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PHARMACOGENETIC QUALITY ASSESSMENT WITH *IN-VITRO* ANTIOXIDANT, ANTI-INFLAMMATORY, CYTOTOXICITY, AND ANTI-BACTERIAL POTENTIAL AGAINST PERIODONTAL PATHOGENS

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ABSTRACT: Background: Herbal extracts have beneficial effects on humankind, so scientific research on their characteristics is necessary. **Objective:** Our investigation aims to analyze the pharmacognostical, phytochemical constituents, standardization, free radical scavenging activity, *in-vitro* anti-inflammatory activity, and cytotoxicity activity of the HAE of Ashvakatri, an herbal drug. **Material and Method:** The studies were based on botanical and pharmacognostical investigation, including standardization, chromatographic evaluation, absorption maxima, and compatibility of the drug, *in-vitro* antioxidant evaluation, anti-inflammatory evaluation, and brine shrimp lethality assay were performed. **Results:** Microscopic studies of rhizomes revealed the presence of multicellular trichomes, ground tissue, epidermis, dictyosome, tracheids, and phloem. Likewise, the powder microscopy verified the presence of parenchyma cells, trichomes, tracheids, epidermal cells, etc. Proximate analysis was evaluated by total ash value, acid-insoluble ash value, and water-soluble ash value. The HAE yield was found to be 22.839% w/v. The phytochemical screening in HAE revealed the presence of alkaloids, tannins, glycosides, flavonoids, polyphenols, gums, and mucilage. The chromatographic evaluation of HAE was performed and resulted in the presence of alkaloids, flavonoids, glycosides, and tannins. Significant anti-oxidant and anti-inflammatory properties as compared to standard drugs were evaluated. The LC50 value of cytotoxicity was found to be more than threefold higher than the standard. Also, the MIC of HAE was assessed against periodontal pathogens and compared with the standard. **Conclusion:** Our investigation concluded that pharmacognostical, followed by anti-oxidant assay, anti-inflammatory activity, cytotoxicity, and antibacterial assay, provided a promising claim for potent activities of Ashvakatri against Periodontitis.

INTRODUCTION: Plants have been used to heal illness in people since the beginning of human civilization. According to a report, medicinal herbal drugs are used to address around 80% of the world's population's general health care problems.

Plants have long been utilized in the treatment of cancer, diabetes, arthritis, dental infections, and other neurological disorders. At different stages of development, several active ingredients from plants have been employed in the treatment of various diseases¹.

The ayurvedic medical system makes various uses of medicinal herbs with all diseases related. The primary advantages of using traditional medicine are its affordability, no toxicity or adverse effects or any resistance, and cost-efficient treatments². It also fulfills the need for more personalized health

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care. According to WHO, nearly 21,000 plant species are utilized as medicinal herbs. *Ashvakatri* also called oak fern or oak fern basket fern is a species of *Aglaomorpha* belonging to the Polypodiaceae family. It is also known by the synonym *Attukal kizhangu* L. The fern is also known by the name gurar, koi hin, kabkab, kabkaban and uphatkarul. This herb is helpful against rheumatoid arthritis and is also used against pain from traumatic injuries such as sprains and contusions with swelling and bruising.

As this is the first study of its kind, our research aims to investigate the medicinal use of *Ashvakatri* HAE from macroscopic to cytotoxic activity. Also, this research focuses on phytochemical constituents, free radical scavenging activity, standardization, *in-vitro* anti-inflammatory activity, and cytotoxicity activity.

MATERIAL & METHODS:

Procurement of Herbs & Identification: The live plants of *Ashvakatri* were collected from the western ghats of Kerala, India in July 2023. The plant parts were identified and authenticated by Dr Jasmeet Singh, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, India. The voucher specimen with reference number DG/22-23/563 has been deposited in the same department.

The rhizome was cut, shade-dried, and powdered by an electrical mixer grinder, followed by stored in an airtight container until further studies.

Extraction of the Drug: The powder (2kg) of *Ashvakatri* dried rhizome was extracted with 3 liters of ethanol: water (6:4) by using a maceration process for 72 hours³. After this, the mixture was filtered and evaporated using a water bath to afford a brownish sticky mass (256.78 gm) and then freeze-dried using a lyophilizer. Until usage, the dried HAE was kept at -4 °C^{4, 5}. The extraction yield was calculated as

$$\text{Yield \%} = \text{Extracted} / \text{Total} \times 100$$

Standardization of Drug:

Morphology: The morphological evaluation was carried out as per standard protocol. It includes all organoleptic characteristics such as shape, size, type, color, odor, flavor, etc. were observed⁶.

These criteria have been investigated according to the WHO guidelines and qualitative assessment of crude drugs was carried out accordingly.

Microscopy:

Transverse Section Study: Small portions of the *Ashvakatri* rhizome were manually thin-cut in the transverse section using the sharp platinum blades for microscopy⁷. The sections were then dyed using Safranin and Phloroglucinol HCL using light microscopy (OMAX- XM82ESC02) attached with Nikon COOLPIX P950 in a bright field light. The scalebars referred to magnifications.

Powder Microscopy: After being shade-dried and powdered, a part of the rhizome was put in a sealed container for powder microscopy and then passed through sieve no. 60⁷. The powder was then placed in glycerine on a sterile micro-slide and examined under light microscopy (OMAX- XM82ESC02) attached with Nikon COOLPIX P950 in a bright field light.

Proximate Analysis:

Investigation of Total Ash Value: A dried crucible was filled with precisely weighed 5 gm powdered rhizome of *Ashvakatri*. It was incinerated at 450°C until carbon-free, after which it was cooled. The total ash weight was measured and the percentage was determined concerning the air-dried sample⁸.

Analysis of Acid-Insoluble Ash Value: The ash collected previously was heated for 5 minutes with 25 ml of 2N hydrochloric acid. It was then filtered, and the insoluble residue was collected on filter paper, with less ash. The residue was thoroughly cleaned with hot water, burned in a crucible that had been covered with tar, and allowed to cool, and the resulting residue was weighed⁹.

Calculation for Water-Soluble Ash Value: The total amount of ash was heated in 25 ml of water for 15 minutes. By using ash-free filter paper, the insoluble material was collected, cleaned with hot water, and burned for a few minutes at a temperature of less than 450 °C. The estimation of water-soluble ash is represented by weight difference concerning air-dried drugs¹⁰.

Calculation of Alcohol and Water-Soluble Extractive Value: 20 mg of air-dried, and coarsely

powdered *Ashvakatri*. Macerated the rhizome in 100 ml ethanol for 24 hours, continued stirring during the first 6 hours, and then let it stand for 18 hours. After that, quickly filtered and precaution was taken to prevent the loss of alcohol. From that, 25 ml of filtrate was dried to 105 °C in a china dish with a flat surface at the bottom and weighed accordingly. The extracts percentage that are soluble in ethanol was calculated for air-dried drugs. The same technique was carried out, except instead of ethanol, chloroform water was used.

Determination of Moisture Content: 5 mg of powdered rhizome of *Ashvakatri* was measured precisely and placed on a porcelain plate. It was held at 105 °C to 110 °C for 30 minutes in a hot air oven. The moisture content percentage was then determined concerning air-dried drugs at different intervals of time ¹¹.

Preliminary Histochemical Screening: During the preliminary histochemical analysis, various phytochemicals including saponin, flavonoids, steroids, alkaloids, phenols, and other components were identified.

Alkaloids were detected by dissolving the herbal aqueous extract (HAE) in diluted hydrochloric acid (HCL) and using alkaloid reagents for evaluation. The formation of yellow-cream precipitation during Mayer's test and brown or reddish precipitation during Wagner's test indicated the presence of alkaloids ¹².

For flavonoid detection, the HAE was treated with a 10% lead acetate solution, resulting in yellow precipitation. Proteins and amino acids were detected using Millon's test and Biuret test. Additionally, the presence of amino acids was indicated through the Ninhydrin test. Glycosides detection involved various tests such as Modified Borntrager's test, Legal test, Baljit's test, and Liebermann-Burchard's test. Saponin and phytosterol were also detected through specific tests, while phenolic compounds and tannins were identified using FeCl₃ test, Gelatin test, lead acetate tests, alkaline reagent test, vaniline test and shinoda test. The presence of fixed oils and fats were determined by stain test and soap test ^{11, 12}. For carbohydrate detection, the Molisch test, Benedict's and Fehling's tests were performed, and

the presence of carbohydrates was confirmed by a violet ring for the Molisch test, crimson red precipitate for Benedict's test (indicating reducing sugar) and a mixture of orange-red colour for Fehling's test. Finally, gums and mucilage in HAE were detected by the alcohol precipitation test and the ruthenium red test ¹².

Chromatographic Evaluation of Drug: Thin-layer chromatography is a very efficient method for separating and identifying the chemical compounds from an extract. When comparing the potency of medicinal plants, the TLC profile created for an HAE forms a specific solvent system, and other parameters may be used as a fingerprint ¹³.

TLC is a significant analytical technique for separating, identifying, and estimating several categories of natural products. In this method, the solute migrates differently between the stationary phase and a mobile phase to separate the various components. The stationary phase serves as an adsorbent which is based on the adsorption principle ¹³.

Separation of Components: The HAE was dissolved in suitable solvents separately and with a calibrated capillary tube, spotting was done at 1cm above on prepared TLC plate's bottom.

The spots were similar in size and ranged in dimension from 2 to 3 mm.

Mobile Phase Selection: The solvent and mobile phase selection were done based on:

- a) Substance's nature to be separated
- b) Stationary phase's nature (polar/non-polar)
- c) Chromatographic method as (reverse/normal)
- d) Separation's extent to be achieved by (analytical/preparative)

Selection of Stationary Phase: Precoated prepared TLC plates by (E- Merck) were used for this research.

Standardization of Drug: It is the initial stage in the systematic fabrication of any dosage form. Preformulation study can be defined as the study of a drug substance's physical and chemical

characteristics both by itself and in combination¹⁴. The pre-formulation study aims to generate information for formulating suitable stable and bioavailable dosage forms.

Absorption Maxima Study: The identification of the drug was done by absorption maxima study. The chromatographic component of the molecule was determined by using UV-visible spectrophotometry. After exposed to the light in the UV visible spectrum region, the organic molecule absorbs light of a certain wavelength based on the electronic transition involved in absorption. A standard cuvette was used to collect the HAE solution in distilled water (5, 10, 15, 20, and 25 µg/ml), which was then scanned between a range of 200-800 nm using a UV-visible spectrophotometer¹⁴.

Compatibility Study: The Fourier Transform Infrared Spectroscopy (FTIR) is a technique to examine materials that are in the solid, liquid, or gaseous phase. It is based on the electromagnetic radiation and vibrations of chemical bonds among atoms that compose matter. Not every molecule's potential vibrations will produce an IR absorption spectrum. Identification of structural clarification and functional groups of compounds is accomplished using IR spectroscopy. Additionally, it is employed to determine whether a particular sample of organic substance may be distinct from another. It is also used for quantitative analysis, impurity detection, and reaction progress¹⁴.

With the use of a sample holder, the sample may be positioned directly in the direction of the infrared beam, and with 128 scans, the resolution of 4 cm⁻¹ for individual measurements for the spectral range of 500-4000 cm⁻¹ was noted.

Free Radical Scavenging Activity Determination:

Reducing Power Activity: The HAE of various dosages were combined to get (10µg, 20µg, 40µg, 80µg, and 100µg) in different concentrations, and control, in 1ml of distilled water (without any test sample). As a positive control, l-ascorbic acid (Balaji traders pt. ltd, Varanasi) was employed. They were combined with potassium ferricyanide (2.5 ml) and phosphate buffer (2.5 ml, 0.2M, pH 6.6). The mixes were incubated for 20 minutes at

50°C. Then mixtures were mixed with a quantity (2.5ml) of 10% v/v trichloroacetic acid, and the combination was centrifuged at 3000 rpm for 10 minutes. UV-visible spectroscopy was used to measure the absorbance (OD) at 700nm after adding distilled water (2.5ml) and ferric chloride (0.5ml, 0.1%) to the top layer of the solution (2.5ml) increasing reducing power shown by the reaction mixtures increased absorbance. Calculated percentage inhibition was used to represent this activity as an effective concentration of 50 (EC₅₀)^{3, 14}.

The calculation of percentage inhibition was calculated by using:

$$\text{Reducing power} = \text{Test OD} - \text{Control OD} \times 100$$

Superoxide Anion Scavenging Activity: In a test tube, 100µl of riboflavin solution (10 mg/ml of riboflavin in 0.1 M NaOH forms clear to orangish solution), 0.2 ml of EDTA solution (11.7g EDTA in 100ml water → slowly adjusted pH 8.0 with 2M NaOH), 0.2 ml of ethanol and 0.1 ml of nitro blue tetrazolium (NBT) solution were combined. The reaction mixture was then diluted with phosphate buffer by up to 3 ml (pH 7.4). After exposure for 15 minutes, the solution's absorbance was measured at 560 nm using phosphate buffer as a blank. This was used as a reference reading¹⁴.

Different concentrations of HAE and L- ascorbic acid (positive control) in 100 mL were combined with 100µl of riboflavin solution, 200µl of EDTA solution, 200µl of ethanol, and 100 µl of nitroblue tetrazolium (NBT) solution in test tubes. The reaction mixture was diluted with 3 mL phosphate buffer. After 15 minutes of illumination at 560 nm, the solution's absorbance was assessed using UV-visible spectroscopy¹⁴. The calculation of percentage inhibition was calculated by using:

$$\% \text{ Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

DPPH Radical Scavenging Activity: By utilizing the Gulchin *et al.* technique (2002) and 1,1-Diphenyl-2-picryl hydrazil (DPPH, HiMedia Labs Pvt. Ltd. India), the free radical scavenging activity of hydroalcoholic was assessed. Firstly, 0.1mM of DPPH solution in ethanol was formulated. Thereafter, 3 ml of Hydroalcoholic solution and L-Ascorbic acid (Positive control) solution at various

dosages (20-100µg) were combined with 1 ml of this solution. The mixture was briskly shaken before being let to stand at room temperature for 30 minutes. UV-visible spectroscopy was used to measure the absorbance at 517 nm. More free radical scavenging activity was shown by lower absorbance.

Calculated as the percentage of DPPH free radical inhibition, this activity was reported as 50 percent inhibition concentration (IC₅₀).

$$\% \text{Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Total Phenol Content: The Follin-Ciocalteu (F-C) technique reported by Rosa M. *et al.*, was used to calculate the total phenolic content of the HAE. 100µl of the HAE solution/standard was combined with 200 µl of the 10% (v/v) F-C reagent. 800 µl, 700 mM Na₂CO₃ was added into each test tube. And incubate at room temperature for 2 hours. 200 ml sample or blank was transferred from the assay tube to a clear 96-well microplate and the absorbance of each well was taken at 765 nm. Gallic acid at 765 nm was used to generate the standard curve, and total phenolics were converted to gallic acid equivalents using the regression equation using gallic acid standards¹⁵.

Total Anti-Oxidant Assay: According to the procedure outlined by Zhou *et al.*, a total antioxidant capacity test was carried out. On a screw cap test container containing 3 ml of reagent solution (0.6 M sulfuric acid, 28 ml sodium phosphate, and 4 ml ammonium molybdate in a 1:1:1 ratio), added to 0.3 ml HAE of various concentrations (50, 100, 150, 200, 250 g/ml). The screw cap container was incubated for 90 minutes at 90°C in a water bath and then cooled to room temperature. After that absorbance was taken by using UV visible spectroscopy at 700nm in comparison to blank. Equal concentrations of ascorbic acid were used as a standard drug to express the overall anti-oxidant capacity¹⁶.

Total Flavonoid Content: By using the aluminium chloride (AlCl₃) calorimetric technique, the total flavonoid concentration of the HAE was ascertained. 1.5ml of methanol, 0.1ml of 10% AlCl₃, 0.1 ml of 1 M potassium acetate, and 2.8ml of distilled water were combined with 0.5ml of HAE. Spectrum was used to measure the reaction

mixture's absorbance at 415nm after 30 min of incubation at room temperature.

In-vitro Anti-Inflammatory Activity: The following recognized techniques were utilized to assess the *in-vitro* anti-inflammatory activities of crude HAE of *Ashvakatri* as:

Inhibition of Egg Albumin Denaturation: Protein denaturation causes inflammatory and arthritic disorder, and in some cases, it is caused by to production of an autoantigen¹⁶. Therefore, the agents with the ability to denaturation can be utilized as an anti-inflammatory drug.

The reaction mixture (5ml) contained 0.2 ml of fresh hen's egg albumin, 2.8 ml of phosphate-buffered saline (PBS, pH 6.5), and 2 ml of HAE at different concentrations of 100, 200, 300, 400, and 500 g/ml. As a standard, the same concentrations of Aceclofenac sodium (Aristo Pharmaceutical Pvt. Ltd, India) were used to determine the absorbance. The solutions were incubated for 15 minutes at 37 °C in a BOD incubator (Lab-line Technologies) and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Protein denaturation inhibition percentage was determined using follows:

$$\% \text{Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Membrane Stabilization Test: A human red blood cell (HRBC) membrane stabilization assay was carried out with 10 ml of fresh whole human blood. The centrifuge tubes were cleaned three times with an equivalent volume of normal saline after being HRBC centrifuged for 10 minutes at 3000 rpm. Blood volume was calculated, then reconstituted as a 10% v/v suspension with normal saline¹⁶.

Heat-induced Hemolysis: In one test tube, 2ml of the reaction mixture, 10 % RBC suspension, and 1ml of the test sample (HAE) were added at various concentrations (50-400µg). In another test tube, saline was added instead of the test sample as a control test tube. Aceclofenac sodium (10mg), from Aristo Pharmaceutical Pvt. Ltd., was used as a standard drug. The reaction mixture in each centrifuge tube was incubated in a water bath at 56 °C for 30 minutes. The tubes were cooled under running water after incubation. After centrifugation

at 2500 rpm for five minutes, the absorbance of the supernatants was measured at 560 nm. For each test sample, the experiment was run in triplicate¹⁶.

Cyclooxygenase-1 and Cyclooxygenase-2 Inhibition Assay: To measure percentage inhibition at a dose of 100 g/ml concentration, the potency of the HAE for COX-1 and COX-2 were evaluated by using a calorimetric assay kit (Caspase 9 calorimetric assay kit) purchased from Elabscience, USA. Firstly, HAE was dissolved in DMSO and diluted to obtain concentrations of 100 µg/ml for the sample. Various dosage (10-100 µg) of Ketorolac Tromethamine (Ketorol- DT, Dr. Reddy's, India) and Aceclofenac sodium (Aristo Pharmaceutical Pvt. Ltd., India) were used as a positive control solution. Our method was used individually to suppress COX-1 and COX-2 inhibition¹⁷. According to manufacturer instructions, percentage inhibition was measured, and activity was reported as inhibition concentration (IC₅₀).

Brine Shrimp Lethality Assay/Cytotoxicity Study: In artificial seawater made from 38 g/L of commercially available sea salt, the eggs of brine shrimp (*Artemia saline*) were hatched. To attract the hatched shrimp, a lamp was placed above the open side of the tank. The shrimp were ready to assay after 24 hours as they matured into *Artemia Salina nauplii*. The brine shrimp lethality assay was carried out of HAE of Aswakirti rhizome using a standard protocol¹⁸.

The stock solution was prepared by dissolving 10 mg of HAE in five labelled test tubes and DMSO solution with varying concentrations (1 mg/ml, 0.1 mg/ml, 10 µg/ml, and 1 µg/ml) was added to it. Afterward, 10 brine shrimp were added to the premarked test tubes.

After 24 hrs, each test tubes were inspected and the number of dead shrimps via a magnifying glass. Vincristine sulphate was taken as a positive control. The percentage of lethality of brine shrimp was calculated for each concentration of solution. The value of LC₅₀ was calculated by concentration and percentage mortality. Linear regression correlation was plotted for concentration against percentage¹⁹.

$$\% \text{ Death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{Number of live nauplii}} \times 100$$

In-vitro Anti-bacterial Assessment:

Microorganism Source: The standard bacterial strain of *Aggregabacter actinomycetemcomitans* (ATCC 29523) and *Porphyromonas Gingivilis* (ATCC 33277) were procured from Promochem, Bengaluru, Karnataka.

MIC: The MIC is the lowest concentration of the extract that will prevent the growth of test microorganisms. 0.5 McFarland standard was used to compare the growth turbidity to prepare 1.5 × 10⁸ CFU/ml of bacteriological suspension. BHI broth supplemented with horse serum and vitamin K was utilized as a media for both bacterial strains. For MIC, the stock solution was prepared by dissolving 500 mg HAE in 1 ml of 50% DMSO. Six dilutions (50%, 25%, 12.5%, 6.25%, 3.125%, and 1.562%) of the above solutions were prepared and tested against both bacterial strains by broth dilution method as per CLSI guidelines. Additionally, six dilutions (2%, 1%, 0.5%, 0.25%, 0.125%, and 0.0625 %) Chlorhexidine (Merck Co. Ltd., Sigma Aldrich) were prepared and used as a positive control. Afterward, the tubes were stored in the bacteriological incubator at 37 °C for 48 hours and their turbidity was observed. The anaerobic jar was used to incubate *P. gingivilis* while the CO₂ desiccator was used to incubate *A. actinomycetemcomitans*. Growth was indicated by turbidity in tubes and MIC was determined by recording the lowest concentration that remained clear. The mean value was recorded and all the tests were performed in triplicate²⁰.

Statistical Analysis: The data were analyzed as mean ± SEM using one-way or two-way analysis of variance (ANOVA) and Friedmann's test was employed as a test of significance. The minimum threshold of significance was considered as P value ≤ 0.05. IBM-SPSS statistical software version 20.0 was used to conduct all statistical analyses.

RESULTS & DISCUSSION:

Morphological Observation: The young, fleshy rhizome of the *Ashvakatri* plant, can reach a maximum length of 18 cm and maximum breadth of 8 cm. The tough, dry rhizome is uneven in shape and almost flat and measures up to 12×6×12 cm. It is covered in a velvet-like scale that is copper in color. After cutting, the inner surface is creamish and has longitudinal wrinkles that are visible with

fracture splintery having a puny in sliced surface, sweetish odor, and bitter flavour as described in Fig. 1.



FIG. 1: MACROSCOPICALLY IMAGE OF ASHVAKATRI RHIZOME

Microscopical Observations:

Transverse Section Study: Detailed microscopy of *Ashvakatri* rhizome has wavy ridges and furrows.

There is a single-layered, wavy outer epidermis with a few multicellular trichomes and winged scales emerging from the furrows. Long, lanceolate scales with an extended, tapering tip are present.

Rectangular epidermal cells are filled with a brownish substance followed by a broad region of ground tissue made up of rectangular parenchyma cells with thin walls that are highly packed. In addition, a stele that is dispersedly distributed and the ground tissue also has starch grains. Fig. 2 suggests the microscopical representation of the large number of heteromorphic meristemes produced by heavily dissected protostele. Endodermis and pericycle are each present in a separate layer on top of each stele. The meristemes have solitary xylem content that is surrounded by phloem. The xylem is composed of thick-walled angular end arch metaxylem and exarch protoxylem components that are encircled by smaller phloem content.

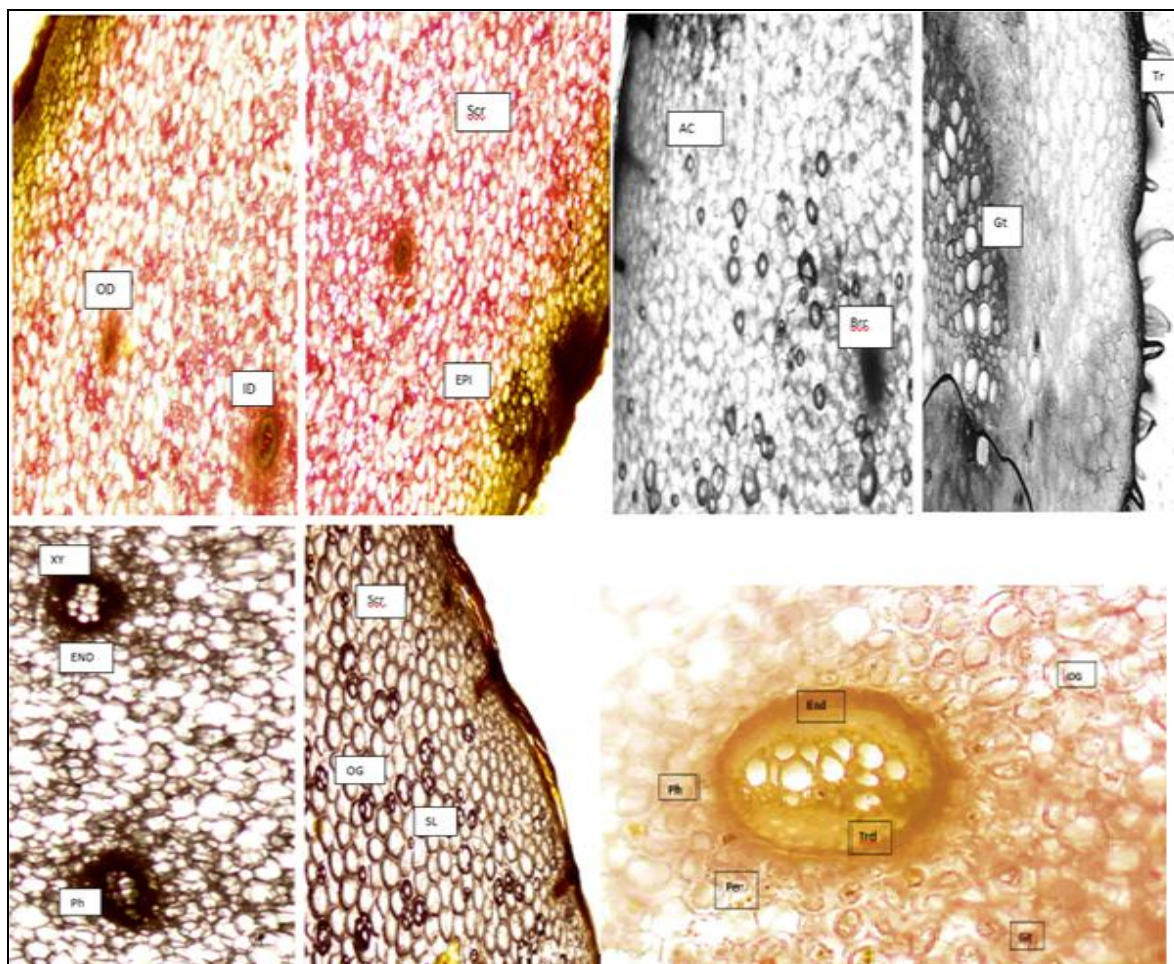


FIG. 2: MICROSCOPICAL REPRESENTATION OF TRANSVERSE SECTION OF ASHVAKATRI RHIZOME. Scr- Silica crystal, OD- Outer dictyostele, EPI- Epidermis, ID- Inner dictyostele, AC- air canal, Gt- ground tissue, Tr- Trichome, Brc- Brown content, Xy- Xylem, END- endodermis, Ph- Phloem, OG- oil gland, SL- scaly leaves, Trd- Tracheid, Per- Pericyclic, Gt- Ground tissue.

Powder Microscopy: The powdered rhizome has a coffee-brown appearance, a slightly distinctive odor, and a bitter taste. When powdered rhizome was examined under a microscope, scaliform, and

reticulate tracheid, trichomes, many parenchyma cells with brown content, lignified fibres with silica crystals, etc. were all seen in **Fig. 3**.

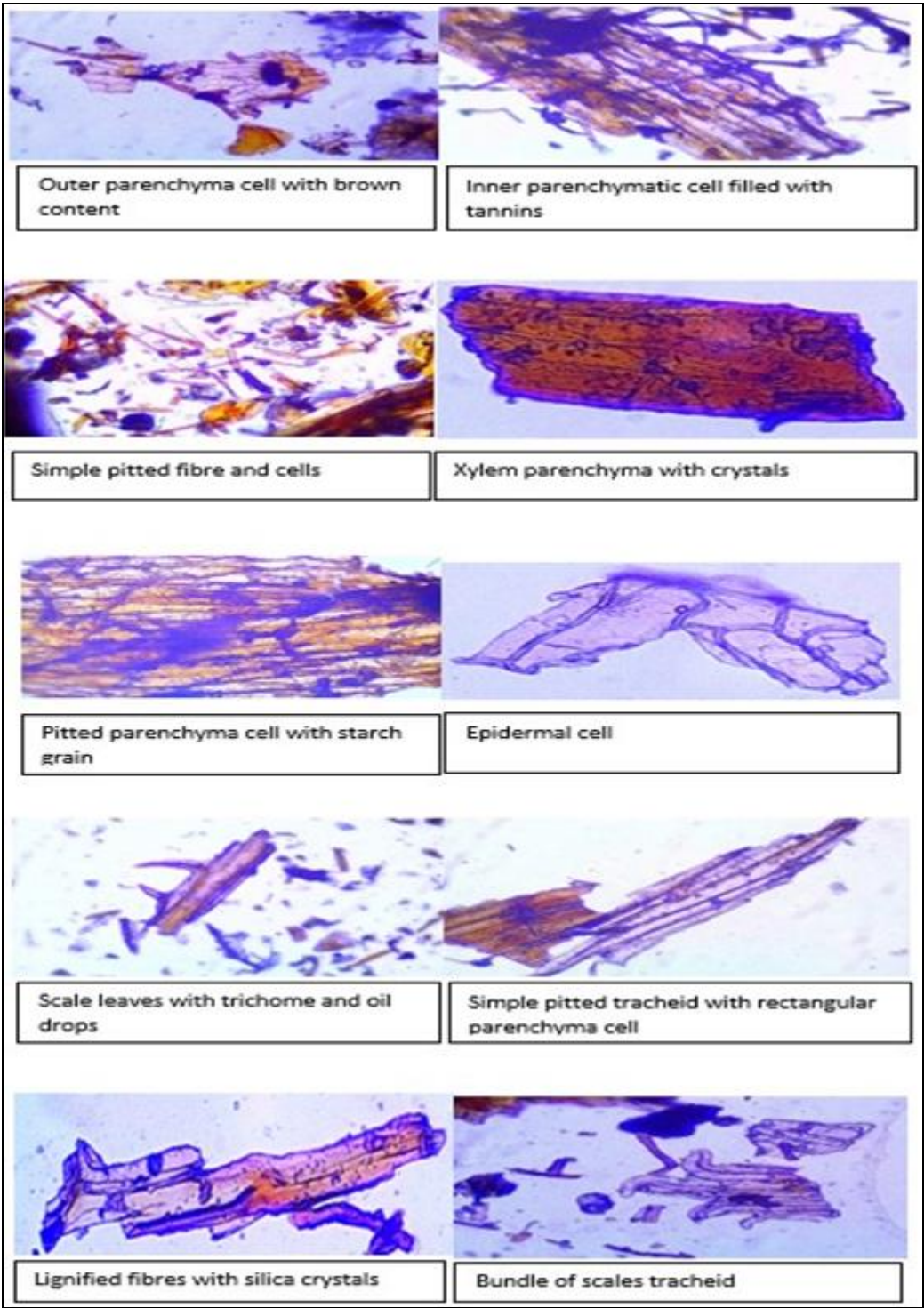


FIG. 3: POWDER MICROSCOPIC EVALUATION OF ASHVAKATRI RHIZOME. Brc- Brown content, Xy- Xylem, END- endodermis, Ph- Phloem, OG- oil gland, SL- scaly leaves.

Proximate Analysis Results: The total ash value, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value,

and moisture content of the *Ashvakatri* rhizome were evaluated and described in **Table 1**. The main purpose of all these parameters is to regulate the

quality of the crude drug. The total ash value was found to be 9.88 %, followed by acid insoluble ash value was 4.36%, and the water-soluble ash value was obtained as 6.08%. Ash value indicates the amount the foreign material such as inorganic salts or silica present in crude drug as an adulterant, which may also be used to evaluate the purity of the crude drug obtained. Ash content also known as ash value, is the residue that remains after burning plant/rhizome material.

TABLE 1: RESULTS OF PROXIMATE ANALYSIS

Plant Name	Total ash (%)	Acid insoluble ash (%)	Water soluble ash (%)	Ethanol soluble extractive value (%)	Water soluble extractive value (%)
Ashvakatri crude drug	9.88	4.36	6.08	9.90	14.06

TABLE 2: RESULTS OF THE MOISTURE CONTENT OF ASHVAKATRI

Moisture content	
Time (minutes)	Moisture content (%)
30	12.52
45	10.86
60	7.65
75	5.54
90	5.49

HAE Yield: The HAE was made by the maceration technique, and the blackish-brownish color of *Ashvakatri* was found to be 22.839% w/v. The obtained mass was then freeze-dried using a

It simply means that inorganic salts either naturally occur in crude drugs or are purposefully added to them as a means of adulteration. These ash values are an important pharmacognostical tool for standardizing the crude drug. The extractive value for ethanol was found to be 9.90% w/w and 14.06% w/w for water. Likewise, **Table 2** describes the moisture content in the crude drug was found to be 12.52% for 30 minutes, followed by 5.49 % after 90 minutes.

lyophilizer and kept under refrigeration until further investigation.

Phytochemical Screening Observation: The phytochemical analysis of the hydro-alcoholic extract of *Ashvakatri* rhizome is displayed in **Table 4** and **Fig. 4**. Tannins, terpenoids, alkaloids, anthraquinones, glycosides, saponins, flavonoids, gums and mucilage, saponin, phytosterols, and carbohydrates were all present in the sample. Henceforth, *Ashvakatri* rhizome has a greater number of chemicals present.



FIG. 4: PHYTOCHEMICAL TEST EVALUATION OF HAE

Phytochemical screening is the most effective method to identify new sources of therapeutic and industrially valuable compounds that may also have significant medicinal properties.

Many therapeutic plants have a chemical component that specifically affects the human body. The most significant plant bioactive components include flavonoids, alkaloids, tannins, saponins, and phenolic compounds.

There is also evidence that these phytochemicals have a variety of functions, some of which may contribute to the prevention of chronic illness.

Alkaloids, for instance, offer protection against long-term illness, whilst saponins have strong anti-bacterial properties and also prevent hypercholesterolemia. **Table 3** represents the result obtained by phytochemical screening of powdered rhizome.

TABLE 3: PHYTOCHEMICAL SCREENING OF ASHVAKATRI RHIZOME

Histochemical screening of drug		
Test		Hydro-alcohol HAE
Alkaloid		
Mayor test		+
Wagner test		+
Dragendorff's test		+
Hager's test		+
Favanoids		
Lead acetate test		+
Glycosides		
Mod. Bontrager's test		-
Legal's test		+
Liebermann-Burchard test		+
Baljet test		-
Saponin		
Forth test		+
Phytosterols		
Liebermann-Burchard Test		+
Phenolic & Tannins		
Ferric Chloride test		+
Gelatin test		-
Lead acetate test		+
Alkaline reagent test		-
Shinoda test		-
Vanillin HCL test		+
Protein And Amino Acid		
Millions test		+
Biuret test		-
Ninhydrin test		+
Fixed Oils & Fats		
Stain test		+
Soap test		-
Carbohydrates		
Molisch's test		+
Benedict's test		+
Barfoed's test		+
Gums & Mucilage		
Alcohol ppt test		+
Ruthenium test		+

Chromatographic Evaluation: To validate the presence of several phytoconstituents in the drug, thin-layer chromatography was performed on an HAE of *Ashvakatri*. The hydroalcoholic potential R_f values are shown in **Table 4**. The alkaloid had three spots with R_f values of 0.4, 0.8, and 0.9, respectively. Likewise, flavonoids had four spots

with 0.4, 0.72, 0.96, and 0.85 R_f values, respectively. Additionally, the glycoside had R_f values of 0.38, 0.64, and 0.49, respectively, with three spots in TLC plates. Tannins were observed with two spots with R_f values of 0.6 and 0.82 respectively as indicated in **Fig. 5 (A, B, and C)**.

TABLE 4: CHROMATOGRAPHIC PARAMETERS FOR HYDRO-ALCOHOLIC HAE COMPONENT

Sr. no.	Chemical constituents	Mobile Phase	Compositions	Detecting method	No. of Spots	R _f values
1	Alkaloids	Toluene: Ethyl acetate: Diethyl amine	7: 2: 1	UV at 366 nm	3	0.4, 0.8, 0.9
2	Flavonoids	Chloroform: Methanol	8: 2		4	0.4, 0.72, 0.96, 0.85
3	Glycoside	Ethyl acetate: Methanol: Water	5: 2.5: 2.5		3	0.38, 0.64, 0.49
4	Tannins	Toluene: Ethyl acetate: Formic acid	6: 2: 2		2	0.6, 0.82

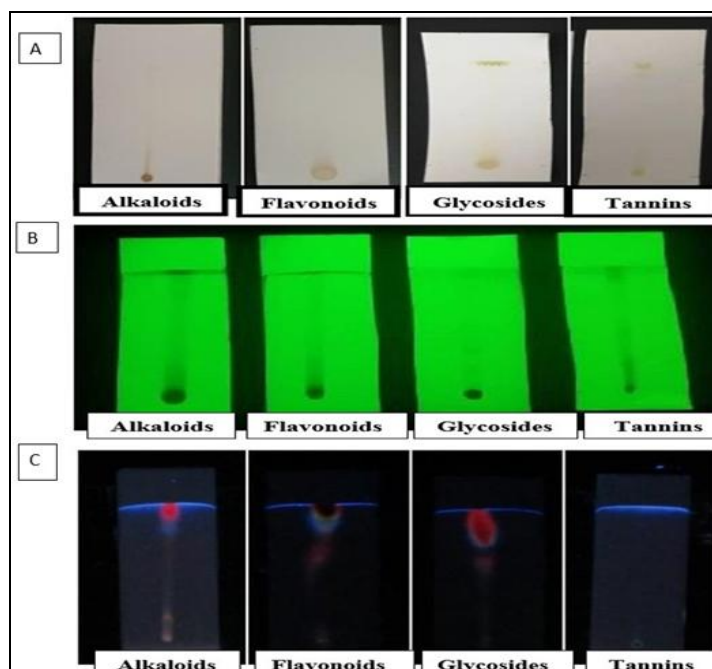


FIG. 5: (A)- TLC CHROMATOGRAM OF ASHVAKATRI AND ITS COMPONENTS, (B)- CHROMATOGRAM AT UV VISIBLE AT 254NM, (C): CHROMATOGRAM SPOT ON UV AT 366NM

Standardization of Drugs: Standardization is the most important part of any drug, ensuring its quality, safety, and reproducibility. It involves the whole pre-formulation and ethnography process from crude drug procurement to developing the finished product. The important parameters of pre-formulation studies are solubility, melting or boiling point, absorption maxima, and compatibility studies.

Solubility: The drug was completely soluble in ethanol and sparingly soluble in water.

Melting point: With a melting point of 218-220°C the drug produced a brownish needle-like crystal.

Absorption maxima (λ max) of HAE: By using a UV spectrophotometer (Shimadzu), the absorption maxima were produced. Two peaks were observed from the HAE of *Ashvakatri* rhizome as shown in Fig. 6. One peak was observed at 218.96 nm with an absorbance unit of 4.400 and the second peak was obtained at 282.6 nm with an absorbance unit of 1.949 AU.

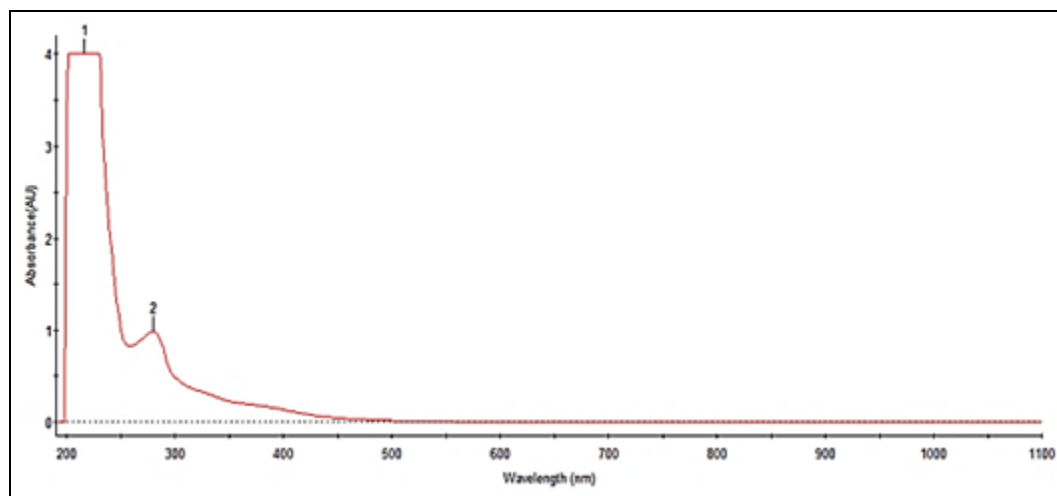


FIG. 6: UV SPECTRUM GRAPH OF HAE OF ASHVAKATRI

Compatibility Study: FTIR study was performed for the HAE of *Ashvakatri*. The vibrational spectrum of any HAE or drug is believed to

represent its special and unique physical characteristics. Table 5 represents the absorption spectra of the *Ashvakatri* hydro-alcoholic HAE that

were collected within the range of 4000-400 cm^{-1} . Asymmetric N-H stretching was observed at 3380.68 cm^{-1} . Similarly, three dominant C-H stretching in the spectrum were observed at 2974.56, 2925.75, and 2899.84 cm^{-1} respectively. Also, an asymmetric stretching of vibration was

seen at 1923.76 cm^{-1} . Furthermore, C=C bands were observed in IR spectra at 1649.08 cm^{-1} . The obtained finding demonstrated in **Fig. 7** the existence of several functional groups from the HAE of *Ashvakatri*.

TABLE 5: INTERPRETATION OF IR SPECTRUM OF HAE

Sr. no.	Interpretation	
	Wave number (cm^{-1})	Assignment
1.	3380.68	N-H stretching
2.	2974.56, 2925.75, 2899.84	C-H stretching
3.	1923.76	C-H stretching
4.	1649.08	C=C stretching
5.	1451.35, 1406.97	C-C stretching (in-ring)

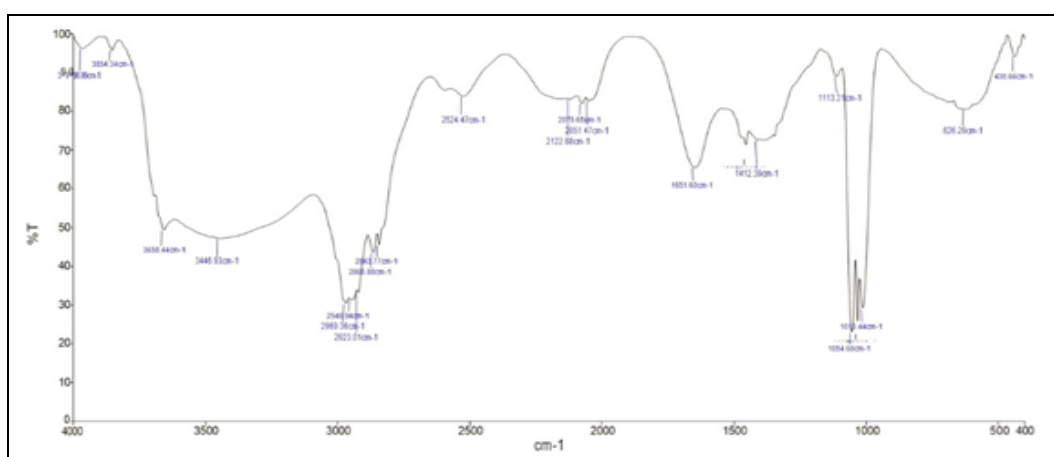


FIG. 7: IR SPECTRA OF HAE OF ASHVAKATRI

Determination of Antioxidants: The reducing power, superoxide, and DPPH free radical scavenging activities of the HAE were compared to standard L-ascorbic acid. **Table 6** outlines the results of these comparisons. In the measurement of reducing power capacity, the HAE sample showed greater activity compared to the control, with an IC_{50} value of 46.85 μg (as shown in **Table 7**). The HAE also demonstrated greater dose-dependent superoxide anion scavenging activity, with an IC_{50} value of 55.8 μg , indicating higher NBT-superoxide activity than the standard (as indicated in **Table 8**). In the DPPH radical scavenging activity test, the HAE exhibited hydrogen-donating capability at different doses compared to a standard antioxidant drug. The IC_{50} value for the HAE was found to be 59.4 μg , which was greater than the standard drug L-ascorbic acid. Additionally, the total antioxidant activity represented in **Fig. 8** showed an IC_{50} value of 53.6 μg , higher than that of the standard drug. The study concluded that the increasing concentration of the antioxidant activity

of HAE compared with standard drug L-ascorbic acid had a correlation coefficient (r^2) value of 0.9435. Furthermore, the presence of flavonoids and polyphenols in the HAE may result in its free radical scavenging activity, as demonstrated in **Fig. 8**. The total flavonoid content in the HAE of *Ashvakatri* was found to be 73.8 mg/g (as indicated in **Table 8**), and the total phenol content in HAE was found to be 63.24 mg/g. Flavonoids, which are widely found in plants, release free polyphenols in the gastrointestinal system and are essential for the human body as antioxidant compounds. They are composed of several polyphenol or phenol hydroxyl groups joined to ring structures. Furthermore, during inflammation, the release of lysosomal enzymes can cause various illnesses. Non-steroidal drugs act by either stabilizing the lysosomal membrane or by inhibiting these enzymes. The RBC membrane damage can increase the cell's susceptibility to subsequent damage from lipid peroxidation induced by free radicals.

TABLE 6: % INHIBITION OF DPPH RADICAL SCAVENGING ACTIVITY

Sr. no.	Sample	% Inhibition of DPPH radical scavenging activity		
		Concentration (µg)	Optical Density at 517 nm	Inhibition (% RSA)
1	L-Ascorbic acid	Control	0.52	-
		20	0.218	58.076
		40	0.194	62.692
		60	0.102	80.384
		80	0.088	83.076
		100	0.078	85
2	HAE	Control	0.52	-
		20	0.208	60
		40	0.337	35.192
		60	0.298	42.692
		80	0.247	52.5
		100	0.178	65.769

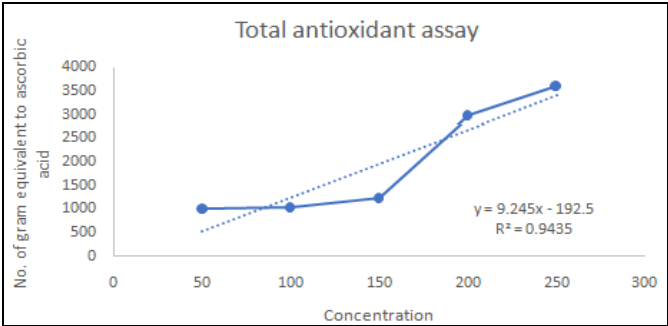


FIG. 8: TOTAL ANTI-OXIDANT ASSAY OF HAE

TABLE 7: IC₅₀ VALUE OF *IN-VITRO* ANTI-OXIDANT ACTIVITIES

Sr. no.	Sample	IC ₅₀ value of <i>In-vitro</i> Anti-oxidant activities (µg)			
		Reducing power activity	Superoxide anion scavenging activity	DPPH scavenging activity	Total antioxidant activity
1.	Ascorbic acid	11.45	8.1	3.9	4.3
2.	HAE	46.85	55.8	59.4	53.6

TABLE 8: DATA INTERPRETATION FOR TOTAL ANTI-OXIDANT ASSAY

Total antioxidant assay		
Concentration (µg)	Optical Density at 517 nm	No. of gram equivalent to ascorbic acid
Control	0.124	-
50	0.252	1000
100	0.367	1025
150	0.429	1225
200	0.716	3000
250	0.848	3625

***In-vitro* Anti-inflammatory Result:** Protein denaturation is a process by which proteins are subjected to external stressors or compounds, such as heat, an organic solvent, a strong acid or base, or concentrated inorganic salts. As a result, proteins lose their secondary and tertiary structures. When denatured, the majority of biological proteins lose their functions. One of the well-established causes of inflammation is protein denaturation. The current study has demonstrated the inhibition of thermally induced protein (Albumin) denaturation in a dose-dependent manner. The IC₅₀ value of albumin denaturation assay was found to be 18.47 µg which was higher than the standard drug Aceclofenac sodium as described in **Table 9**.

TABLE 9: TOTAL FLAVONOID & PHENOL CONTENT

Sr. no.	Observation	Standard drug	Standard drug quantity (µg)	Results (mg/g)
1.	Total Flavonoid content	Quercetin	100	76.89
2.	Total Phenol content	Gallic acid	100	63.24

Furthermore, since the erythrocyte membrane and lysosomal membranes are similar, it has been possible to evaluate the *in-vitro* anti-inflammatory effect using the stabilization of the HRBC membrane, which suggests that the HAE may also stabilize lysosomal membranes. By inhibiting the release of bacterial enzymes and protease, which are lysosomal components of activated neutrophils that further cause tissue inflammation and damage upon extracellular release, lysosomal stabilization

plays an important role in reducing inflammatory responses. In the present study, the IC₅₀ value (18.60 µg) of HAE of *Ashvakatri* showed somewhat similar activity compared to Aceclofenac sodium (18.53 µg) as demonstrated in **Table 10**. Therefore, the result revealed that HAE of *Asvakatri* inhibiting against both albumin as well as heat-induced hemolysis henceforth, it may be useful against acute and chronic inflammation.

TABLE 10: IC₅₀ VALUE (µG) OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY

Sr. no.	Sample	IC ₅₀ value (µg) of <i>in-vitro</i> Anti-inflammatory activity (µg)	
		Albumin Denaturation Assay	Inhibition of Heat-induced Hemolysis Assay
1.	Aceclofenac sodium	8.62	18.53
2.	Hydroalcoholic solution	18.47	18.60

Inhibition of COX-I and COX-II Assay: The two significant enzymes that catalyze the production of mediators involved in the inflammatory process are cyclooxygenase and 5-lipoxygenase. The inhibition of COX is a modern treatment to reduce pain, inflammation, and fever. Our study aims to assess the potential of HAE to suppress COX-I and COX-

II in *In-vitro* assay. **Table 11** suggests the comparative inhibition of COX-I and COX-II with NSAID drugs as a standard. The result revealed the significant effect of HAE by inhibiting *in-vitro* COX-I and COX-II enzymes to reduce inflammation and pain.

TABLE 11: IC₅₀ VALUES OF HAE BY COX I AND COX II INHIBITION ASSAY

Samples	IC ₅₀ value (µg) ± SEM of <i>In-vitro</i> COX Inhibition Assay	
	COX-I	COX-II
HAE	08.56±0.002	09.28±0.003
Ketorolac Tromethamine	0.018±0.004	0.12±0.005
Aceclofenac sodium	04.74±0.002	05.49±0.004

Brine Shrimp Lethality Assay: The LC₅₀ value of HAE and Vincristine was found to be 15.83 and 0.839 µg/ml, respectively, as shown in **Table 12**. An appropriate linear correlation was observed in **Fig. 9** by plotting a graph between percentage

mortality against the LC₅₀ value obtained by the effect of HAE concentration on brine shrimp. *Ashvakatri* HAE's cytotoxicity was promising, indicating the presence of potent bioactive compounds.

TABLE 12: CYTOTOXICITY ASSAY RESULT

Sr. no.	Sample	LC ₅₀ (µg/ml)	Regression equation	R ² value
1.	Vincristine	0.839	Y= 34.02x + 52.58	0.9521
2.	HAE	15.83	Y = 33.03x + 35.145	0.9909

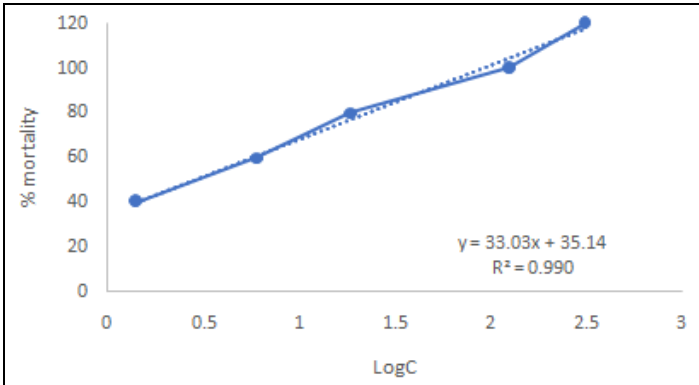


FIG. 9: LETHALITY ASSAY OF HAE AFTER 24 HOURS

Anti-bacterial Activity: The anti-bacterial screening of HAE of *Ashvakatri* was observed against the two most common periodontal pathogens, and the results are summarised in **Table 13**. Among the two strains tested with HAE, *P. gingivitis* was the more sensitive organism with a

MIC value of 1.84 mg/ml against chlorhexidine gluconate at 0.0625 mg/ml, whilst *A. actinomycetemcomitans* had less susceptibility with a MIC value of 2.71 against the positive control at 0.25 mg/ml.

TABLE 13: MIC VALUE OF HAE OF ASHVAKATRI

Sr. no.	Periodontal pathogens	MIC of test agents (mg/ml)	
		HAE	Chlorhexidine gluconate
1	<i>A. actinomycetemcomitans</i>	2.71	0.25
2	<i>P. gingivitis</i>	1.84	0.0625

CONCLUSION: Herbal drugs can easily be adulterated or replaced with lower quality from the same plant or others. Therefore, appropriate collection, identification, authentication, and standardization of crude drugs are crucial factors for the quality assurance of herbal medicine (Szerlauth *et al.*, 2019). The fundamental factors for standardizing plant-based medication are macroscopic, microscopic, and powder microscopic assessment, absorption maxima, solubility, melting point, compatibility study, *etc.* (Okareh *et al.*, 2018). Our study concluded that the HAE of *Ashvakatri* promises all these standardization factors in all manner. Also, the physiochemical and quantitative proximate analysis revealed the product purity and identified no adulterant or substitute present. This plant is safe to use and no toxicity was found from our investigation.

Phytochemical screening also provided important information regarding the existence of phytoconstituent in the *Ashvakatri* rhizome. Our research also concluded that HAE has decent flavonoid and phenolic content as compared to standard drugs. Additionally, the conclusion of this investigation confirmed the extract's ability to protect against oxidative damage and act as a potent anti-oxidant drug. Thus, it is also concluded that compounds with the ability to stabilize membranes such as our drug, may provide greater protection against cell membranes against harmful enzymes to reduce inflammation. Furthermore, the HAE of *Ashvakatri* also has modest cytotoxic action which ensures the presence of bioactive compound in our drug. Also, the current investigation evaluated the antibacterial activity of HAE against ATCC standard strains of two putative periodontal pathogens from 1996 like *A. actinomycetemcomitans* and *P. gingivitis*.

It has been demonstrated that the existence of these bacteria can serve as a helpful marker for both active illness and a higher risk of gingival attachment loss.

Declaration:

Ethics Approval: Not applicable.

Data Availability: The corresponding author can make the data used to verify the article's results accessible upon reasonable request.

Funding: Not applicable.

Author Contributions: P.D. and N.M. conceptualized and designed the experiment. P.D. wrote the manuscript. N.M. reviewed the whole manuscript. S.G. and K.P.T. also validated the experiment. All authors are approved for the submission.

CONFLICTS OF INTEREST: The authors state they have no known competing financial or personal relations that could have influenced their research.

REFERENCES:

1. Akhtar MS, Rafiullah M, Shehata WA, Hossain A & Ali M: Comparative phytochemical, thin layer chromatographic profiling and antioxidant activity of HAEs from some Indian herbal drugs. *Journal of Bioresources and Bioproducts* 2022; 7(2). <https://doi.org/10.1016/j.jobab.2022.01.001>
2. Bairagi J, Katore V, Chourey B, Delouri A & Nema S: Evaluation of *in-vitro* anti-inflammatory activity of leaves of *Pongamia pinnata*. *Asian Journal of Dental and Health Sciences* 2023; 3(1). <https://doi.org/10.22270/ajdhs.v3i1.35>
3. Chaiyana W, Charoensup W, Sriyab S, Punyoyai C & Neimkhum W: Herbal HAEs as potential antioxidant, anti-aging, anti-inflammatory, and whitening cosmeceutical ingredients. *Chemistry and Biodiversity* 2021; 18(7). <https://doi.org/10.1002/cbdv.202100245>

4. Shakthi A, Linoj J, Suresh V, Hussein MA, Selvamani M and Sakthivel A: Evaluation of antibacterial and antioxidant activities of Suaedamonoica extract for its potential application. *Cureus* 2024; 28: 16(1).
5. Ginovyan M, Hovhannisyan S, Javrushyan H, Sevoyan G, Karabekian Z, Zakaryan N, Sahakyan N & Avtandilyan N: Screening revealed the strong cytotoxic activity of *Alchemilla smirnovii* and *Hypericum alpestre* ethanol HAEs on different cancer cell lines. *AIMS Biophysics* 2022; 10(1). <https://doi.org/10.3934/BIOPHY.2023002>
6. Kagawad P, Gharge S, Jivaje K, Hiremath SI & Suryawanshi SS: Quality control and standardization of Quercetin in herbal medicines by spectroscopic and chromatographic techniques. *Future Journal of Pharmaceutical Sciences* 2021; 7(1). <https://doi.org/10.1186/s43094-021-00327-y>
7. Liu H, Specht CD, Zhao T & Liao J: Morphological Anatomy of Leaf and Rhizome in *Zingiber officinale* Roscoe, with Emphasis on Secretory Structures. *Hort Science* 2020; 55(2). <https://doi.org/10.21273/HORTSCI14555-19>
8. Manubolu K, Peeriga R, Bonthu MG, Vatchavai BR, Korukola N, Gope ER & Kadirvel D: Evaluation of Anti-inflammatory Activity of *Millingtonia hortensis* Leaf HAE. *International Journal of Chemical and Biochemical Sciences* 2023; 24(6).
9. Nithin MK, Veeramani G & Sivakrishnan S: Phytochemical screening and gc-ms analysis of rhizome of *Drynaria quercifolia*. *Research Journal of Pharmacy and Technology* 2020; 13(5). <https://doi.org/10.5958/0974-360X.2020.00408.4>
10. Hasan H, Jadallah S, Akrim Z, Ali F and Saber M: The anti-cancer, anti-inflammatory, antibacterial, antifungal, anti-oxidant and phytochemical investigation of flowers and stems of *Anacyclus clavatus* Plant Extracts. *AlQalam Journal of Medical and Applied Sciences* 2025; 9: 415-27.
11. Pace R & Martinelli EM: The phytoequivalence of herbal HAEs: A critical evaluation. *Fitoterapia* 2022; 162. <https://doi.org/10.1016/j.fitote.2022.105262>
12. Prashanth GK, Giresha AS, Lalithamba HS, Aman M, Rao S, Ravindra KN & Chaturvedi V: Sustainable bio-fabrication of Ni/Mn co-doped ZnO nanoparticles using *Simarouba glauca* leaf extract: Evaluation of non-cytotoxic, anti-carcinogenic, anti-tubercular, anti-bacterial properties, anti-oxidant and hyaluronidase inhibition activities. *Inorganic Chemistry Communications* 2025; 171: 113592.
13. Shubayr N: Phytochemicals properties of herbal HAEs for ultraviolet protection and skin health: A narrative review. *Journal of Radiation Research and Applied Sciences* 2023; 16(4). <https://doi.org/10.1016/j.jrras.2023.100729>
14. Sukweenadhi J, Yunita O, Setiawan F, Kartini Siagian MT, Danduru AP & Avanti C: Antioxidant activity screening of seven Indonesian herbal HAE. *Biodiversitas* 2020; 21(5). <https://doi.org/10.13057/biodiv/d210532>
15. Govindaiah PM, Ruban SW, Kiran M, Mohan HV & Prabha R: Exploring the *in-vitro* anti-oxidant and antimicrobial properties of pomelo fruit extract in goat meat: a promising alternative for preservative and health benefits. *Scientia Horticulturae* 2024; 337: 113534.
16. Syukri Y, Purwati R, Hazami N, Anshory Tahmid H & Fitria A: Standardization of specific and non-specific parameters of propolis HAE as raw material for herbal product. *EKSAKTA: Journal of Sciences and Data Analysis* 2022; 128.
17. Tran TTX, Pham TV, Lam LT, Hua BT, Nguyen NB, Do BH & Tran BG: Anti-oxidant and anti-bacterial effects of Diospyros kaki leaf extract in Vietnam. *Ho Chi Minh City Open University Journal of Science-Engineering and Technology* 2025; 15(1).
18. Tmušić N, Ilić ZS, Milenković L, Šunić L, Lalević D, Kevrešan Ž, Mastilović J, Stanojević L & Cvetković D: Shading of medical plants affects the phytochemical quality of herbal extracts. *Horticulturae* 2021; 7(11). <https://doi.org/10.3390/horticulturae7110437>
19. Zhou X, Razmovski-Naumovski V, Mendoza R, Truong J & Chan K: A comparative study of *Salvia miltiorrhiza* Radix & Rhizoma raw material and granule products using chromatographic analysis and antioxidant activity. *Journal of Traditional Chinese Medical Sciences* 2022; 9(3). <https://doi.org/10.1016/j.jtcms.2022.06.004>
20. Alaguvél S and Sundaramurthy D: Unveiling the anti-cancer, anti-inflammatory, anti-oxidant and anti-bacterial properties of in situ phyto-fabricated silver nanoparticles: an *in-vitro* approach. *New Journal of Chemistry* 2024; 48(25): 11366-76.

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