elements, phloem parenchyma, thick-walled lignified fibers, sclereids, and numerous medullary rays. Pitted vessels and pigment-containing cells were also observed. These anatomical features confirm the identity and purity of *T. arjuna* bark as per standard pharmacognostical markers.

The transverse section of *Picrorhiza kurroa* revealed an outer cork (phellem) of several layers of thick-walled, tangentially elongated cells, followed by a narrow cork cambium (phellogen) of 1-2 layers of rectangular meristematic cells. The secondary cortex (phelloderm) contained parenchymatous cells with starch grains, pigment cells, and occasional calcium oxalate crystals. A well-developed vascular region showed thick-walled xylem vessels in radial rows, xylem parenchyma, and fibers, surrounded by phloem with sieve elements, fibers, and parenchyma. Medullary rays traversed the vascular tissue, while the central medulla comprised large, starch-rich parenchyma cells and pigment cells.

4.1.3 Powder Microscopy

Terminalia arjuna

The powder exhibited numerous cork cells, which appeared polygonal, brownish, and tangentially elongated with thick walls, often seen arranged in groups. Abundant lignified phloem fibers were present, identified by their long, thick walls and narrow lumen. Stone cells or sclereids were prominently seen, characterized by their broadly oval to polygonal shape, thick lignified walls, wide lumen, and distinct pits, occurring singly or in small groups. Numerous parenchymatous cells were also noted, which were thin-walled, polygonal to oval, and contained brownish contents.

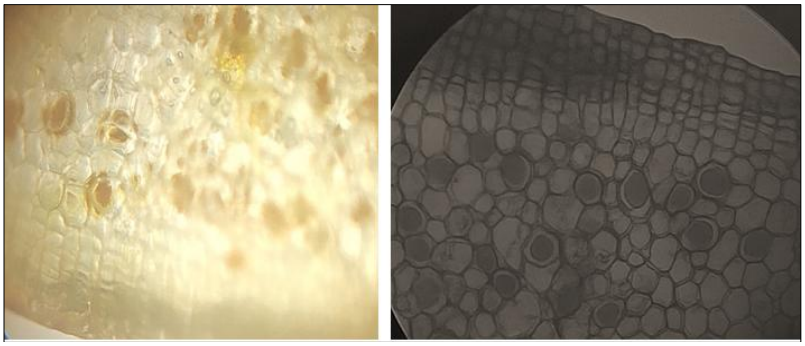

Fig 1: Transverse section of (A) *Terminalia arjuna* (B) *Picrorhiza kurroa*

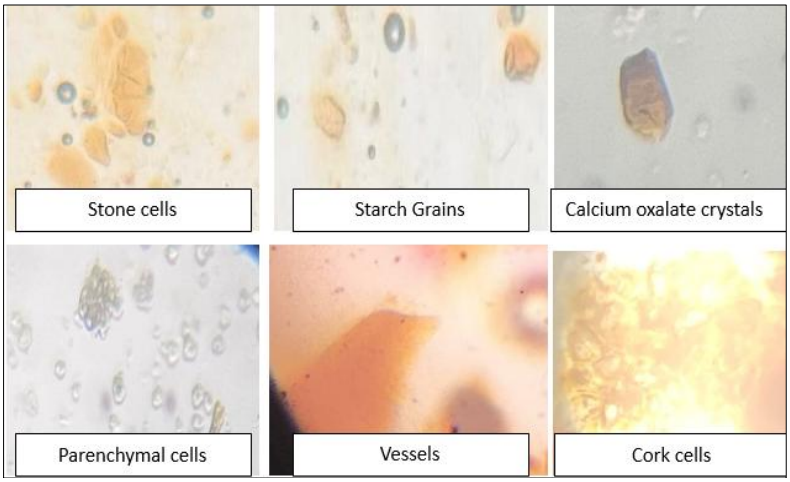

Fig 2: Powder Microscopy of T. arjuna

Picorhiza Kurroa

The microscopy revealed abundant thin-walled parenchymatous cells containing simple and compound starch grains. Lignified xylem vessels with simple and bordered pits, thick-walled tapering fibers, and tracheidal elements were observed. Cork cells appeared polygonal, thick-walled, and pigment-filled. Occasional calcium oxalate crystals and scattered brown pigment cells were also present, confirming diagnostic features of the bark.

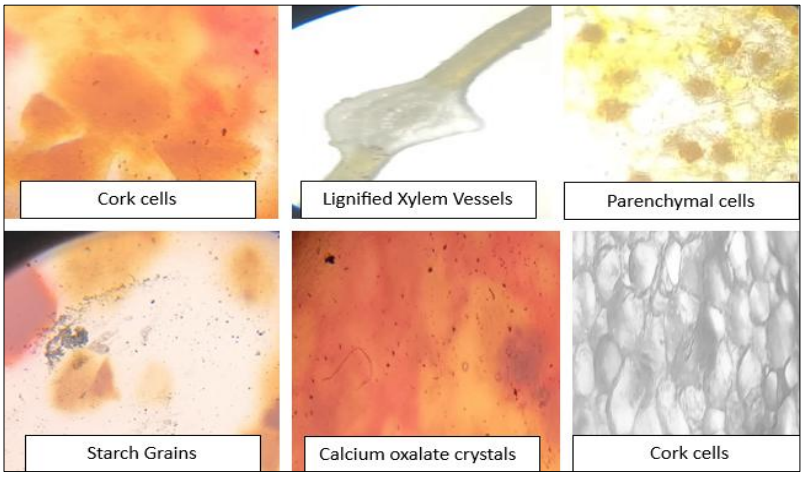

Fig 3: Powder Microscopy of *Picrorhiza kurroa*

4.1.4 Determination of physicochemical parameters

The physicochemical evaluation of *Picrorhiza kurroa* rhizomes revealed a total Ash value of 9.20 ±0.24% w/w, indicating the total inorganic residue content. Acid soluble ash and water-insoluble ash values were recorded as 1.05 ±0.11% w/w and 2.64 ±0.01% w/w respectively, reflecting the amount of acid-soluble minerals and insoluble siliceous matter. The sulphated ash value was found to be 9.48 ±0.14% w/w, confirming the presence of stable inorganic components. Alcohol and water-soluble extractive values were 18.01±0.20% w/w and 27.44 ±0.4% w/w respectively, indicating better extractability of phyto-constituents in aqueous medium. The foreign matter content was minimal at 0.22 ±0.04% w/w, confirming the purity of the sample. Moisture content was 6.51±0.33% w/w, within acceptable limits for crude drugs. The crude fibre content was 12.11 ±0.5% w/w. The drug was confirmed bitter in taste with a swelling index of 1.4, reflecting its mucilage or

hydrophilic content. These parameters confirm the identity, purity, and quality standards of *P. kurroa* rhizomes.

The physicochemical evaluation of *Terminalia arjuna* bark revealed a total ash value of 16.01 ±0.5% w/w, indicating the total inorganic residue present after incineration. The acid soluble ash was 1.09 ±0.11% w/w, representing the amount of acid-soluble mineral content, while water-insoluble ash stood at 4.82 ±0.7% w/w, reflecting the presence of siliceous matter. The sulphated ash value was high at 17.98 ±0.1% w/w, confirming the presence of stable inorganic components after sulphation. The alcohol soluble extractive value was 12.14 ±0.8% w/w, while water soluble extractive value was 18.14 ±0.03% w/w, indicating better solubility of constituents in aqueous medium. The foreign matter content was found to be minimal at 0.39 ±0.1% w/w, denoting good sample purity. Moisture content was recorded at 4.35 ±0.5% w/w, which is within acceptable limits. Crude fibre content was considerably high at 22.45 ±0.6% w/w, suggestive of its fibrous texture. The swelling index was 3.15, indicating notable water-absorbing capacity, while the foaming index was observed to be less than 100, pointing to the limited presence of saponins. These results confirm the identity, purity, and quality of *T. arjuna* bark as per standard pharmacognostic parameters.

4.2 Determination of Microbial Contamination of Crude Drug

Microbial contaminations of crude drug were found within limit in case of total bacteria and total fungi. *Entrobacteria, E. coli, Salmonella species, Staphylococcus* and *Pseudomonas aeruginosa* were found absent.

Table 1: Microbial contamination of *Picorhiza kurroa* rhizomes and *Terminalia arjuna* roots.

Microbial Counts	*Picorhiza kurroa* rhizomes	*Terminalia arjuna* roots
Total bacteria	3.2×10^3	2.8×10^3
Total fungi	1.5×10^2	1.1×10^2
E. coli	Absent	Absent
Salmonella species	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent
Staphylococcus aureus	Absent	Absent
Clostridium species	Absent	Absent

4.3 Element Analysis

In both selected plants *Picorhiza kurroa* (kutki)and *Terminalia arjuna* bark elements such as sodium, potassium copper, magnesium, iron, zinc, calcium was found in recommended daily allowances limit as shown in table 2-3.

Table 2: Different Element analysis of *Picorhiza kurroa* (kutki) with recommended daily allowances limits

Elements	mg/100kg of dry matter	(RDA) recommended daily allowances
Calcium (Ca)	950-1250	1000 mg/day (adults)
Magnesium (Mg)	480-610	310-420 mg/day (adults)
Potassium (K)	6200-8400	3400-4700 mg/day (adults)
Sodium (Na)	150-320	<2000 mg/day
Iron (Fe)	18-25	17 mg/day (males), 21 mg/day (females)
Zinc (Zn)	7.8-10.4	12 mg/day (males), 10 mg/day (females)
Manganese (Mn)	2.2-3.8	1.8-2.3 mg/day
Copper (Cu)	0.6-1.1	0.9 mg/day
Lead (Pb)	ND-0.02	Not more than 0.01 mg/kg (WHO)
Cadmium (Cd)	ND	Not more than 0.003 mg/kg (WHO)
Arsenic (As)	ND	Not more than 0.01 mg/kg (WHO)
Mercury (Hg)	ND	Not more than 0.001 mg/kg (WHO)

Table 3: Different Element analysis of *Terminalia arjuna* bark with recommended daily allowances limits

Elements	mg/100kg of dry matter	(RDA) recommended daily allowances
Calcium (Ca)	820-1150	1000 mg/day (adults)
Magnesium (Mg)	410-590	310-420 mg/day (adults)
Potassium (K)	5900-7900	3400-4700 mg/day (adults)
Sodium (Na)	120-280	<2000 mg/day
Iron (Fe)	22-30	17 mg/day (males), 21 mg/day (females)
Zinc (Zn)	6.5-9.8	12 mg/day (males), 10 mg/day (females)
Manganese (Mn)	2.4-4.2	1.8-2.3 mg/day
Copper (Cu)	0.5-1.0	0.9 mg/day
Lead (Pb)	ND-0.02	Not more than 0.01 mg/kg (WHO)
Cadmium (Cd)	ND	Not more than 0.003 mg/kg (WHO)
Arsenic (As)	ND	Not more than 0.01 mg/kg (WHO)
Mercury (Hg)	ND	Not more than 0.001 mg/kg (WHO)

4.4 Extraction and Percentage Yield: The initial weight was 200g of *Picorhiza kurroa* rhizomes and 200g of *Terminalia arjuna* roots. The percentage yield of both the extracts are shown in table 4.

Table 4: % Yield of *Picrorhiza kurroa* rhizomes & *Terminalia arjuna* roots

Sample	Extracts	Colour	Percentage yield (%)
Picrorhiza kurroa rhizomes	Ethanol	Light brown	22.06±0.2
	Hydroalcoholic	Light brown	18.34±0.6
	Methanol	Brown-Black	24.8±0.8
	Aqueous	Brown	21.01±1.5
Terminalia arjuna roots	Ethanol	Dark Brown	13.22±0.03
	Hydroalcoholic	Dark Brown	16.05±0.21
	Methanol	Brown	21.1±0.41
	Aqueous	Brown-Black	15.52±0.1

4.5 Phytochemical Screening

Picrorhiza kurroa rhizomes & *Terminalia arjuna* roots were used under study were subjected for qualitative and qualitative phytochemical screening.

4.5.1 Qualitative analysis: In qualitative analysis preliminary screening of prepared extracts using different solvents like ethanol, hydroalcoholic, methanol and aqueous of *Picorhiza kurroa* rhizomes & *Terminalia arjuna* roots.

Table 5: Phytochemical Screening of *Picorhiza kurroa* rhizomes extracts

S. No.	Tests	Ethanol	Hydroalcoholic	Methanol	Aqueous
1	Alkaloids	+	+	+	-
2	Glycosides	+	+	+	+
3	Phenolic Compounds	+	+	+	+
4	steroids and sterols	+	+	+	-
5	Saponins	-	+	+	+
6	Carbohydrates	+	+	+	+
7	Flavonoids	+	+	+	+
8	Tannins	+	+	+	+
9	Proteins and Amino acids	-	+	+	+
10	Mucilage	-	+	+	+
11	Fixed oils and volatile oil	-	-	-	-
12	Resins	+	+	+	-

Where; - indicates absent; + indicates present

Fig 4: Phytochemical screening of *P.kurroa*

Table 6: Phytochemical screening of *Terminalia arjuna* roots extracts

S. No.	Tests	Ethanol	Hydroalcoholic	Methanol	Aqueous
1	Alkaloids	+	+	+	-
2	Glycosides	+	+	+	+
3	Carbohydrates	+	+	+	+
4	steroids and sterols	+	+	+	-
5	Saponins	+	+	+	+
6	Proteins and Amino acids	-	+	+	+
7	Flavonoids	+	+	+	+
8	Tannins	+	+	+	+
9	Phenolic compounds	+	+	+	+
10	Resins	+	+	+	-
11	Fixed oils and volatile oil	-	-	-	-
12	Mucilage	-	+	+	+

Where; - indicates absent; + indicates present

Fig 5: Phytochemical screening of *T. arjuna*

4.5.2 Quantitative Analysis (Spectrophotometric Method)

(1) Determination of Total Phenolic Contents (TPC)

The total phenolic content was calculated by plotting the standard calibration curve. The result of the TPC in the extracts was expressed as mg of Gallic Acid Equivalent per gm Dry Weight. The slope of line and goodness of fit (R2) value were calculated to identify the line equation. Gallic acid equivalents (mg GAE/g) of *Picorhiza kurroa* (kutki) ethanol, hydroalcoholic, methanol and aqueous extracts were found to be 62.44±0.28, 76.57± 0.65, 84.06± 0.54 and 52.15± 0.21 respectively. The mg of Gallic acid equivalents (mg GAE/g) of *terminalia arjuna roots* ethanol, hydroalcoholic, methanol and aqueous extracts were found to be 92.14±0.54, 106.44±0.16, 134.04±0.88 and 83.77±0.17 respectively. Methanol extracts generally yield higher TPC and TFC due to efficient extraction of polar phytoconstituents. *Terminalia arjuna* consistently shows higher total phenolics and flavonoids than *Picrorhiza kurroa* across solvents, owing to its rich tannin and flavonoid profile. As shown in figure 3, Gallic acid was used to prepare a standard curve as linearity curve where y=0.0089x-0.0017; (R^2=0.9954) where; y is the absorbance; x is the solution concentration). The results were expressed as mg of gallic acid equivalents (GAE) per g of powdered crude drug.

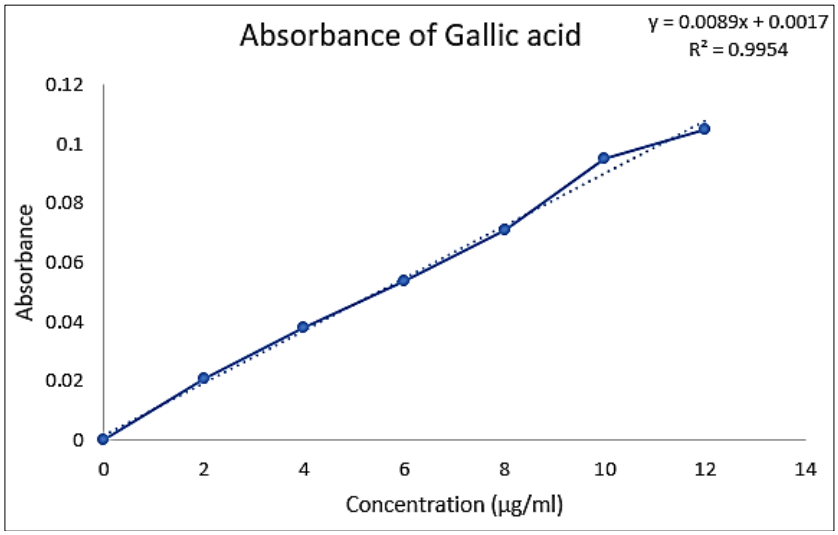

Fig 6: Linearity graph for gallic acid concentration v/s absorbance

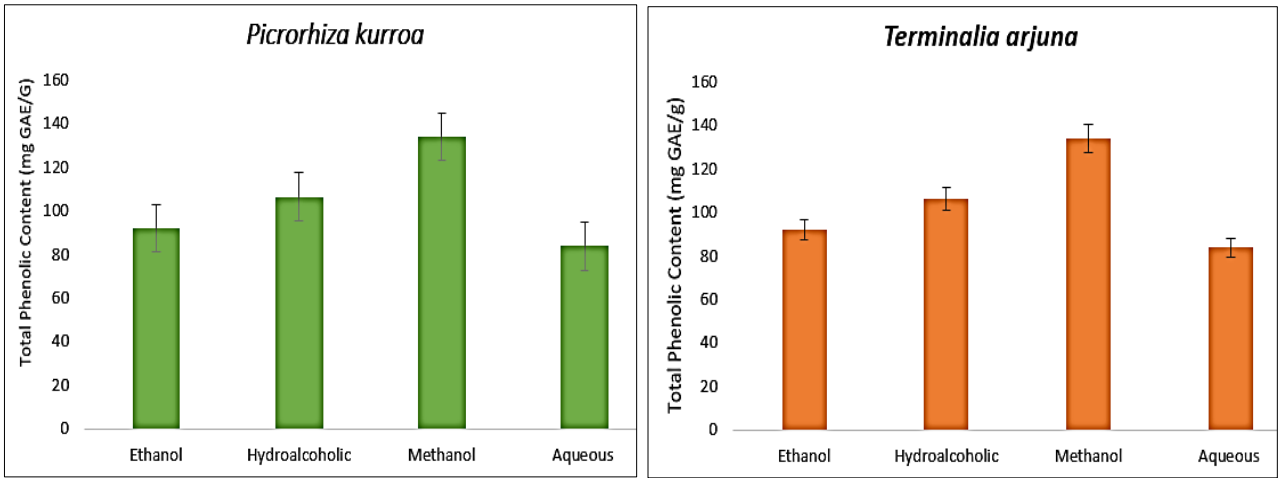

Fig 7: Total phenolic content of *Picrorhiza kurroa* rhizomes *& Terminalia arjuna*

2) Determination of Total Flavonoid Contents (TFC)

A calibration curve of Quercetin was plotted and the total flavonoid content of the extract was calculated from the line of regression and expressed as mg Quercetin Equivalent per gm. For the estimation of TFC a calibration curve was plotted with a standard concentration. The slope of line and goodness of fit (R2) value were calculated to identify the line equation. As shown in figure 5, Quercetin was used to prepare a standard curve as linearity curve. *Picrorhiza kurroa* rhizomes using different solvents like ethanol, hydroalcoholic, methanol and aqueous extract showed maximum total flavonoid content 126.81±1.10, 154.01±0.13, 184.35±1.34 and 141.71±1.03 mg QE/g. *Terminalia arjuna* roots using different solvents like ethanol, hydroalcoholic, methanol and aqueous extract showed maximum total flavonoid content 152.01±1.18, 181.61±0.13, 206.53±1.74 and 170.25±1.67 mg QE/g.

y=0.137x + 0.0093; (R^2=0.9987) where; y is the absorbance; x is the solution concentration).

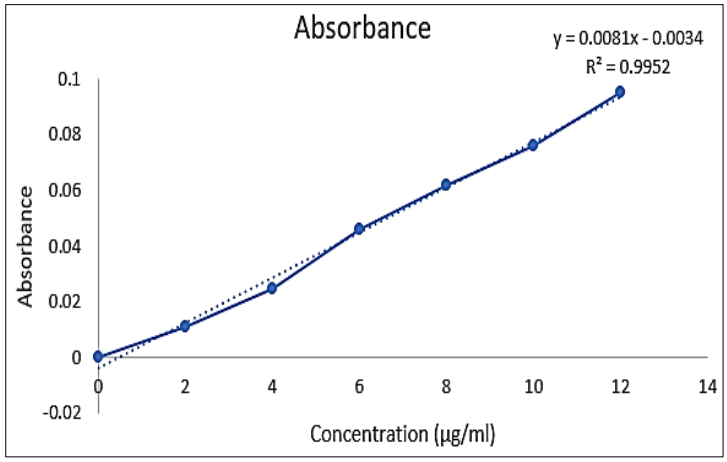

Fig 8: Linearity for quercetin concentration vs absorbance

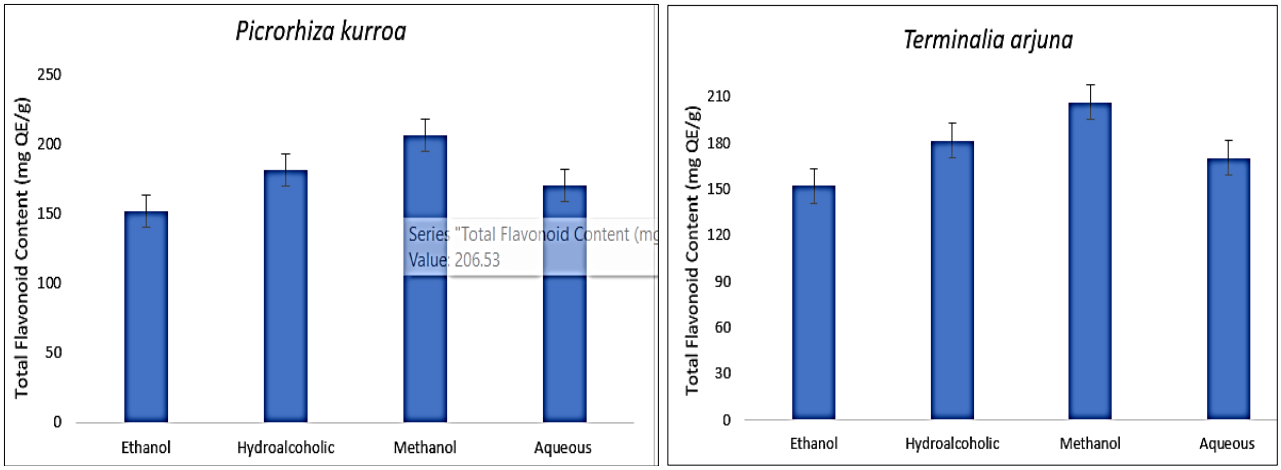

Fig 9: Total phenolic content of *Picrorhiza kurroa* rhizomes & *Terminalia arjuna*

4.5.3 Thin layer chromatography analysis

TLC of the plant extracts were carried out for qualitative analysis of various components using various solvents. The results of TLC analysis are represented in Figure 7-9. In TLC profile of *P. kurroa* rhizomes and *T. arjuna* roots extract in mobile phase Toluene-Acetone-Formic acid (4.5: 4.5: 1) v/v, ethanol showed no spot Under UV light 254 nm, and Under UV light 365 nm.

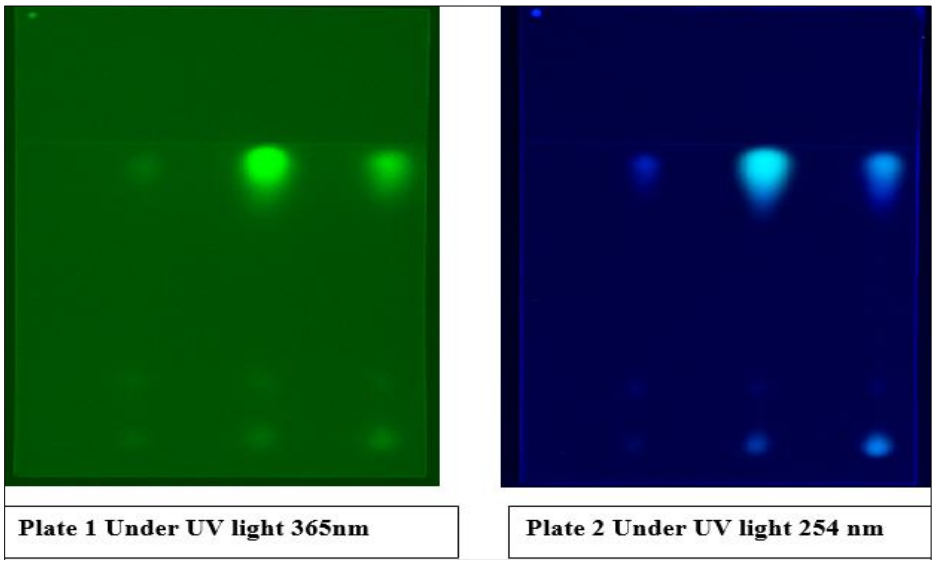

Fig 10: Representative images of TLC analysis of *P. kurroa* leaves: in Solvent System: Toluene-Acetone-

Formic acid

In TLC profile of *Terminalia arjuna* roots extract in mobile phase Toluene-Acetone-Formic acid (4.5: 4.5: 1) v/v, methanol, hydoalcoholic, methanol and aqueous extract showed one spot (0.14) Under UV light 254 nm, and Under UV light 365 nm.

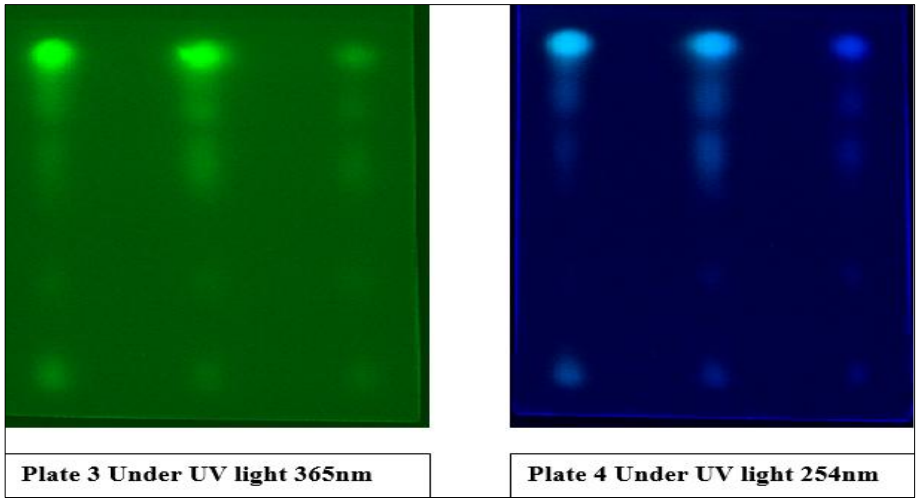

Fig 11: Representative images of TLC analysis of *T. arjuna* roots extracts: under UV light (365nm); Solvent System: Toluene-Acetone-Formic acid

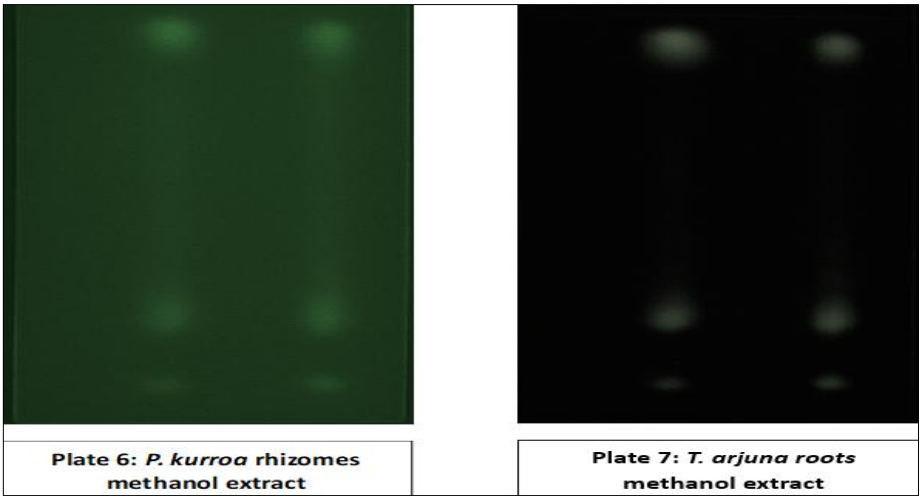

Fig 12: Representative images of TLC analysis of *P. kurroa* rhizomes and *T. arjuna* roots methanol and aqueous extracts: under UV light (365nm); Solvent System: Toluene-ethyl acetate-Glacial acetic acid (3:4:0.5) v/v

5. Antioxidant Activity

5.1 DPPH (1,1 diphenyl-2-picryl hydrazyl) radical scavenging activity

Ascorbic acid exhibited the lowest IC_{50} value of 28.00 µg/mL, confirming its potent antioxidant property. Among the plant extracts, *Terminalia arjuna* methanolic extract showed the most significant antioxidant activity with an IC_{50} value of 36.50 µg/mL, followed by its hydroalcoholic and ethanolic extracts. *Picrorhiza kurroa* methanolic extract also demonstrated appreciable free radical scavenging activity with an IC_{50} value of 49.58 µg/mL. The aqueous extracts of both plants showed comparatively lower antioxidant potential, with IC_{50} values exceeding 60 µg/mL, which may be attributed to the lower solubility of phenolic and flavonoid compounds in water.

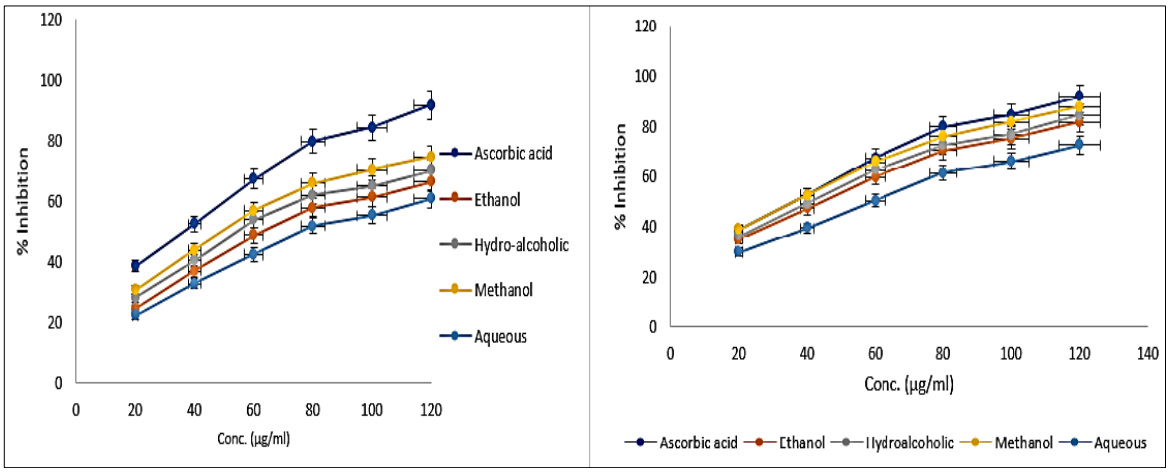

Fig 13: DPPH radical scavenging activity of *P. Kurroa* & *T. Arjuna* rhizome extracts

5.2 Hydrogen peroxide scavenging activity

The methanol extract of *Terminalia arjuna* showed the highest antioxidant activity with an IC_{50} value of 31.55 µg/mL, followed by its hydroalcoholic (36.73 µg/mL), ethanolic (40.39 µg/mL), and aqueous (57.89 µg/mL) extracts. This enhanced activity can be attributed to the high content of polyphenolic compounds, tannins (like arjunolic acid, arjunic acid, ellagic acid), and flavonoids extracted more efficiently in polar solvents like methanol and hydroalcoholic mixtures. In comparison, *Picrorhiza kurroa* exhibited moderate scavenging potential, with the methanol extract displaying the best IC_{50} value of 49.20 µg/mL, followed by hydroalcoholic (57.01 µg/mL), ethanolic (64.80 µg/mL), and aqueous (79.10 µg/mL) extracts. The aqueous extracts showed the least activity, which aligns with the known lower solubility of phenolic constituents in water.

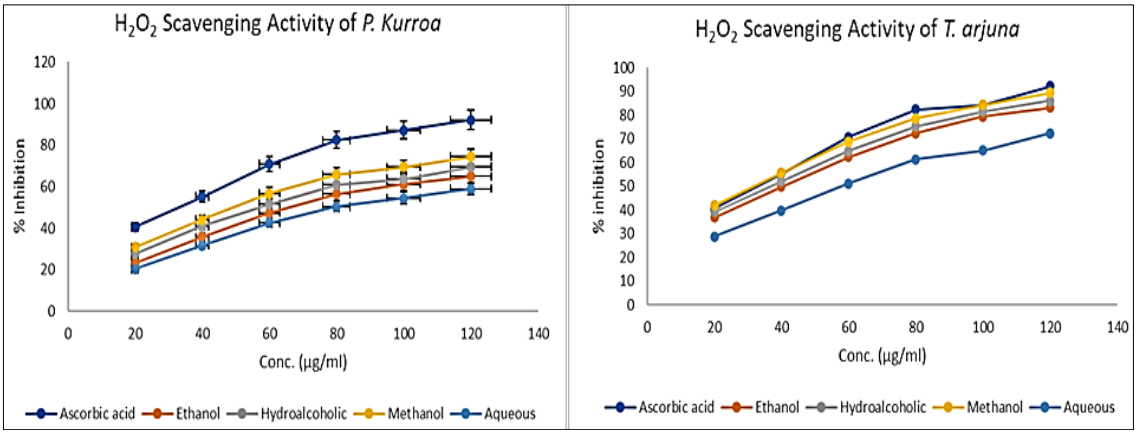

Fig 14: H_2O_2 scavenging activity of *P. Kurroa & T. arjuna* extracts

6. Conclusion

This study successfully established a systematic pharmacognostical, physicochemical, and phytochemical profile for *Picrorhiza kurroa* and *Terminalia arjuna*, confirming their authenticity, quality, and therapeutic potential. The comprehensive evaluation revealed that methanolic extracts of both plants yielded superior phenolic and flavonoid contents, correlating with significant antioxidant activity. Physicochemical parameters and microbial analyses were within pharmacopoeial limits, affirming the crude drugs' safety and purity for medicinal use. Additionally, elemental analysis validated that the essential minerals and trace elements in both plants remained within recommended daily allowances, ensuring their non-toxic nature. This study not only reinforces the traditional claims surrounding these medicinal plants but also provides essential standardization data that can serve as a reference for future pharmacological, clinical, and formulation-based research. These findings promote the safe, effective, and scientifically validated integration of *T. arjuna* and *P. kurroa* into contemporary phytotherapy.

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