Antioxidant and Chemometric Profiling of phytochemicals from *Eupatorium triplinerve Vahl*'s leaf extract and *In-silico* screening of potential inhibitors of hemorrhoids

Gayathri Murugesan[1], Madhan Shankar S R[2*] Bhargavi Srinivasan[2]

[1] Department of Biotechnology, PSG College of Arts and Science, Coimbatore 641014, Tamilnadu, India.

[2] Department of Biotechnology, Kongunadu Arts and Science College (Autonomous), Coimbatore 641029, Tamilnadu, India.

ABSTRACT

Eupatorium Triplinerve Vahl commonly used in traditional medicine to treat hemorrhoidal diseases. It is considered one of the most prevalent gastrointestinal diseases with high risk and prevalence. The present systematic research aims to express the prodigious antioxidant phytoconstituents by evaluating their chemometric analysis and molecular docking for the identification of potential molecules against haemorrhoids. The leaves were extracted using five different solvents and screened for qualitative and quantitative phytochemical analysis and antioxidant potentials using DPPH and FRAP assay. Image-based chemometric analysis of High–Performance Thin Layer Chromatography (HPTLC) was assessed by Principal Component Analysis (PCA), and Hierarchical Cluster Analysis (HCA) – Heat maps. Further, *In-silico* studies were used to identify the bioactive phytocompounds against haemorrhoids causing protein target. Qualitative and quantitative phytochemical analysis of the leaf extracts exhibited a richness for variegated phytocompounds. Antioxidant potential showed significantly high for DPPH IC_{50} (µg/ml): Methanol - 51.53±0.71 and Acetone - 57.73±9.06 and reducing power capacity with a value of FRAP (µM FeE/g): Methanol - 163±0.7, Acetone - 184±1.6 respectively. Chemometric data acquirement demonstrated secondary metabolites alkaloids and phenol exhibit the high intensity and close clusters with methanol and acetone leaf extract. Further, *Insilico* molecular docking studies found good binding affinity (-8.027 kcal/mol) towards the Nitrogen Oxide Synthase (NOS) protein corresponding to other ligands which might be a hydrogen interaction formed with amino acids residues (ARG367, ALA448, TRP449). The present study indicates that leaf extracts exhibit a strong correlation between the various phytochemical constituents and have better potential towards the inhibitory enzyme to serve as an effective for hemorrhoid activity.

KEYWORDS

Eupatorium triplinerve Vahl; hemorrhoids; phytocompounds; molecular docking; Chemometric profiling.

INTRODUCTION

Haemorrhoids are a pathological manifestation in the gastrointestinal tract which are commonly seen in the anal canal cushion region and can be characterized as enlargement and distal displacement in the vasculature.[1] According to the report of the National Center for Health Statistics, the prevalence of hemorrhoids is 3.82% in the United States and 0.36% in India. The 21st - century scenario reports that 14.4%, 38.9%, and 16% prevalence of hemorrhoids were among the adult population in the age group of 35-45 years in South Korea, Austria, and Israel respectively. Hemorrhoid is the fourth leading proctological disease and is the main cause of hematochezia, colorectal cancer, Inflammatory Bowel Disease (IBD), and colitis.[2-3] The generation of free radicals is the primary reason for the initiation of many physiological and pathological disorders like hemorrhoids[4–6] leading to cancer and related disorders. Colon and anal cancer are related to hemorrhoids which cause similar symptoms and might be with rectal bleeding or lumps in the anus. Hemorrhoids can be treated with surgical and non-surgical methods. The current management to treat hemorrhoids at the various stages is classified depending on four - degrees as follows: Grade I, II, III, IV) that including open and closed hemorrhoidectomy, Doppler-guided hemorrhoidal artery ligation, and stapled hemorrhoidopexy are some of the surgical procedures followed under pronic conditions.[7-8] Side effects and potential risks of surgical methods differ based on the person's health condition and can be damage to sphincter muscle, urinary retention, stool leakage, and narrowing of stenosis due to scar tissue. Non-surgical methods include lifestyle and dietary modification including topicals, oral medication, fiber supplements, adequate hydration, and straining avoidance to anal muscle does not damage any tissue and muscles. Drug therapy usually consists of the administration of topical formulations of herbal bioactive compounds, corticosteroids, local anesthetics, antiseptics, or oral venotonics, such as flavonoids.[9–11]

Several numbers of plant species are used as raw material in the form of natural drug extracts having biologically active compounds that serve as prolific resources for medicinal purposes. Bioactive compounds are capable of preventing and reversing cellular level damage and inflammation in humans caused by the release of free radicals.[12-13] The plants used in traditional medicine are a great source of natural antioxidants, anti-inflammatory, and anticancer agents which serve as a base for the development of novel drugs.[14] WHO's (World Health Organisation) 2019 and 2021 refer Traditional, Complementary, and Alternative Medicine (TCI) to board set of health care practices to anthroposophic medicine and the

Indian Ayurveda developed model and criteria for assessment in the integration of herbal medicines in the form of herbs, herbal products, and its preparation into health system which contains all ingredients from plant parts and plant materials as active elements for many infectious diseases which can treat.[15]

Eupatorium triplinerve vahl belongs to the Asteraceae family and is locally known as Ayapana in Tamil, Mrithasanjeevani in Malayalam, and Ayaparna in Hindi. The plant is an ornamental erect perennial and slender herb with a semi-woody base growing up to 1m in height.[16] Traditionally, the leaf extracts are consumed orally to reduce hemorrhage and it also heals induced ulceration in any place within or inside gout. It helps in ailments like gastric and duodenal ulcers, Crohn's disease, and haemorrhoids.[17–19] The leaves are very useful in reducing pain and inflammation. The plant serves as a natural therapeutic agent for neurodegenerative and hepatotoxic disorders due to the presence of unique bioactive components like coumarin, thymohydroquinone, umbelliferone, herniarin, stigmasterol, sabinene. Coumarin as (7-methoxy coumarin) is used as an anti-tumor agent and is considered to be a component of the general defense response to abiotic and biotic stresses which exhibit anti-inflammatory activity.[16–21] Several research reports state that the plant has various medicinal properties like antimicrobial, antioxidant, antineoplastic, sedatives, analgesic, antiseptic, and anti-hemorrhoid activity.[21–23] The obtained leaf ethanolic extract results showed analgesic, antibacterial, antifungal, and antiseptic activity in the treatment of haemorrhage,[24-25] and methanolic extracts of leaf procured hepatoprotective and antioxidant effects. And the plant is highly used for antinociceptive and anti-inflammatory activity.[14,26]

The present study aims to investigate and compare the five different solvents (Petroleum ether, Methanol, Ethanol, Acetone, and Hexane) in leaf extracts for the analysis of phytochemical content, particularly secondary metabolites, phytoconstituents present in the extract were analyzed for qualitative and quantitative assessment, antioxidant analysis, and HPTLC image-based chemometric profiling. Further, followed by *in-silico* docking studies to determine the potent compound which can be used to treat haemorrhoid, inflammatory cytokines, and epithelial cell markers.

Materials and methods

Reagents, Chemicals, and reference standards

Petroleum ether, Methanol, Ethanol, Acetone, Hexane solvents used from SIGMA-Aldrich, DMSO, $FeCl_3$, $AlCl_3$, Benedict's reagent, Fehling's reagent, KI, $MgCl_2$, gelatin, NaCl,

NaOH, Sulphuric acid, glacial acetic acid, chloroform, ninhydrin reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), $FeSO4.7H_2O$, Quercetin, catechol, BSA, D-glucose, gallic acid, oleanolic acid, para coumaric acid, colchicine.

Collection and authentication of plant material

The leaves of *Eupatorium triplinerve vahl* were collected from the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore in the month of December 2017. The collected plant was authenticated by the Botanical Survey of India (BSI), Coimbatore, Tamilnadu, India. The Voucher number of the plant specimen is BSI/SRC/5/23/2019/TECH/250.

Preparation of plant leaf extracts

The leaves of *E. triplinerve Vahl* were washed thoroughly in running tap water and finally washed with sterile distilled water. It was then shade dried and ground using a mortar and pestle into coarse powder and stored at room temperature until further use. The powdered leaf (20g) was subjected to soxhlet using various solvents based on the polarity index units as Petroleum ether (0.1), Hexane (0.1), Acetone (5.1), Methanol (5.1), Ethanol (5.2). The leaf extract was collected in a round bottom flask for about 16-24 hours and the solvents were allowed to evaporate at room temperature and filtered through Whatman No. 1 filter paper. Further, the crude extract was collected, aliquoted, and stored at 4^{o}C. The percentage yield for the extracted sample was calculated and tabulated [27] (Supplementary Table S1).

Characterization of phytoconstituents from the leaf of *E. triplinerve Vahl*

Qualitative Phytochemical analysis

For the phytochemical analysis, different organic solvents extracts prepared earlier were used to screen for the presence of various secondary metabolites. The extracts were individually dissolved in analytical grade DMSO to qualitatively analyze secondary metabolites such as alkaloids, flavonoids, phenols, coumarin, glycosides, carbohydrates, proteins, terpenoids, phytosterols, tannins, and saponins based on their chemical reactions, precipitation, and color changes following the standard protocol as described by *Trease and Evans and Harborne*.[28,29]

Quantitative Phytochemical analysis

From the different solvents leaf extracts of *E. triplinerve vahl*, for Total Phenol Content,[30] Total Protein Content determined by Bradford's method,[31] Total Flavonoids Content,[32-

33] and Total Carbohydrates Content by anthrone method [31] were estimated expressed in graphical representation (Supplementary Table S2).

Statistical analysis

Linear regression analysis was performed for the secondary metabolites estimated from five different solvents leaf extracts. Results were expressed as mean ±SD and triplicates (n=3) respectively. The analysis was tabulated and graphical representation was performed exhibited using Graphpad Prism version 8.0, USA.

Determination of Antioxidant activity by Spectrometric techniques

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay

The antiradical powers of substances were determined in different leaf extracts using the DPPH assay with minor modification by spectrometric analysis.[24] For the analysis, different concentrations (20-100 µg/ml) of the five different solvent leaf extracts were taken and added 1.5ml of samples were added to each of the tubes containing freshly prepared 1.5 ml of 6×10^{-5} M DPPH. After vigorous shaking, it was incubated for 30 min at room temperature in the dark and the absorbance value was read at 517 nm by using a spectrophotometer (UV-VIS Shimadzu) against the reagent solvent as blank. Ascorbic acid (1mg/ml) was used as the reference standard. The percentage inhibition for DPPH activity was calculated using the formula:

$$\text{Inhibition (\%)} = [(A_C - A_S) / A_C] \times 100$$

Where A_C is the absorbance of the control, and A_S is the absorbance of the sample.

The IC_{50} value of the plant extract sample, Log dose inhibition curve calculated by using the concentration of the sample required to inhibit 50% of the DPPH free radical. The lower absorbance of the reaction mixture indicated higher free radical activity.

Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing antioxidant powers of the different solvent leaf extracts were estimated spectrophotometrically following the modified method.[25] The method is based on the reduction of a colorless Fe^{3+} TPTZ complex to blue colored Fe^{2+} - tripyridyltriazine complex formed by the action of electron-donating antioxidants must be performed in low pH. Add 1.5 ml of the different leaf extracts (100 µg/ml) was mixed thoroughly with 1.5 ml freshly prepared FRAP reagent [containing 300 mM acetate buffer (3.1 g CH_3COONa in 16 ml glacial acetic acid) at pH 3.6, a solution of 10 mM TPTZ in 40 mM HCl and 20 mM $Fecl_3.6H_2O$ solution in the proportion of 10:1:1 (v/v/v) at 37°C]. Further, the reaction mixture was incubated in a water bath for 30 min at 37°C in dark and its plotting the

absorbance was measured at 593 nm. The ferric reducing power of the different leaf extracts was analyzed relating to the calibration curve for standard ascorbic acid and concentration of $FeSO_4$ (2-200 µM) and results were expressed in µM FeE/g. Higher value absorbance of the leaf extracts indicates a greater ferric reducing capacity, and was subjected to a standard nonlinear calibration curve method.

Statistical analysis:

To analyze the antioxidant capacity parameters (DPPH and FRAP analysis), the non-linear regression analysis was performed to calculate the dose-response relation of five solvent leaf extracts and the Pearson, r two-tailed was evaluated to find out the significant correlation coefficient and the value $p < 0.001$ which was considered. The statistical and graphical evaluations were performed by Graphpad Prism version 8.0, USA. The observed statistical data of the FRAP study were enclosed in the Microsoft Excel format as a mean and standard value. Results were expressed as mean ± SD ($n=3$).

High-Performance Thin Layer Chromatography (HPTLC) fingerprinting analysis

To determine the content of alkaloids, flavonoids, phenols, terpenoids, and coumarin present in the five different leaf extracts HPTLC was performed with standards for the development of the characteristic chromatogram data profile. Chromatography was performed on10×10 cm TLC plate pre-coated with the silica gel 60 F_{254} (E.Merck, Mumbai, India) and loaded using a Hamilton syringe to form a 6 mm band length in a semiautomatic CAMAG LINOMAT 5 instrument with a concentration of 10 µg of sample and standards. Mobile phase plate was developed by linear rising progression using a 20×10 cm CAMAG Twin tank with their respective solvent mixtures of Ethyl acetate: MeOH: Water (10:1:35:1, v/v/v); Ethyl acetate: MeOH: Formic acid: Water (3:0.9:0.9:0.5); Toluene: Ethyl acetate: MeOH: Formic acid (6:6:1:0.1); Toluene: Ethyl acetate: MeOH: Acetone (14:4:1:1) saturated up to 70.0mm for 20 min at room temperature. The plates were dried in an oven at 60ºC for 5 min and formerly mentioned anisaldehyde sulfuric acid reagents (Anisaldehyde sulphuric acid; 1% Ethanolic $AlCl_3$; 5% Alcoholic $FeCl_2$) sprayed as a post derivation agent and dried at 120ºC for 20 min. The images were documented at UV 254 nm, and 366 nm using CAMAG TLC scanner 3 and Visualizer for Spectro densitometric analysis linked with WinCATS software.[34]

Fingerprint image analysis using chemometric techniques

Chemometric can be used as a multivariate tool to find out the cluster format distance and relationship between variables for HPTLC plate data set for the chemical profile present in all solvent leaf extracts according to the process described by *Ristivojevic et al.*,2017.[35] The

image of the HPTLC plate was processed by the Image J program, inbuilt FIJI version 1.52v (NIH, Wisconsin, USA) freely available on the java platform. The images were cropped (selection/Image/Crop) and are split into three filter channels: primary colors red, green, and blue filter channels (Image/Color/Split channels) were deionized through median filter function, baseline drift removed and rectangular selection tool utilized for outline the image. Finally, the line profile plots (Analyze/Plot profile) were achieved for each sample, and the profile plots were combined to display a single-channel two-dimensional graph, X-axis representing intensities by y-axis representing pixel distance along the line with their plot profiles with R_f value for each object and considered as independent variables.[36-37]

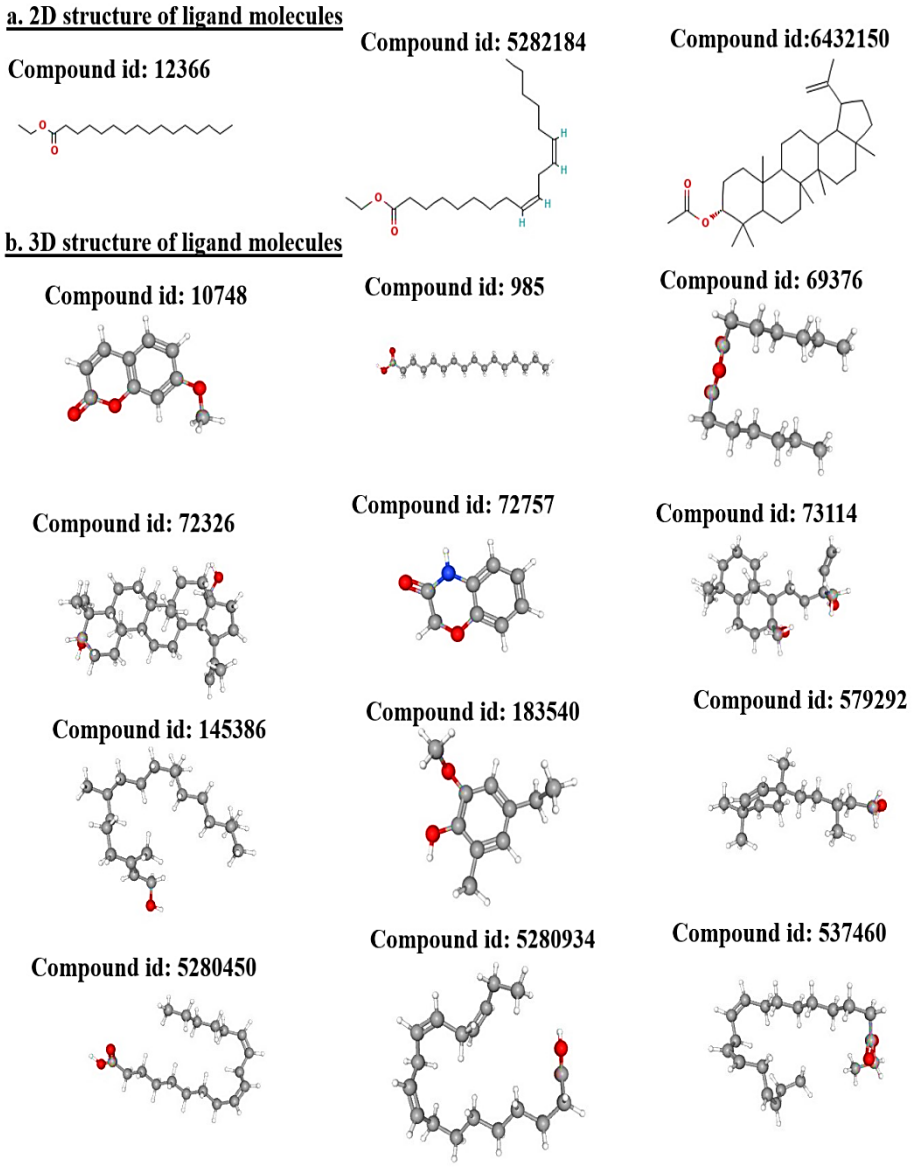
Principal Component Analysis (PCA) was carried out to reduce the dimensionality of a data set including a wide range of interrelated variable hyperspace and Hierarchical Cluster Analysis (HCA) and Heat maps applying for euclidean distance as measured between the sample clustering based on their chemical fingerprint variabilities. The data matrix was constructed using Microsoft Excel and PCA was performed using the JMP 15.1.0 statistical discovery software (SAS Institute Inc., NC, and USA). The agglomerative Hierarchical Clustering Analysis (HCA) and Heat maps were performed by NCSS 2020 20.0.1 (Utah, USA).

In-silico model for anti hemorrhoid activity

Ligand preparation

All the 90 ligand molecules from five different solvent leaf extracts of *E. triplinerve vahl* were selected for an *in-silico* docking study to demonstrate their hemorrhoids activity. 2D and 3D structures of the 90 ligand molecules were obtained from the PubChem database, as in structure-data file (SDF) format (Figure 1). The OPLS3 force field was selected for energy minimization and possible protonation and ionization states were assigned to each ligand. Possible stereoisomers, tautomers states, and metal-binding sites were generated. The ligands were prepared using the LigPrep 2.3 module of the Schrodinger 9.0 suite which was used for the optimization potentials for ligand datasets.[38]

Figure 1: 2D and 3D structure of the ligand molecules from natural plant *Eupatorium triplinerve vahl* leaf compounds

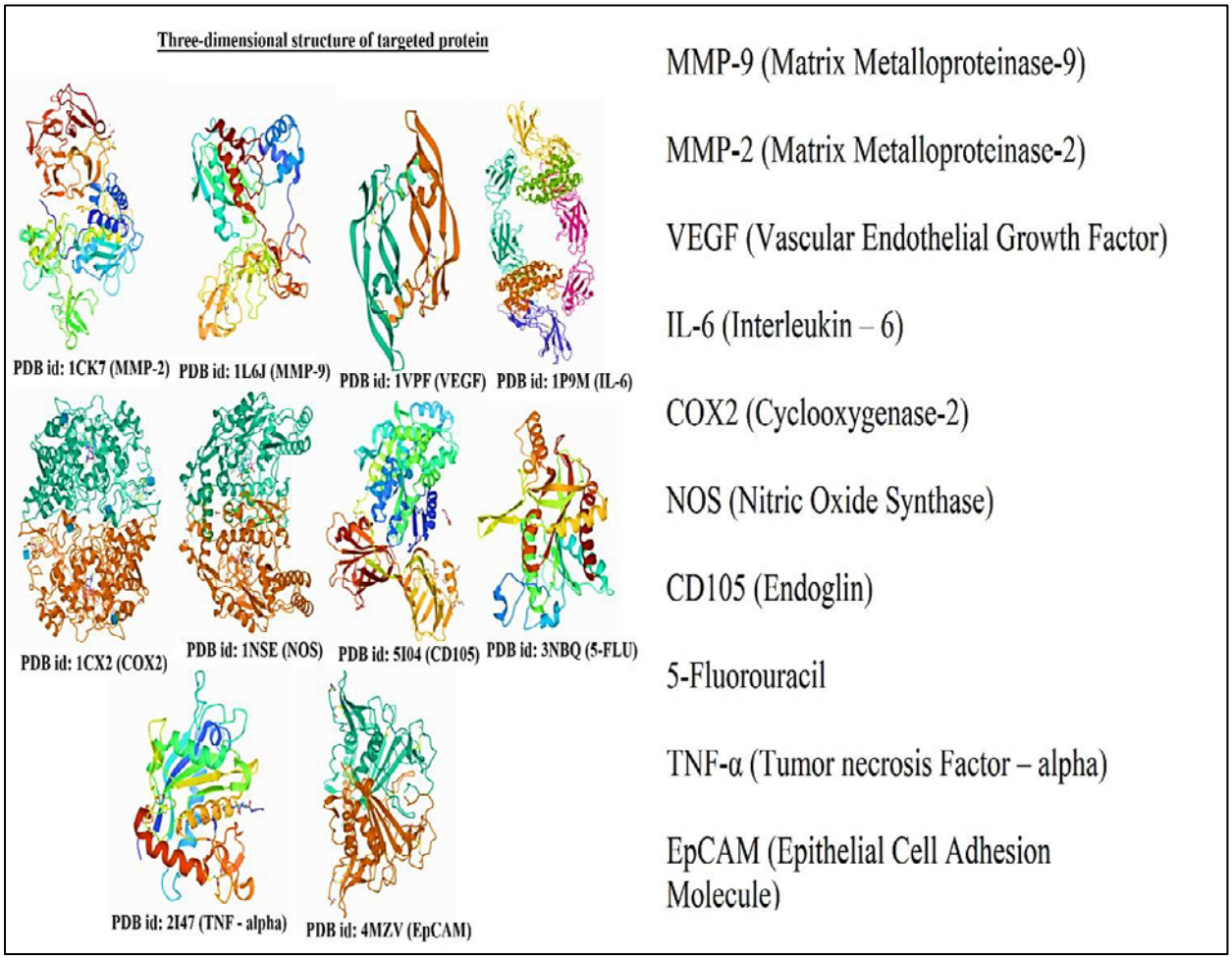


n- Hexadecanoic acid	Ethanol	985
2H-1-Benoxazin 3(4H)-one	Methanol	72757
Hexadecanoic acid (or) Palmitic acid, ethyl ester	Ethanol	12366
Heptanoic acid, anhydride	Ethanol	69376
Betulin (or) Betulinol	Methanol	72326
2H-1-Benzopyran-2-one, 7-methoxy	Acetone and Methanol	10748
1-Naphthalenepropanol	Methanol	73114
Phytol Isomer (natural)	Methanol	145386
2-methoxy-4-ethyl-6-methylphenol	Methanol	183540
3-methyl-5-(1,4,4-trimethylcyclohex2-enyl) pentan-1-ol	Ethanol	579292
9,12, octadecadienoic acid	Methanol	5280450
9,12,15- Octadecatrienoic acid (or) Alpha-Linolenic acid(z,z,z)-	Methanol	5280934
Ethyl Linoleate/ethyl (9Z, 12Z)-9,12-octadecadienol	Ethanol	5282184
9,12,15-octadecatrienoic acid, ethyl ester	Ethanol	5367460
Lup-20(29)-en-3-ol, acetate, (3.beta)-	Hexane and Methanol	6432150

Target Protein preparation

Three-dimensional structure targets proteins of hemorrhoids such as Matrix Metalloproteinase (MMP-2 and 9) (1CK7 and 1L6J), Nitric Oxide Synthase (NOS) (1NSE), Vascular Endothelial Growth factor (VEGF) (1VPF), cyclooxygenase2 (COX2) (1CX2), and inflammatory and epithelial cell marker such as Interleukin-6 (IL-6) (1P9M), Tumor Necrosis factor-alpha (TNF-α) (2I47), Endoglin (CD105) (5I04), Epithelial Cell Adhesion Molecule (EpCAM) (4MZV) and standard drug 5-Fluorouracil (5-Flu) (3NBQ) were obtained in PDB format from Protein Data Bank (Figure 2). The proteins were prepared and refined using the protein preparation wizard in Mestro Schrodinger suite 9.0. Bonds and hydrogen orders were assigned and added to heavy atoms as well as water molecules were detected and side chains were adjusted.

Figure 2: Three Dimensional structure of targeted protein and representation with PDBID.



ADME and Toxicity Prediction

The pharmacokinetics and pharmacodynamics of each 90 ligand molecule were analyzed using the Qikprop program (Schrodinger software). The ADME/T is a comprehensive tool to determine the absorption, distribution, metabolism, excretion, and toxicity (ADME/T) approach useful for studying the mechanism of chemical compounds.[39]

Docking studies

Compounds having the best 15 scores of ADMET property were targeted against proteins for the i*n-silico* model with the grid glide-based ligand docking program of the Schrodinger suite.[40] Only active molecules have available access to accurate hydrophobic contact between rigid, flexible docking for predicting the binding affinity, ligand efficiency, and inhibitory constant were analyzed for the target hemorrhoids study. The determination of the ligand-binding affinity was calculated using the lig score and dock score to estimate the ligand-binding energies.

Results

Qualitative phytochemical analysis of the *E. triplinerve vahl* leaf extract

The qualitative phytochemical analysis of the extracts is based on color change, precipitation, and chemical reaction of presence and absence of secondary metabolites were summarized in Table 1. The results assessed that the methanol, ethanol, and acetone extracts showed a high presence of coumarin, flavonoids, carbohydrates, terpenoids, and moderate presence of alkaloids, phenolic compounds, tannins, phytosterols, and phenol respectively. Glycoside, quinone, and proteins were moderately present in all the solvent extracts, and for petroleum ether and hexane, a moderate presence in flavanones and tri-terpenoids was observed.

Table 1: Qualitative phytochemical analysis of *E. triplinerve vahl* leaf extracts.

Sl.No	Phytochemical group	Phytochemical Test Performed	solvent leaf extractions				
			Petro. ether	Methanol	Ethanol	Acetone	Hexane
1.	Alkaloids	Dragendroff's test	+++	+++	+++	++	+++
		Mayer's test	+	++	+	++	-
		Wagner's test	-	+	+	-	-
2.	Terpenoids	Salkowski's test	+	+++	+++	+++	+++
		Tri- terpenoids	+	-	-	-	+
3.	Coumarin	Coumarin	++	+++	+++	+++	++
4.	Phenolic Compound And Tannins	Ferric chloride	++	+++	++	+++	+
		Gelatin	-	+	+	-	-
		Lead Acetate	++	++	+	++	+
5.	Flavonoids	Shinoda	++	++	++	+++	++
		Alkaline Reagent	++	+++	+++	++	++
6.	Anthraquinone	Borntrager	-	-	-	-	-
	Glycosides	Keller - Kelliani	+	++	+	++	-
		Legal test	-	-	-	-	-
7.	Saponins	Froth	+	++	+	++	+
8.	Phytosterols	Salkowski's test	+	+++	+++	+++	+++
		Liber Burchard's	+	++	++	-	-
9.	Phenol	Ferric chloride	++	+++	++	+++	+
10.	Test For Protein	Ninhydrin test	++	++	++	++	+
		Biuret test	-	-	-	-	-
11.	Steroids	Liber Burchard's	+	+	+	+	+
12.	Flavanones	Test - I	++	++	++	+	++
		Test – II	-	-	-	-	-

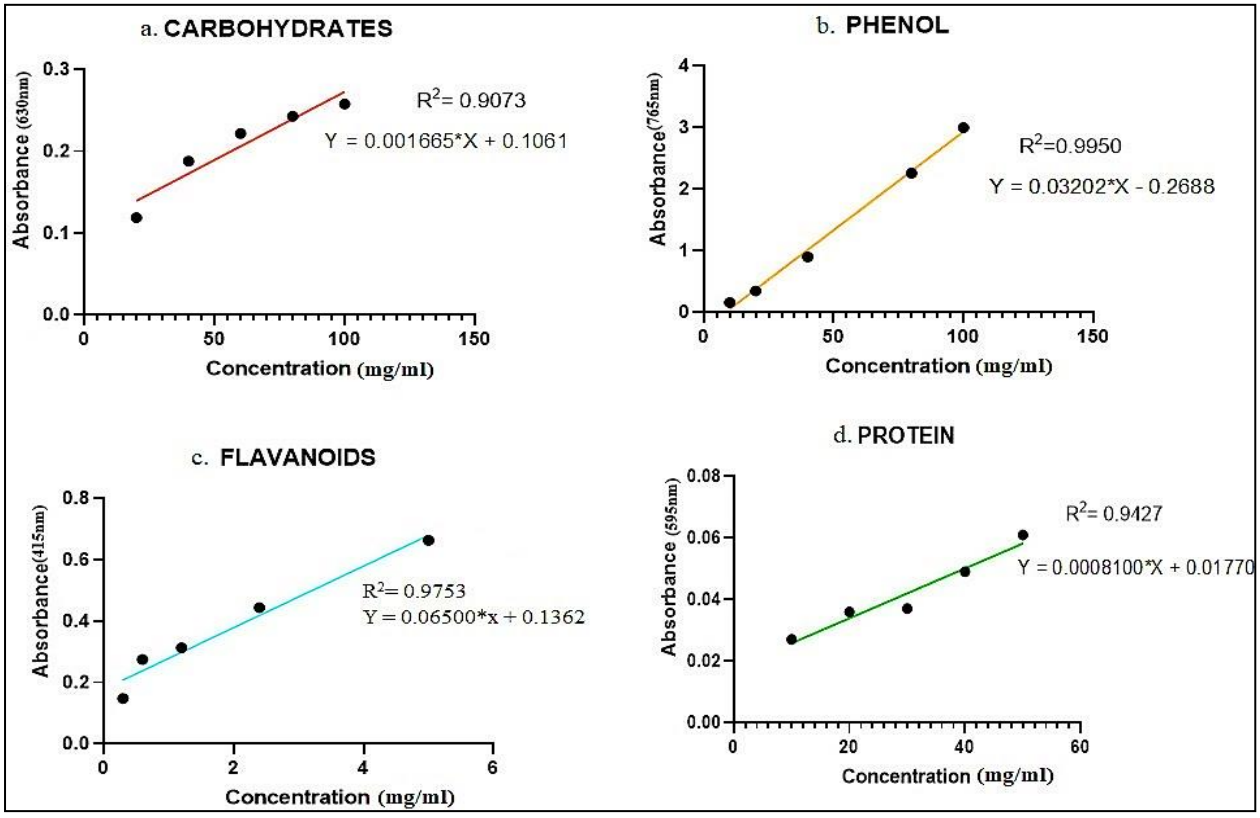
13.	Carbohydrate	Molisch's test	-	-	-	-	-
		Fehling's test	+	+++	+++	+++	+
		Benedict's reagent	-	+	++	++	-
14.	Quinones	Quinones	+	++	++	+++	+

+++ High, ++ Moderate, + low, - Absent; the experiments were conducted in triplicates and showed the active phytochemical classes, and classification was based on the intensity of color and the amount of precipitate formed.

Quantitative phytochemical analysis of the *E. triplinerve vahl* leaf extract

The quantitative phytochemical analysis of the five different solvent leaf extracts was estimated for total phenol, protein, flavonoids, and carbohydrate content by UV visible spectrophotometric method in Figure 3. From the graph, carbohydrates were found to be 0.26 mg/ml, protein at 0.49 mg/ml, phenol at 0.31 mg/ml, and flavonoids at 0.72 mg/ml as determined as a total content in the methanol leaf extracts respectively (Supplementary Table S2).

Figure 3: Phytochemical Quantitative estimation of *E. triplinerve vahl* methanol leaf extracts.



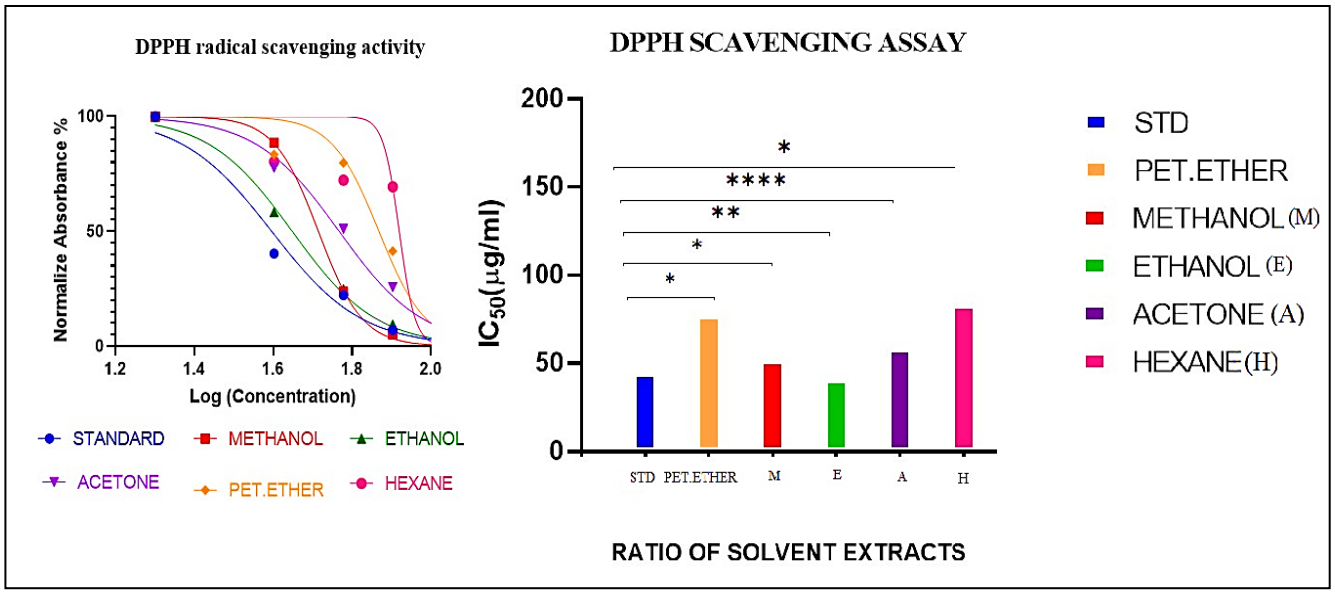
Quantitative estimation by plotting standard calibration curve with the mean value for the five different solvent leaf extracts.

Determination of antioxidant assay

DPPH radical scavenging activity

The antioxidant activities of the five different solvent leaf extracts were analyzed with standard ascorbic acid are visualized. The high IC_{50} value indicates minimum scavenging activity for petroleum ether (76.97 µg/ml) and hexane (83.03 µg/ml) and standard ascorbic acid (44.24 µg/ml). The IC_{50} value indicates moderate scavenging activity for methanol (51.53µg/ml), ethanol (40.80 µg/ml), and acetone (57.73 µg/ml) respectively. According to the antioxidant concentration, the fractional representation for each sample tested to the reaction kinetics was plotted in Figure: 4. The Pearson Correlation Coefficient -showed a high correlation between the standard ascorbic acid and the different leaf extracts. Out of all the extracts, significant correlation (p<0.0001) was exhibited for petroleum ether R^2 =0.9368, methanol R^2= 0.9993, ethanol R^2= 0.9894, acetone R^2= 0.9713 and hexane R^2= 0.8074 respectively.

Figure 4: Graphical representation of dose-response fit for the five different solvent leaves extracts with the standard ascorbic acid.



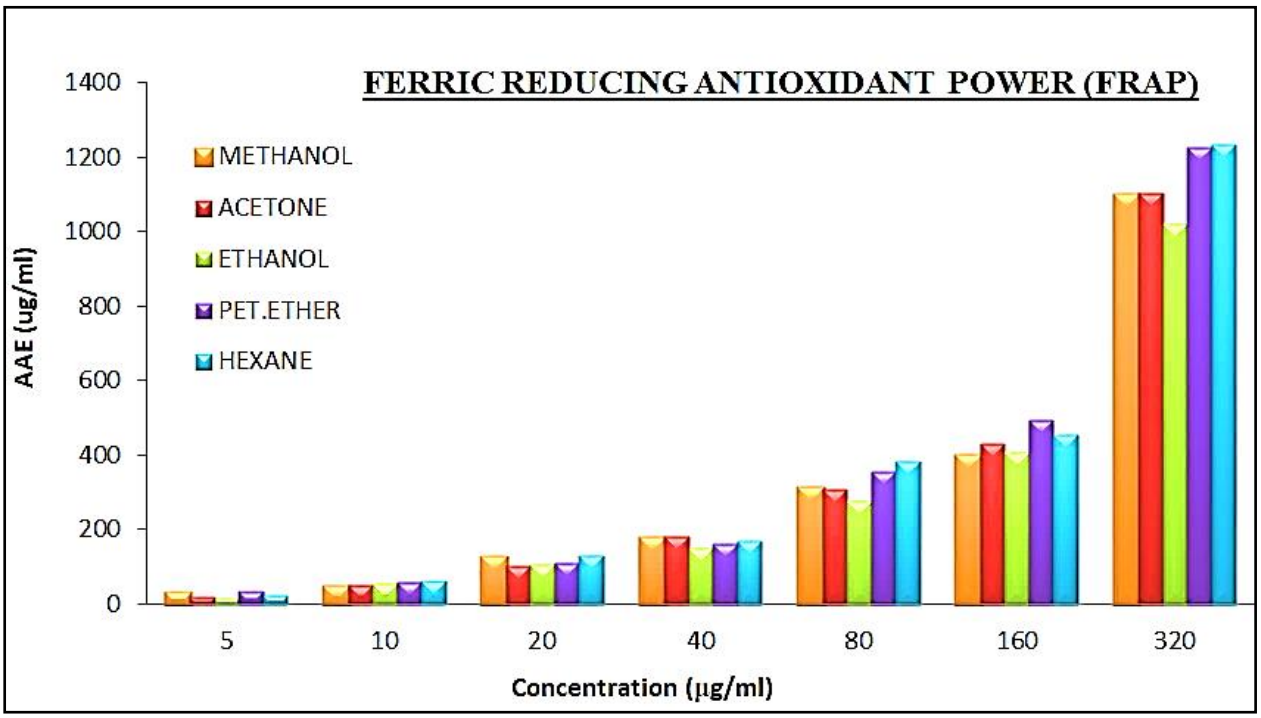
To evaluate the antioxidant activity for the leaves extract of petroleum ether (PET.ETHER), methanol, ethanol, acetone, and hexane by Dose-response inhibition with regrading standard ascorbic acid mean value ± SD (n=3). b. Bar graph representation of DPPH Scavenging assay for the leaf extracts with its IC_{50} values denoted with Pearson's correlation coefficient (R^2): *p< 0.05, **p< 0.01, ***p< 0.001, ****p<0.0001 with standard ascorbic acid.

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP reducing the ability of leaf extract of *Eupatorium triplinerve vahl* in comparison to standard ascorbic acid is visualized in Figure 5. The results revealed that $FeSO_4$ equivalent

calibration curve for ethanol R^2= 0.9624, acetone R^2= 0.9455, and Ascorbic acid R^2=0.9535. Among the extract, ferric reducing antioxidant activity showed high for acetone sample (184±1.6 µM FeE/g of extract) and ethanol sample (180±0.5 µM FeE/g of extract), and methanol (163±0.7 µM FeE/g of extract) respectively. Ascorbic acid a greater rate of ferric reducing capacity (178±0.9 µM FeE/g of extract).

Figure 5: Comparative FRAP- Ferric Reducing Antioxidant Power Assay of leaf extracts.



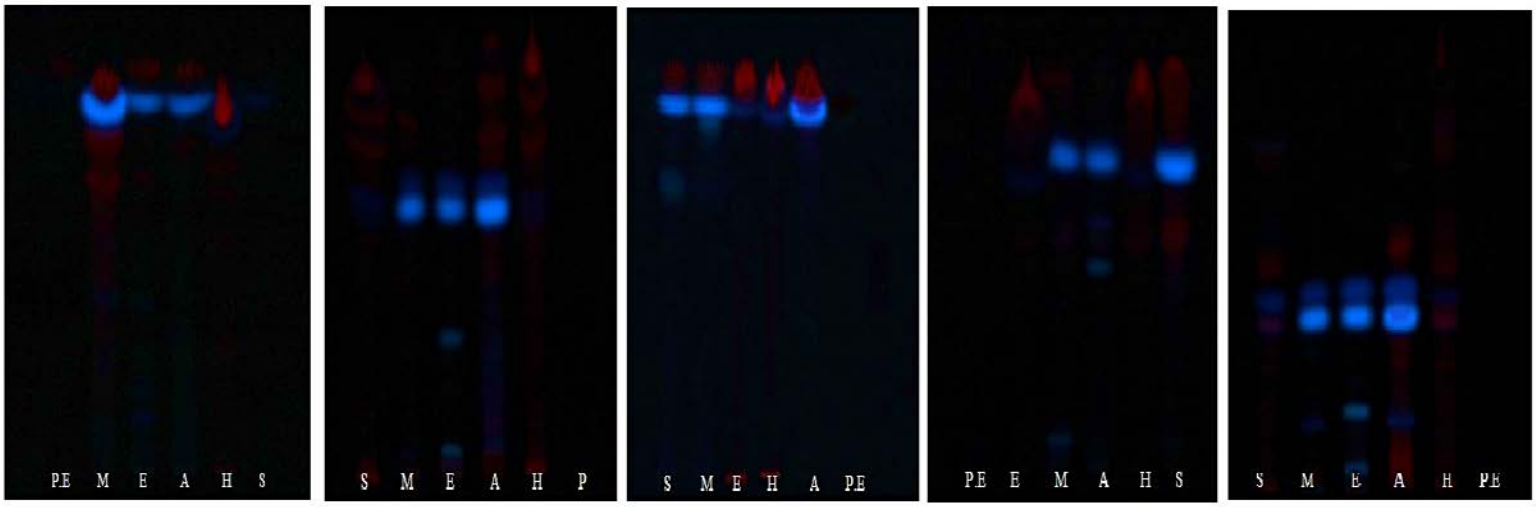
The scatter graph and bar graph represented the significant FRAP potential in a dose-dependent manner in Microsoft excel as mean (±) standard value (*n=3*) with a comparison of different concentrations among standard ascorbic acid.

HPTLC – Chemometric analysis of different solvent leaf extracts

HPTLC analysis for the five solvent leaf extracts demonstrated a quality of fingerprint obtained for the hemorrhoid potential secondary metabolites with their standard respectively Colchicine, Quercetin/Rutin, Gallic acid, Para coumaric acid, Oleanolic acid visualized under UVspectrometer at 366nm (Supplementary Figure S1). The HPTLC image J software was processed and depicted in Figure 3. Each sample chromatogram is split into three filter channel line profile plots as Red (R), Green (G), and Blue (B) in track order to perform full chemometric analysis by refining their increased selectivity and differentiate compounds for the spots that are equivalent according to fluorescence color. The filter channel was selected for each of the constituents based on their observed bands in an image processing for

petroleum ether (red), methanol (yellow), ethanol (green), acetone (blue), hexane (pink) to analyze the line profile plots of the samples with standard (black).

Figure 6: Chemometric analysis of HPTLC fingerprints for five different leaf extracts along with its standard.

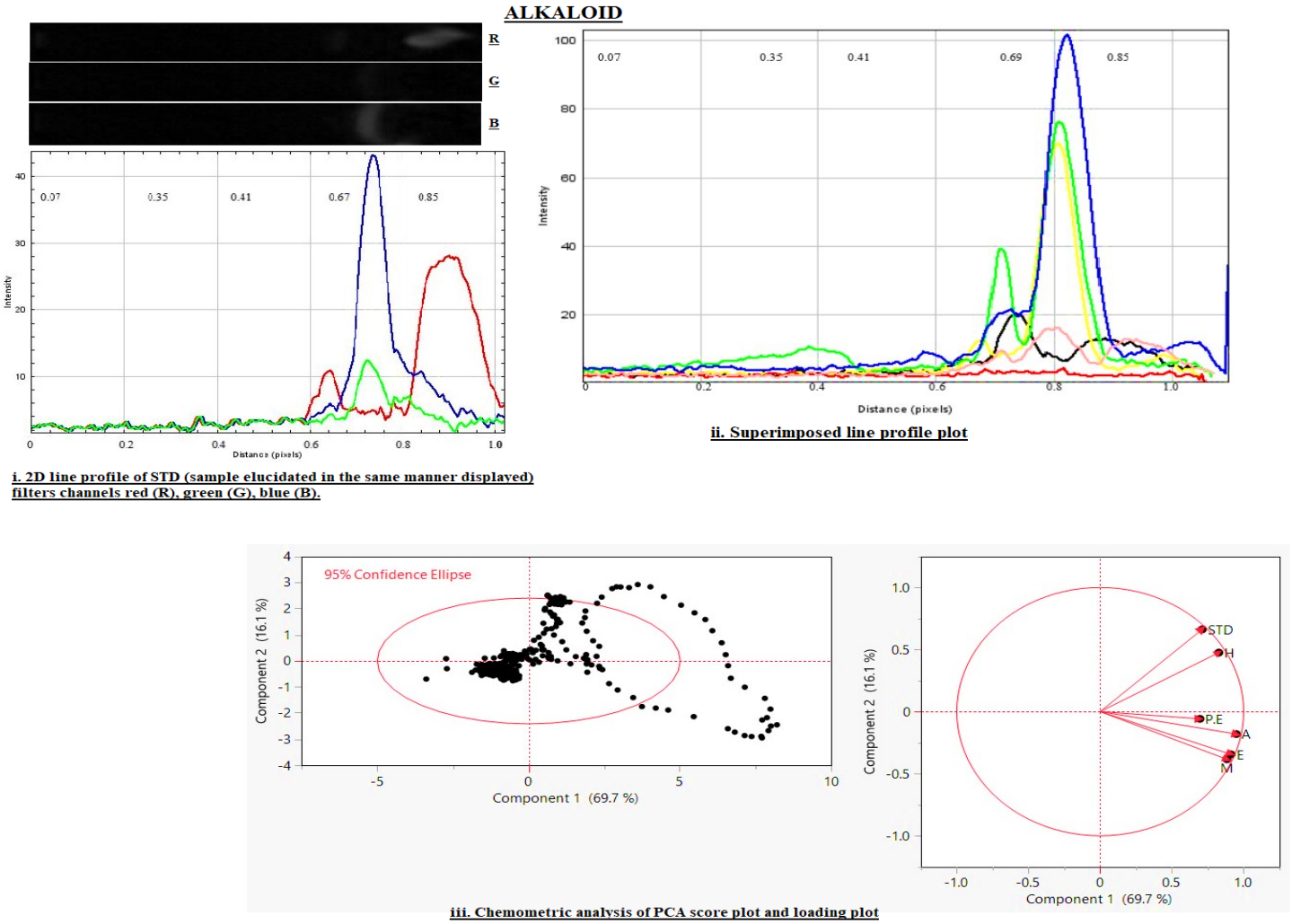


i. HPTLC profile of *Eupatorium Triplinerve Vahl* (finger print scanned at wavelength)

HPTLC chromatogram (UV 366nm) Alkaloids S (STD): Colchicine; Coumarin (STD): Para coumaric acid; Flavonoids (STD): Quercetin/ Rutin; phenol (STD): Gallic acid; terpenoids (STD): Oleanolic acid for five different solvent leaf extract (petroleum ether, ethanol, methanol, acetone, hexane).

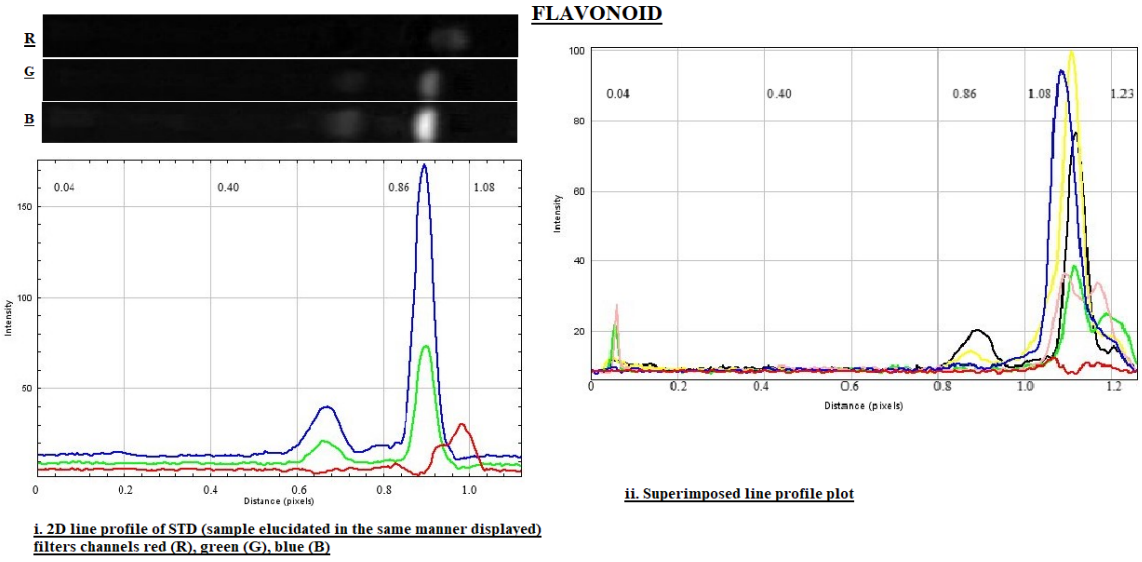
From the HPTLC chromatogram, mean intensities of pixels and mean values were calculated for each zone of the different solvent samples and standards, alkaloids (5 samples × 422 variables), flavonoids(5 samples × 399 variables), phenol(5 samples × 412 variables), terpenoids (5 samples × 428 variables) and coumarin (5 samples × 428 variables) are used as the dataset for chemometric analysis.

Figure 7: a. Chemometric analysis of HPTLC alkaloids fingerprints for five different leaf extracts along with its standard.



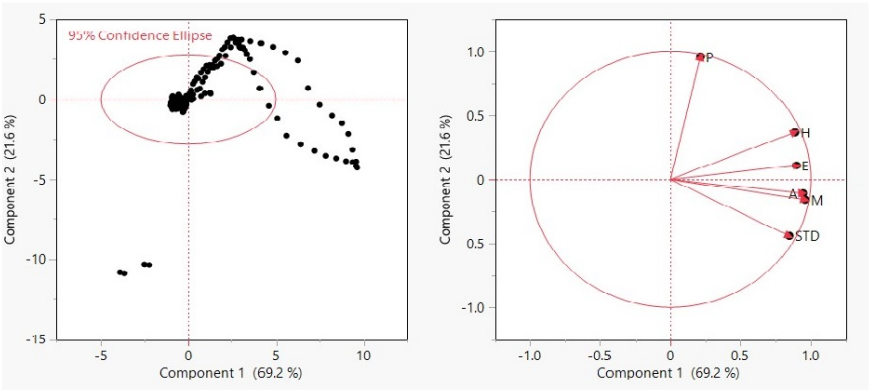
i. 2D line profile of STD (sample elucidated in the same manner displayed) filter channels red (R), green (G), blue (B), ii. Superimposed line profile plot for comparing the different solvent leaf extracts with R_f distance value (Red- petroleum ether; Yellow- methanol; Green- ethanol; Blue- acetone; Pink- hexane), iii. The data analysis of the leaf extracts provides the intensities of the pixels and PCA loading plot for the projection of samples chromatogram for their data variance as PCA score plot of PC1 and PC2 with the source of variability in secondary metabolites.

Figure 7: b. Chemometric analysis of HPTLC flavonoids fingerprints for five different leaf extracts along with its standard.



i. 2D line profile of STD (sample elucidated in the same manner displayed) filters channels red (R), green (G), blue (B)

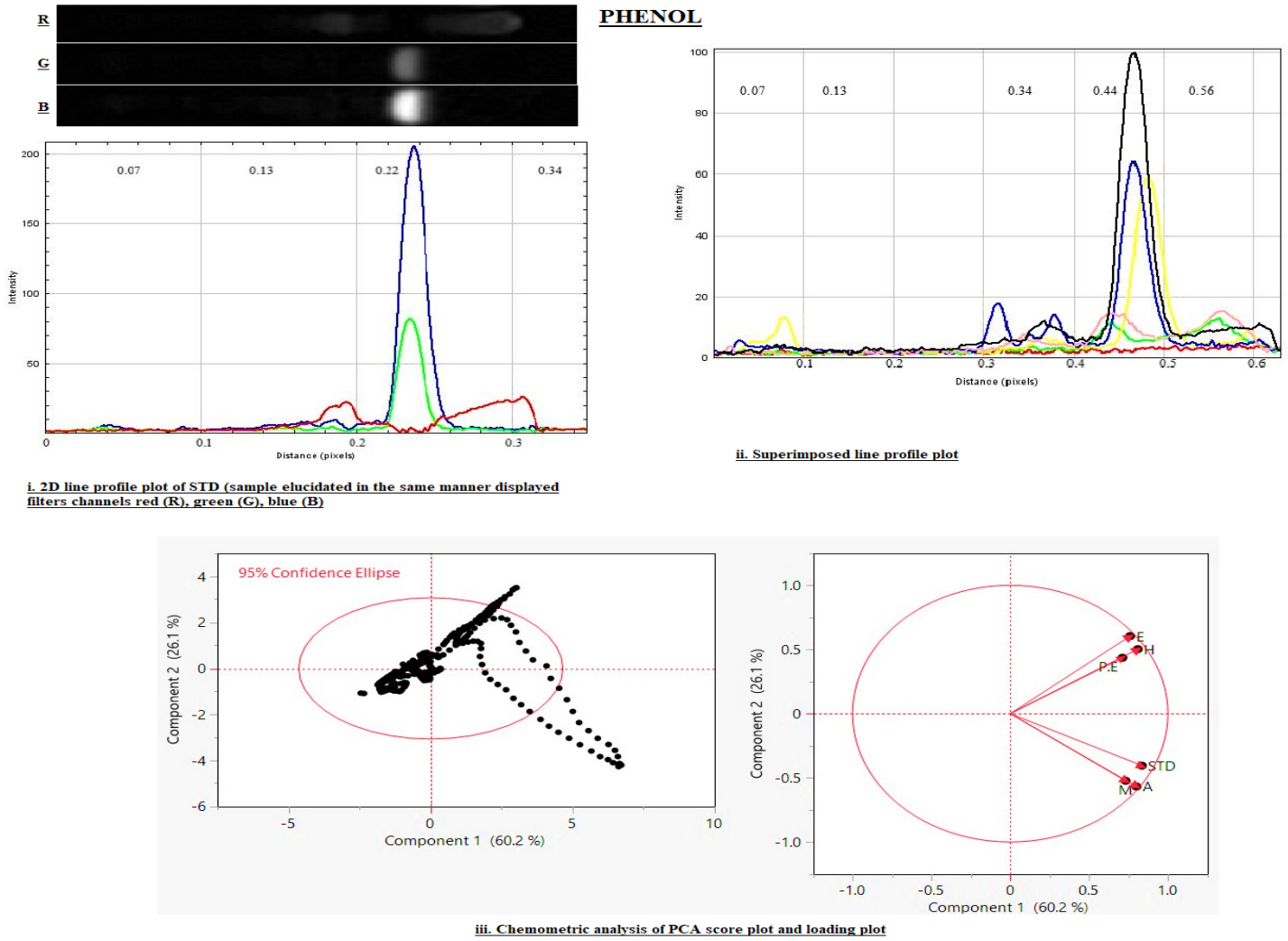
ii. Superimposed line profile plot



iii. Chemometric analysis of PCA score plot and loading plot

i. 2D line profile of STD (sample elucidated in the same manner displayed) filter channels red (R), green (G), blue (B), ii. Superimposed line profile plot for comparing the different solvent leaf extracts with R_f distance (Red- petroleum ether; Yellow- methanol; Green- ethanol; Blue- acetone; Pink- hexane), iii. The data analysis of the leaf extracts provides the intensities of the pixels and PCA loading plot for the projection of samples chromatogram for their data variance as PCA score plot of PC1 and PC2 with the source of variability in secondary metabolites.

Figure 7: c. Chemometric analysis of HPTLC phenol fingerprints for five different leaf extracts along with its standard.



i. 2D line profile plot of STD (sample elucidated in the same manner displayed filters channels red (R), green (G), blue (B)

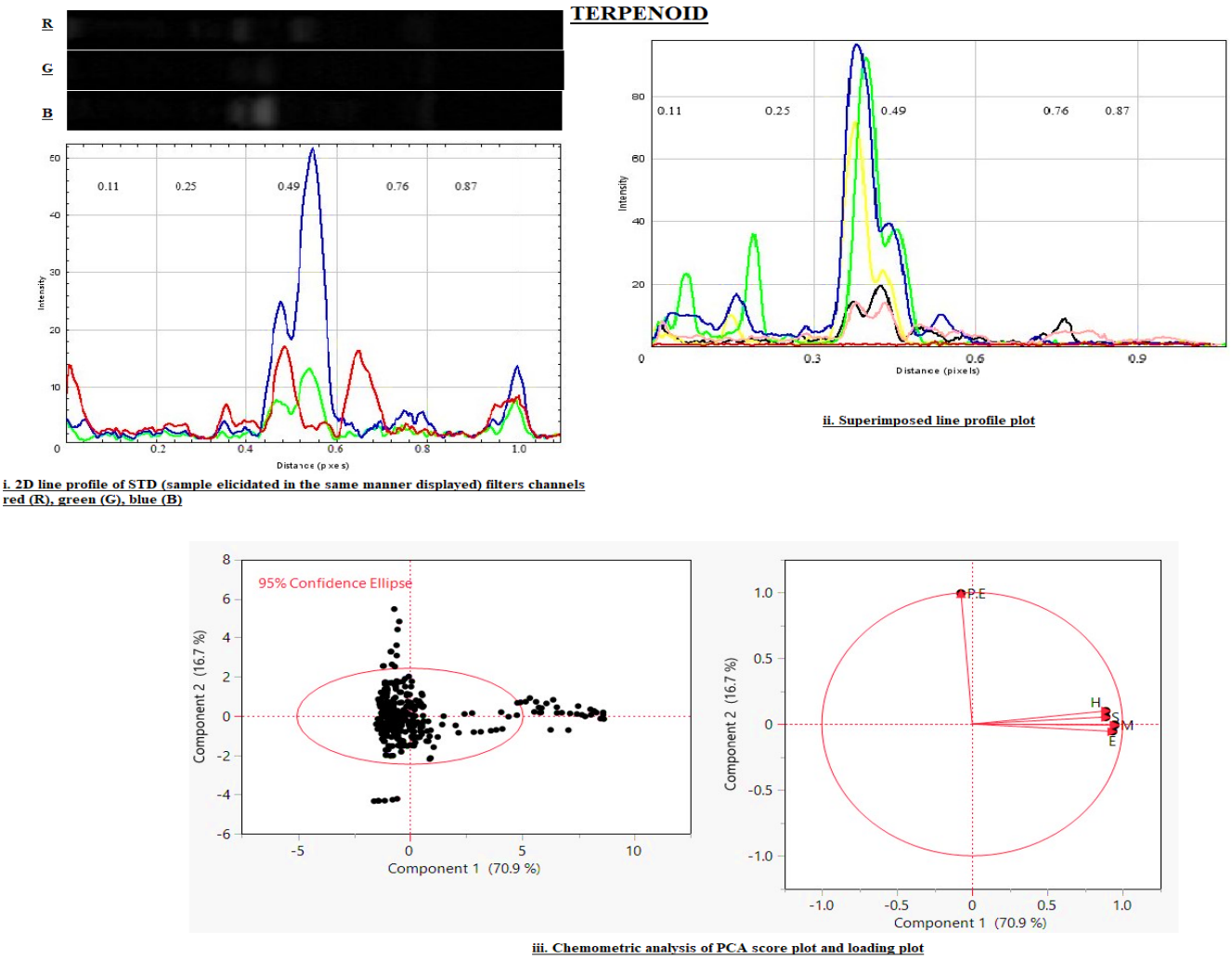
ii. Superimposed line profile plot

iii. Chemometric analysis of PCA score plot and loading plot

i. 2D line profile of STD (sample elucidated in the same manner displayed) filter channels red (R), green (G), blue (B), ii. Superimposed line profile plot for comparing the different solvent leaf extracts with R_f distance (Red- petroleum ether; Yellow- methanol; Green- ethanol; Blue- acetone; Pink- hexane), iii. The data analysis of the leaf extracts provides the intensities of the pixels and PCA loading plot for the projection of samples chromatogram for their data variance as PCA score plot of PC1 and PC2 with the source of variability in secondary metabolites.

370

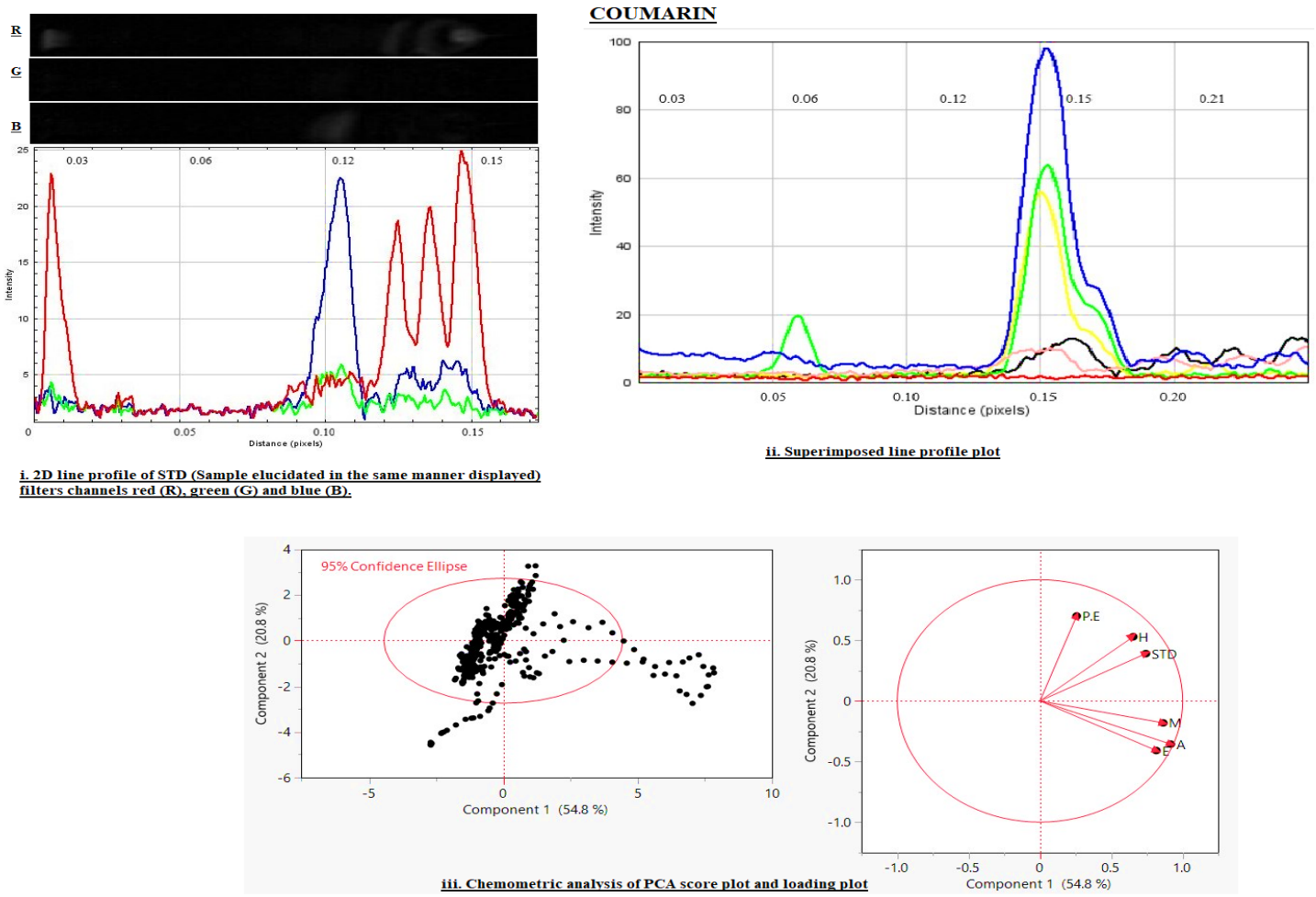
371 **Figure 7: d. Chemometric analysis of HPTLC terpenoids fingerprints for five different**
372 **leaf extracts along with its standard.**



373 i. 2D line profile of STD (sample elucidated in the same manner displayed) filter channels red
374 (R), green (G), blue (B), ii. Superimposed line profile plot for comparing the different solvent
375 leaf extracts with R_f distance (Red- petroleum ether; Yellow- methanol; Green- ethanol;
376 Blue- acetone; Pink- hexane), iii. The data analysis of the leaf extracts provides the intensities
377 of the pixels and PCA loading plot for the projection of samples chromatogram for their data
378 variance as PCA score plot of PC1 and PC2 with the source of variability in secondary
379 metabolites.

380

Figure 7: e. Chemometric analysis of HPTLC coumarin fingerprints for five different leaf extracts along with its standard.



i. 2D line profile of STD (Sample elucidated in the same manner displayed) filters channels red (R), green (G) and blue (B).

ii. Superimposed line profile plot

iii. Chemometric analysis of PCA score plot and loading plot

i. 2D line profile of STD (sample elucidated in the same manner displayed) filter channels red (R), green (G), blue (B), ii. Superimposed line profile plot for comparing the different solvent leaf extracts with R_f distance (Red- petroleum ether; Yellow- methanol; Green- ethanol; Blue- acetone; Pink- hexane), iii. The data analysis of the leaf extracts provides the intensities of the pixels and PCA loading plot for the projection of samples chromatogram for their data variance as PCA score plot of PC1 and PC2 with the source of variability in secondary metabolites.

The R_f value corresponding loading plots are displayed according to each of the component variables among the samples that were administrated to the greater variances. The line profile plots R_f values 0.03, 0.41, 0.69, 0.95, and 1.13 are the variables that have the higher impact

for alkaloids on this PC direction that differentiates with acetone sample in comparison with other leaf extract fingerprints and 0.41 appeared to be standard Colchicine in Figure 7. a. The formatted loading matrix for PC1 which is high to differentiate the sample in order A > E > M > H > STD > P.E and PC2 represented their medium variability impact is shown in (Supplementary Table S2). For flavonoids, similarly, the R_f value represented the greater influence in Figure 7. b. is 0.07, 0.32, 0.67, 0.97, 1.13 values, and 0.97 appeared to be standard Quercetin/ Rutin which was high for methanol and acetone leaf extracts. The formatted loading plot for PC1 in order A > M > E > H > STD > P.E and PC2 of the pixel intensities displayed in the order that differs in the mean value and data matrix as second high impacts are shown in (Supplementary Table S3).

For ordinary intensities of phenolic components, Figure. 7. c. describes 0.25, 0.44, 0.73, 0.79, 1.03 values revealed average rooted intensities for acetone extract leaf sample and specifically distinguishing R_f 0.73 with standard gallic acid. In the hierarchical order, the formatted loading matrix for phenol of PC1 displayed and differentiates its modification in their mean values STD > H > A > E > M > P.E and PC2 variability revealed low impact illustrated for its comparison with a standard (Supplementary Table S4).

The chromatogram of coumarin in Figure 7. d details the R_f peak values 0.03, 0.08, 0.14, 0.17, 0.20 exposed faint intensities of the acetone sample compared to the other extracts and followed by ethanol and an R_f value of 0.14 as standard para coumaric acid was lower for petroleum ether. The formatted loading plot for coumarin, PC1 direction displayed in the order A > E > M > H > STD > P.E and significantly PC2 effect expressed the moderate impact intensity are shown in (Supplementary Table S5). Similarly, for terpenoids, values are represented as excessive intensities as 0.21, 0.43, 0.76, 0.85, 1.12 for acetone leaf extract and followed by methanolic sample, R_f 0.76 as a standard oleanolic acid compared with the other leaf extracts Figure 7. e. The formatted loading matrix was a high impact for PC1 with pixel intensity in order M > A > E > H > STD > P.E and PC2 represented a high variability impact for petroleum ether (Supplementary Table S6).

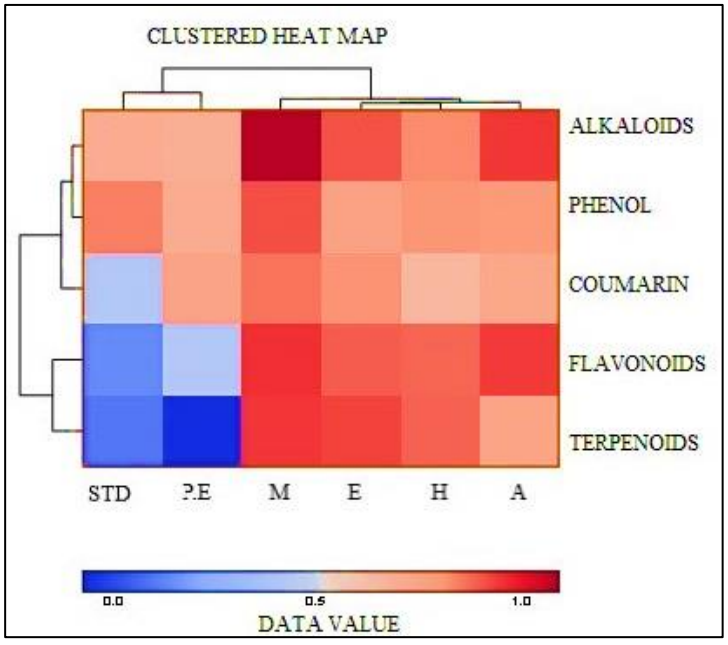
The PCA datasets for secondary metabolites expressed with squared cosine variables and normalized covariance interaction differentiates the different leaf extract samples on comparison and expression in consecutive PCs through the bar graph shown in (Supplementary Table S3-S7). The study revealed that the mutual projection of factor scores for the first two Principle Components (PCs) of all sources of variability, efficacy, and consistency (score plot and loading plot of data matrix) showed the application differences in all solvent leaf extracts and phytoconstituents with its respective standard. The mutual

projection of factor scores for the most important component (PC1) and (PC2) respectively followed up to 69.7% (PC1) and 16.1% for alkaloids, 69.2% (PC1) and 21.6% (PC2) for flavonoids, 60.2% (PC1) and 26.1% (PC2) for phenol, 70.9% (PC1) and 16.7% (PC2) for terpenoids and 54.8% (PC1) and 20.8% (PC2) for coumarin (Supplementary Figure S3 – S7). The PCA is a projection method to transform the data matrix to a 3D (three-dimensional) graphical representation of PCs of its coordinates to show an optimal conception of the distribution; relative to 95% confidence ellipses as represented in Figure 5.

Hierarchical Cluster Algorithm (HCA) - Heatmaps

An agglomerative HCA (Hierarchical Cluster Algorithm) - Heatmaps further elucidate in addition to PCA to cluster with similar objects and more effortlessly with the samples, the results were performed. The best results were obtained by Cophenetic correlation for the variables, with implying group average and Euclidean distance to compare the hierarchical cluster analysis (Figure 8). The Cophenetic Correlation Coefficient for the phytoconstituents sample clusters is CPCC = 0.9829 and for phytocompounds is CPCC = 0.9335 by comparing their dendrograms. It is obvious from the heatmap result that the sample extracts showed close clusters with acetone and ethanol with a distance value (0.1601), petroleum ether (1.0216), methanol (0.4481), and hexane (0.0943) and followed by standard (3.5088) with explaining their distance clustering pattern for the phytoconstituents. The phytoconstituents phenol displayed a distance value of around 0.0401 whereas, 0.3719 for alkaloids and terpenoids, 0.1648 for coumarin, and 0.234 for flavonoids were clustered distantly shown in (supplementary Table S7 and S8).

Figure 8: Hierarchical Clustered Heat-maps for different leaf extracts.



HCA Hetamaps for HPTLC datasets of *E. triplinerve Vahl* leaf extracts with their respective standards based on their peak area of characteristic bands at UV 366 nm. The comparison denotes that as the metabolite concentration levels increases, it can be interpreted by a color change ranging from blue to brown. The clustered dendrogram shows the linkages between the secondary metabolites of the leaf extract samples.

Molecular Docking study

The physicochemical properties for 90 bioactive molecules of *E. triplinerve Vahl* leaf extract such as molecular formula, molecular weight, the volume of the molecules, SASA (Total Solvent Accessible Surface Area), H-bond donor, H-bond acceptor, QPlogPW (Solvation free energy in water), QPlogKp (Predicted skin permeability), percentage human oral absorption were observed using Qikprop tool and results were tabulated in Table: 4. From the ADME/T property table, 90 bioactive molecules showed drug-likeness for 15 compounds (a Table: 2) based on the score range.

Table 2: ADME/T properties of the active plant components by QikProp tool.

Molecule	mol_ MW	volume	SASA	PISA	Donor HB	Accepter HB	QPlog Pw	QPlogKp	EA(eV)	QPlog Khsa	% Human Oral Absorption
985	256.4	1119.1	667.92	0	1	2	2.403	-2.154	-1.152	0.521	87.217
10748	176.1	590.11	369.80	204.6	0	3.25	5.475	-2.038	0.887	-0.581	92.373
12366	284.4	1274.0	760.85	0	0	2	0.59	-1.105	-0.986	1.145	100
69376	242.3	977.10	563.4	0	0	4.5	3.918	-2.229	-0.258	-0.174	100
72326	442.7	1397.9	696.5	20.46	2	3.4	7.468	-2.519	-1.049	1.527	100
72757	149.1	511.17	331.4	189.1	1	3.25	7.041	-2.404	0.342	-0.527	89.241
73114	308.5	1077.8	563.90	31.88	2	1.5	4.766	-1.886	-1.078	0.884	100
145386	296.5	1288.7	736.13	8.47	1	1.7	1.989	-1.202	-0.849	1.225	100
183540	166.2	656.66	409.06	58.60	1	1.5	3.689	-1.854	-0.279	-0.03	100
579292	224.3	919.53	529.26	48.01	1	1.7	3.518	-1.811	-1.065	0.532	100
5280450	280.4	1112.9	618.27	37.30	1	2	2.523	-1.983	-0.75	0.514	87.751
5280934	278.4	1111.8	622.17	49.53	1	2	2.851	-2.076	-0.726	0.552	87.33
5282184	308.5	1314.6	753.94	34.33	0	2	0.807	-0.984	-0.875	1.263	100
5367460	306.4	1274.9	714.89	43.90	0	2	1.001	-1.053	-0.824	1.19	100
6432150	468.7	1515.7	759.50	20.48	0	2	3.498	-2.02	-1.048	2.398	100

The *in-silico* approach helps to predict the binding modes and binding affinities of each ligand in the dataset utilizing docking to x-ray crystallographic structure. And it may design novel potent inhibitors, by deciphering the numerous Hydrogen bond distance interactions between the ligands and enzyme active sites. The bioactive compound exhibited an overall docking score above -6.0(kcal/mol). In the methanolic leaf extract, 2H-1,4 Benzoxazin-

3(4H)-one, the compound showed high affinity to Nitric Oxide Synthase (NOS) hemorrhoid protein target at a glide score of -8.027(kcal/mol), which comes under the benzoxazine group of glycosides and is used the treat various conditions such as inflammation and cancers. The effective prediction indicates that the biological activity of the compound performs better in inhibiting the effect of the Nitric Oxide Synthase (NOS) enzyme target compared with the standard drug 5- Fluorouracil.

Table 3 : Docking results of bioactive molecules with binding energies.

Sl.No	Protein Target	Compound ID	Glide docking score (kcal/mol	Glide ligand efficiency	Glide emodel	Glide energy
1.	NOS (Nitric Oxide Synthase)	72757	-8.027	-0.73	-43.578	-29.611
		10748	-7.463	-0.574	-44.142	-30.398
		183540	-6.821	-0.563	-41.603	-30.013
		579292	-5.575	-0.348	-27.888	-26.639
		5367460	-3.421	-0.155	-40.724	-40.094
2.	COX2 (Cyclooxygenase-2)	10748	-7.051	-0.542	-39.433	-26.859
		183540	-6.895	-0.575	-35.374	-25.276
		579292	-6.537	-0.409	-26.602	-23.918
		75757	-6.426	-0.584	-35.166	-25.265
		5282184	-5.724	-0.26	-20.004	-22.944
3.	TNF-α (Tumor necrosis Factor – alpha)	10748	-7.012	-0.539	-39.525	-28.275
		72757	-6.369	-0.579	-36.253	-26.452
		73114	-5.709	-0.26	-41.98	-32.801
		183540	-5.699	-0.475	-31.788	-24.042
		579292	-5.392	-0.337	-33.94	-29.077
4.	MMP-9 (Matrix Metalloproteinase-9)	10748	-6.734	-0.518	-45.806	-33.034
		579292	-6.52	-0.408	-40.855	-32.778
		72757	-6.509	-0.592	-43.126	-31.12
		73114	-6.305	-0.287	-21.893	-14.858
		183540	-5.959	-0.497	-34.628	-25.397
5.	MMP-2 (Matrix Metalloproteinase-2)	72757	-6.53	-0.594	-42.905	-30.674
		10748	-5.699	-0.438	-39.94	-29.536
		183540	-4.834	-0.403	-35.176	-27.062
		6432150	-4.715	-0.139	-55.102	-45.904
		73114	-4.453	-0.202	-40.7	-31.756
6.	5-Fluorouracil	72757	-6.281	-0.571	-36.734	-26.622
		10748	-6.071	-0.467	-37.753	-27.519
		183540	-5.611	-0.468	-31.497	-26.978
		579292	-5.173	-0.323	-21.383	-17.847
		69376	+0.071	+0.004	-17.035	-23.921
7.	EpCAM (Epithelial Cell Adhesion Molecule)	579292	-6.001	-0.375	-31.894	-23.967
		10748	-5.891	-0.453	-34.229	-24.722
		73114	-5.728	-0.26	-38.138	-30.627
		183540	-5.723	-0.477	-30.647	-21.791
		72757	-5.477	-0.498	-30.091	-21.882
8.	VEGF (Vascular Endothelial Growth Factor)	10748	-5.072	-0.39	-33.981	-25.848
		72757	-4.925	-0.448	-30.501	-23.154
		73114	-4.498	-0.204	-35.59	-28.786
		72326	-3.762	-0.118	-34.756	-28.369
		6432150	-3.498	-0.103	-46.057	-38.367
9.	CD105 (Endoglin)	72757	-4.548	-0.413	-22.519	-17.286
		10748	-3.701	-0.285	-21.286	-18.745
		183540	-3.304	-0.275	-16.929	-13.433
		73114	-2.691	-0.122	-19.406	-16.776

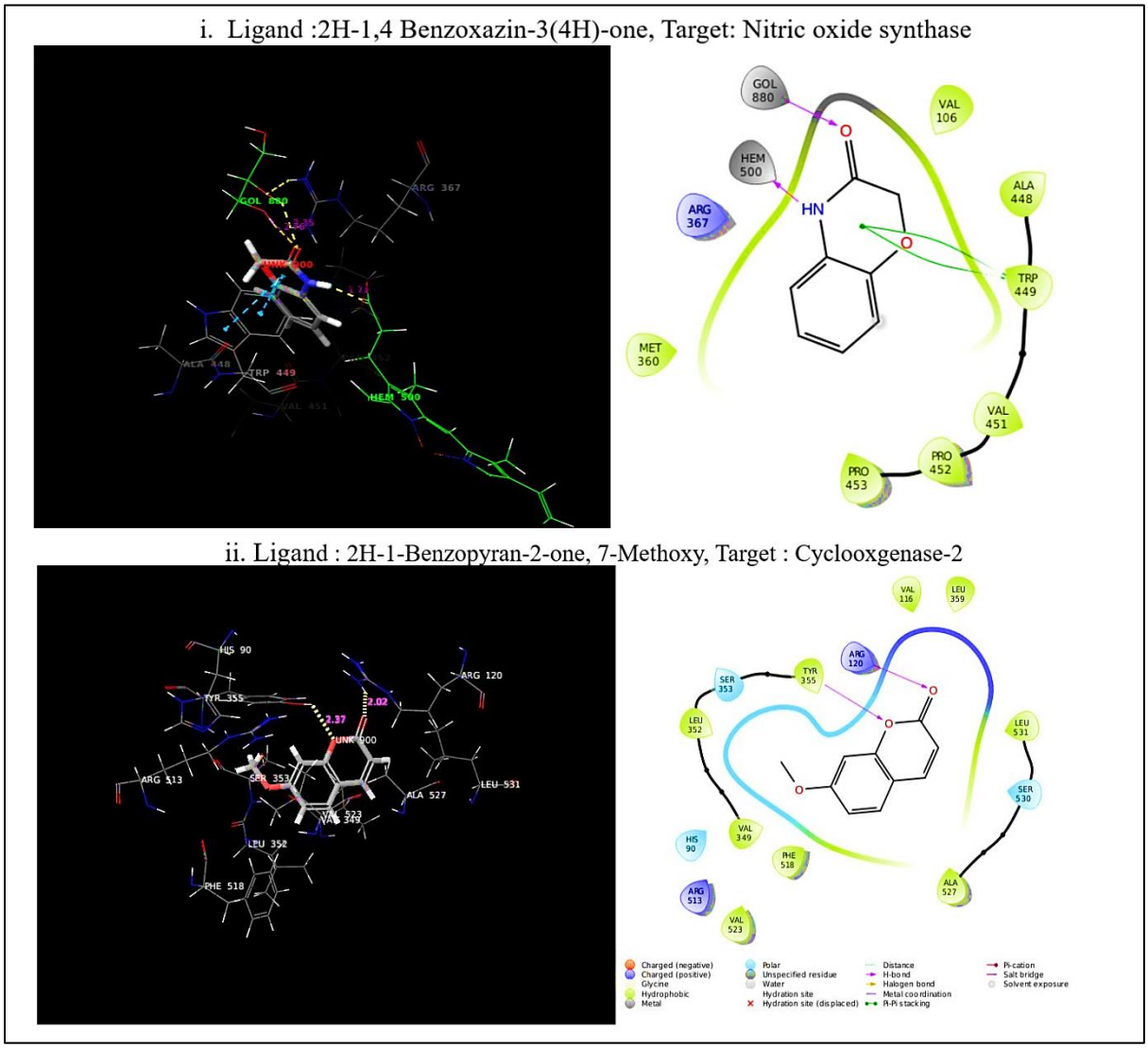
		579292	-1.96	-0.123	-16.405	-15.048
10.	IL-6 (Interleukin – 6)	10748	-4.144	-0.319	-28.953	-24.172
		183540	-4.103	-0.342	-26.975	-21.519
		72757	-4.007	-0.364	-26.656	-21.116
		73114	-3.796	-0.173	-31.123	-25.313
		6432150	-3.066	-0.09	-41.456	-34.965

The Nitric Oxide Synthase (NOS) protein residues interact with the ligand atoms and the surface area were controlled by the complex array of molecular interactions depending on binding sites and outside target binding pocket. The amino acid residues are Valine (VAL106 and 451), Alanine (ALA448), Tryptophan (TRP449), and Proline (PRO452 and 453), Methionine (MET360), Arginine (ARG367). Molecular binding score with residues Hydrogen bonds interaction (1.71 Aº) PRO 452, (2.35 Aº) ARG 367 amino acids residues. TRP449 residue form a contact with the side chain and π-π stacking contacts with Nitric Oxide Synthase protein molecules as depicted in Figure 9. and Cyclooxygenase-2 (COX2) protein interact between hydrogen bonds amino acid residue (2.37 Aº) Histidine (HIS 90) and Tyrosine (TYR 355), (2.02 Aº) Arginine (ARG120) and Leucine (LEU 531), (ARG 513, Serine (SER 353), Alanine (ALA 527), LEU 531, Valine (VAL 523 and 349), Phenylalanine (PHE 518) with 2H-1-Benzopyran-2-one, 7-Methoxy compound.

The phytochemicals obtained from the *E. triplinerve vahl* leaf extract were analyzed by comparing their binding affinity of the ligand towards the binding receptor. To confirm the binding conformation of a lead compound by scoring function with three parameters are usually considered when calculating the results with G-score, H-bond energy, and residual interaction. The potency of a lead compound from the leaf extract with standard drug 5-fluorouracil as receptor was docked with 2H-1-Benzopyran-2-one, 7-Methoxy gives a binding result of -6.281 with the binding pose shown in Table 3.

In the present docking analysis, we notable that these compounds (2H-1-Benzopyran-2-one, 7-Methoxy (other names: Herniarin, coumarin), and 2H-1,4 Benzoxazin-3(4H)-one) have been suitable for molecular properties and predicted pharmacological effect against Nitric Oxide Synthase (NOS) and Cyclooxygenase-2 (COX2) compare to other ligands represented in the Table 3. The results were optimized by Mcule software which is an online drug discovery platform with ID –OE-12111804392D given as a better docking result of 2H-1-Benzopyran-2-one, 7-Methoxy. These observations, further confirm that 2H-1-Benzopyran-2-one, 7-Methoxy may be effective for anti-hemorrhoidal and inflammatory compounds, especially concerning Nitric Oxide Synthase (NOS) protein-mediated to compare a group of highly selective inhibitors of other traditional NSAID drugs.

Figure 9: Molecular interactions of i. Nitric Oxide Synthase (NOS) and ii. Cyclooxygenase-2 (COX2) protein targets.



Residues and hydrogen bond contacts with their distance values in the active site and the 2D template representing the types of contact formed interaction between ligand and target.

Discussion

Traditional medicinal plants are used to treat various diseases and disorders in form of multi-targeted phytotherapy with plant-based herbal preparation as therapeutic uses. The results of the qualitative analysis concluded that the methanol, ethanol, and acetone leaf extracts of *E. triplinerve Vahl* express different levels of phytoconstituents to reduce the hemorrhoidal inflammation with their biological and therapeutic properties. Identification and screening of the phytochemical constituents from the medicinal plants are helpful for the discovery of new therapeutic drugs and also lead molecules for the drug discovery process and are used as a potential source[41] of novel medicines.

E. triplinerve vahl is a medicinal plant and is also used as an important source to treat various diseases and can be developed as a potent chemotherapeutic agent. The phytochemical study revealed that the plant is rich in coumarins. Coumarins are considered to possess potential bioactive components for general defense response to abiotic and biotic stresses and exhibit excellent anti-inflammatory and anti-hemorrhoid activity can act as inhibitors of numerous enzyme systems, thus opening up a plethora of medicinal purposes.[42]

However, Flavonoids are active ingredients of many herbal medicines.[43] It is described that the polarity level and species nature will play a major role in extracting this secondary metabolite.[44] Methanol and Acetone extracts were identified as the most effective solvents for the extraction of terpenoids, flavonoids, phenol, alkaloids, coumarins, and saponins from the medicinal plant and its respective parts from the previous studies conducted and their efficiency in the extraction of the phytoconstituents were proven. A high amount of phenolic and flavonoid content indicated the efficacy of plants for use in the treatment of several inflammatory diseases.[45]

As chemical variation- related to phenological and environmental factors is frequently encountered in aromatic plants, further studies are needed to see if the changes in chemical composition in *E. triplinerve vahl* are due to the cumulative effect of different environmental conditions or if the chemotypes are genetically fixed.

Antioxidants are tremendously important substances that possess the ability to protect the body from damage caused by free radical-induced oxidative stress. Radical scavenging activities of leaf extract *Eupatorium triplinerve* betrayed to prevent the deleterious role of free radicals in oxidative stress. The fluorescence analysis is sensitive and enables precise and accurate determination of the drug with the specific color for each compound . This is due to the presence of flavonoids in plants which acts as antioxidants. According to an earlier report, the antioxidant activity of flavonoids can reduce the free radical formation and scavenge the free radicals.[37-38]

The HPTLC image-based chemometric analysis was observed based on performing the quality, sensitivity, and high resolution of the herbal constituents to its therapeutic potential action on anti-hemorrhoid application and chromatogram by separating their phytocomponents of all solvent leaf extracts. Among phytoconstituents, the total variance suggests that the chromatogram of the extracts is significantly high towards alkaloids, flavonoids, coumarin, phenol, and terpenoids for the sample of methanol, ethanol, and acetone leaf extract respectively.

The principal component analysis (PCA) revealed a score plot with distinct groups that favor the PC1 axis for making compact clustering of the phytocomponents samples and the remaining possible variabilities are fed in low intensities of PC2 and negative impact. The methanolic extract line profile plot explains its high impact on overall secondary metabolites fingerprint, ethanol and hexane showed low intensities for coumarin, acetone had a high value for flavonoids explaining the importance of phytoconstituent improved content through herbal preparation. The study revealed the relationship of Rf value with representation directly to the intensity of the compound and maximum peak height.[46]

HCA elucidate the cophenetic matrix exposed through a double denogram to find variant in the bioactive content. These findings confirmed improvements in efficacy-based chemical profiling of leaf extract samples in a hierarchical order of ethanol and hexane displaying higher concentration and acetone relaying bioactivity and petroleum ether indicating low presence for a required concentration of metabolite fingerprint profile followed by their respective standard.[47-48]

Based on the outcome, we evaluated the residue interaction with the methanol ligand atom and found its maximum level of binding affinities. The bioactive molecules, 2H-1-Benzopyran-2-one also showed good binding affinities in this analysis with Nitric Oxide Synthase (NOS) and Cyclooxygenase-2 (COX2). The bioactive molecules were screened to show the potential and active site against the target protein to determine their suitability as drug molecules for treating hemorrhoid diseases by using computational methods.

Conclusion

In the present study, bioactive constituents were obtained from the leaf extract of *E. triplinerve Vahl* by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The presence of compounds provides for efficacious use of leaves for various aliments by traditional specialists. The obtained secondary metabolites predicted for pharmacological effect and molecular mechanism which act as a potent application of MMP9 and NOS and VEGF expression inhibitor, glucocorticoid antagonist, hemostatic, laxative, sedative, astringent, antipruritic, and antihemorrhagic activity.

The results of HPTLC image-based chemometric fingerprint profiling of secondary metabolites revealed strong intensities and close clustering patterns for ethanol, acetone, hexane, methanol, and petroleum ether based on their fingerprint by PCA and HCA-Heatmaps of the HPTLC dataset. The presence of bioactive compounds provides efficacious use of leaves for various aliments by traditional specialists. Molecular docking study exhibits

that several compounds docked with the receptor and from this 2H-1,4 Benzoxazin-3(4H)-one from the methanol leaf extract holds a lead target formation against hemorrhoids (acting potential inhibitors of NOS and COX2 enzyme) based on minimum hydrogen bond length and maximum docking score.

Furthermore, results showed that the methanolic and acetone leaf extract has a good potential cluster for health benefits with its wide range of therapeutic applications. The present study has set precedence for use of chemometric profiles for quantitative interpreting phytoconstituents. This will be very useful for the serval herbal formulation with predictable treatment procedures and compounds may be developed into multi-target drug candidates.

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Conflict of Interest

The author declares that there is no conflict of interest.

Abbrevation:

DPPH - 2,2-Diphenyl-1-Picrylhydrazyl

FRAP - Ferric Reducing Antioxidant Power

HPTLC - High-Performance Thin Layer Chromatography

PCA - Principal Component Analysis

HCA - Hierarchical Cluster Analysis

DMSO – Dimethyl sulfoxide

SUPPLEMENTARY MATERIAL

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