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Phytochemical Evaluation and Molecular Docking of Hibiscus Flower Extract: An Assessment of Bioactive Compounds

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ABSTRACT –

The study aims to elucidate that hydro alcoholic solvent based extraction provide better and more presence of constituents from the flower part of the plant. Also performed protein ligand simulation studies to investigate the interaction of these compound with therapeutic target proteins.*Hibiscus rosa-sinensis* is a species of plant with flowers belong to the family Malvaceae. It is widely used in traditional medicine across various countries and cultures. The flower of the plant have so many therapeutic properties. The red flower variety is mainly preffred in medicinal uses. It is well defined plant and previous studies suggested that it contain tannin, saponnin, flavonoid and alkaloids.Protein ligand binding for a plant extract use computational simulations to elucidate the interaction between bioactive compound present in the plant extract and target proteins, related to particular diseases. In this technique computationally prediction has been done that how well plant derived molecules can bind to and inhibit the function of proteins associated with diseases, like cancer or microbial infection. In comparison, resins and steroids were present in smaller quantities, with ethanol extracts showing higher resins (0.5% w/w) and water extracts showing lower steroid content (0.3% w/w). This demonstrates that certain phytochemicals like resins and steroids are more readily extracted in ethanol compared to water or hydro-alcoholic solutions.Based on the docking scores and binding energies, Delphinidin 3-sambubioside emerged as the most potent compound for anti-oxidant and anti-fungal activities, while Quercetin-3-diglucoside showed promise for anti-alopecia activity. However, the compounds showed some limitations in terms of ADME properties, which could hinder their oral bioavailability. Further studies, including in vitro and in vivo testing, are required to validate the pharmacological potential of these compounds and to address their bioavailability challenges.

KEY WORDS - *Hibiscus rosa-sinensis,* Phytochemical Screening, Molecular Docking, Anti-Alopecia, Anti-fungal, Anti-Oxidant.

1. INTRODUCTION –

Hibiscus rosa sinensis is a species of plant with flowers belong to the family Malvaceae. It is widely used in traditional medicine across various countries and cultures. The flower of the plant have so many therapeutic properties. The red flower variety is mainly preferred in medicinal uses. It is well defined plant and previous studies suggested that it contain tannin, saponnin, flavonoid and alkaloids. It is also reported that also contain Anthocyanins and flavonoids majorly,also cyaniding-3,-diglucoside, cyaniding-3-sophoroside-5-glucoside, quercein-3,7-diglucoside, quercetin-3-diglucoside[1]. There are more compounds like cyclpeptide alkaloid cyaniding chloride, quercetin, hentriacontane and vitamins like riboflavin, ascorbic acid and thiamine[2]. The flower part contain diglucoside, flavonoids and vitamins, thiamine, riboflavin, niacin and ascorbic acid. Flower extract is a source of many potentially active antioxidants and anticancer constituents such as quercetin, glycoside, riboflavin, niacin, carotene, malvalic acid, Gentisic acid, margaric acid and lauric acid[3-8]. The ethanolic extract of the flower were studied by GC-MS and reported propanol,3,3'-dithiobis(2,2-dimethyl- SS)- or (RR)-2,3-hexanediol, 2-Hydroxy-2-methylbutyric acid, n-Hexadecanoic acid, Heptanoic acid, 2- ethyl- Trans-(2-Ethylcyclopentlyl) methanol, 3-N-Hexylthiolane, SS-dioxide Hexanedioic acid, bis(2-ethylexyl) ester, 1,2-Benzenedicarboxylic acid, dicotyl ester, 1,3-Benzodioxole, 5.5'- (tetrahydro-1H,3H-furo(3,4-c)furan1,4-diyl)bis-, (1S-(1α,3a α,4β,6a α)-Squalene, 2R-Acetoxymethyl-1,3,3- trimethyl-4t-(3-methyk-2-buten-1-yl)-1cyclohexanol[9].

From past years to till now the interest has been focused on its potential in enhancing hair growth and exhibiting antimicrobial activity. The extract of flower with various solvent has shown good results for hair growth as well as for antimicrobial and antioxidant activity. Extraction in water yields 15% and was rich in flavonoids, phenols and saponins which showing moderate antimicrobial activity against *S. aureus* and 80% inhibition in antioxidant tests. Extraction in ethanol gave 12 %, containing flavonoid, alkaloid and tannins with antioxidant activity (IC50: 22ug/ml) and antimicrobial activity against *E. coli*. Methanol produced highest yield at 18%, rich in anthocyanins, phenolic acid and flavonoids, showing antioxidant (85% ZOI) and antimicrobial activity against P. aeruginosa. Hexane (5% yield) extracted terpenoids and steroids that shows weaker antioxidant and antimicrobial property with no hair growth activity. Chloroform extract (8% yield) is primarily rich in alkaloids, flavonoids and terpenoids with medium antioxidant and antimicrobial activity. This study also evaluates the impact of solvent such as ethanol and water. The extraction process or the flower part and use of solvent influence the phytochemical quality and quantity. As in this study the extraction of flower part is performed in hydro alcoholic solvent, the extract contains a mixture and water, which helps extract both polar and non-polar compounds. The constituents found in the extract include flavonoid such as quercetin, kaempferol and anthocyanins, phenolic compound such as phenolic acid, gallic acid and ferulic acid, tannins, saponins, alkaloids, anthocyanins, steroids, terpenoids. They contribute in antioxidant, antimicrobial,anti-inflammatory, astringent and hair growth activity. Mixture of ethanol and water as a extraction solvent particularly effective because they can extract Broderspectrum of phytochemicals compared to water or alcohol alone, provide a well-rounded extract rich in bioactive compounds. This study is performed to

elucidate the phytochemical profile of the flower and evaluate its efficacy in promoting hair growth and combating microbial infection.

Protein legend binding for a plant extract use computational simulations to elucidate the interaction between bioactive compound present in the plant extract and target proteins, related to particular diseases. In this technique computationally prediction has been done that how well plant derived molecules can bind to and inhibit the function of proteins associated with diseases, like cancer or microbial infection. Molecular docking help to determine potential therapeutic compounds from natural source, by analyzing binding affinities and interaction site and optimizing the drug discovery process. It is useful resource in research of phytomedicine, enabling the exploration of plant extracts for pharmaceutical development.[10] It provide encompassing drug discovery, natural product research and understanding the molecular basis o plant based therapeutic effect. This approach holds great promise in leveraging traditional knowledge of medicinal plants for modern pharmaceutical applications.

This research study evaluates the yield and diversity of phytochemicals extracted from *Hibiscus rosa sinensis* flower. The study aims to elucidate that hydro alcoholic solvent-based extraction provide better and more presence of constituents from the flower part of the plant. Also performed protein ligand simulation studies to investigate the interaction of these compound with therapeutic target proteins. The aim is to explore the potential of hibiscus flower extract in the development of novel formulation to provide its various therapeutic effects.

2. MATERIAL AND METHOD –

Chemical and reagents –

Thechemicals were used are Water, ethanol, ferric chloride, acidified alcohol, ammonia, chloroform, acetic acid, Mayer's reagent, Dandruff's reagent, sulfuric acid, sodium hydroxide, Fehling's solution, Molisch's regent, hydrochloric acid, zinc dust, magnesium turnings, biuret reagent, folin-Ciocalteu reagent, sodium carbonate, gallic acid solution, acetone and isopropyl alcohol (IPA), methanol, aluminum trichloride, potassium acetate, etc.

Plant material and extraction –

Hibiscus rosa-sinensis flower were collected from the garden they were cleaned, dried and powdered. The powder was extracting using hydro-alcoholic solvent through Soxhlet extraction. The extract was concentrated using rotary evaporator and stored at 4^0 C for further analysis.

Phytochemical screening –

The comparison of phytochemical constituents in plant extract between water and hydroalcoholic plant extracts, significant differences in the range and concentration of bioactive compounds are observed.The extract containing water as solvent have hydrophilic compound such as polyphenols like flavonoids, phenolic acid, glycosides and alkaloids, which are effective in exhibiting antioxidant, antimicrobial and anti-inflammatory activities. The plant extract are considered more safe and suitable for therapeutic

purpose where hydrophilic compounds are required whereas hydro alcoholic solvent having combination of alcohol and water efficiently extract broader spectrum of phytochemicals, including both hydrophilic and lipophilic compound such as terpenes, essential oils, saponins and alkaloids. The alcohol present in the solvent enhance the solubility of non-polar compounds, provide the extract greater pharmacological activity. So the choice of solvent significantly influences the composition, potency and potential therapeutic effect, its essential to select the appropriate extraction method.

In this study comparative confirmatory qualitative phytochemical screening of plant extract of water and hydro alcoholic solvent was performed to identify the presence of tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids and terpenoids by performing standard protocols. The flower extract was prepared with different solvents- ethanol, water and a hydro-alcoholic solvent system – to determine the presence of various phytochemicals with the help of quantitative and qualitative tests. The result of these test was recorded bases on chemical reaction, such as color changes or precipitation formation, with observation like a yellow precipitate for flavonoids or green black color for tannins. The quantitative test measured the concentration or yield of each phytochemical present in the extract, results expressed as percentage yield (w/w) or mg/gram. The results are given in the given in the table, providing insight into the phytochemical composition of the flower extracts prepared with different solvent and offering valuable data for further analysis and potential application of these extract[11].

Isolation of Quercetin flavonoid from hydroalcoholic flower extract –

Chloroform was utilized as a solvent to extract the hydroalcoholic flower extract in a separate funnel. Exhaustive extraction (EE) employs a range of solvents with varied degrees of polarity to extract as many of the most active components with the highest biological activity as possible. As a result, the extract was divided into two different layers. The two layers separated. The lower, milky-white organic layer was permitted to flow through a silica column, resulting in colored fractions collected one minute apart. A solvent system consisting of hexane and dichloromethane (7:3) was used.

Thin layer Chromatographic Technique –

The original 80% hydroalcoholic extract, the upper and lower organic layers obtained by solvent extraction, and six randomly selected colorless fractions obtained by column chromatography of the hydroalcoholic extract were all submitted to TLC. TLC was carried out with a solvent system that included hexane and dichloromethane (1:1). The card was allowed to run until the solvent had traveled three-quarters of the way across it. The cards were taken out of the solvent. To envision the cards, they were allowed to dry. Rf values were calculated using the visible spots found (Figures 1). The Rf value is calculated as the compound's displacement from the starting point divided by the solvent's displacement from the same location.

High- performance liquid chromatography –

Reversed phase high-performance liquid chromatography (RP-HPLC) is a simple, sensitive, and accurate method for quantifying Delphinidin and Quercetin in *Hibiscus Rosasinensis Linn* flower hydroalcoholic extracts.

Protein Preparation and receptor grid generation –

In this study activities of specific protein inhibitor complexes using well established computational techniques was examined. The antioxidant activity examined, the crystal structure of a protein complexed with dye-decolorizing peroxidise (DyP) and (2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one (PDB ID:3VXI) was retrieved from the Protein Data Bank. The Anti alopecia activity was examined utilizing the crystal structure of androgen receptor ligand binding domain bound to 3-hydrox-2-imino-6-piperidin-1-ylpyrimidin-4-amine (PDB ID: 4K7A). For the antifungal activity, the crystal having structure of cytochrome P450 EryK, co-crystalized with the inhibitor 1-[92-chlorophenyl)-diphenyl methyl] imidazole (PDB ID;2XFH) was selected. The protein structure was carefully prepared using the Protein Preparation Wizard to ensure they were of high quality and suitable for further computational analysis. A grid was created around the active site of each protein using the receptor grid generation tool in Glide v9.1. These preparation steps enabled a comprehensive investigation of theprotein's interaction with their respective inhibitors, providing insight into their roles in driving the targeted biological activities[12-15].

Stock solution and HPLC condition –

To reach a concentration of 1000 µg/mL, 10 mg of standard Delphinidin and Quercetin (99% purity) was accurately weighed and diluted in 10 mL of methanol. This solution served as the Delphinidin and Quercetin stock solution. In an isocratic system for RP-HPLC analysis, the mobile phase consisted of water and methanol (40:60, v/v), with a flow rate of 1.5 mL/min and a detection wavelength of 254 nm. The mobile phase and samples were vacuum-degassed and filtered using a 0.22 µm Dura PVDF membrane aqueous filter and Millex GV filter unit before injection into the instrument.

A chromatogram of standard Delphinidin and Quercetin has been obtained. As well as one of the samples has been run on HPLC. Delphinidin was detected during the second and fourth minutes of a 10-minute cycle and Quercetin was detected during fifth minutes of a 10-minute cycle. The difference in retention time between standard delphinidin, quercetinand hydroalcoholic sample was 0.47%, 0.21 (<5%). The difference in retention duration between conventional and separated delphinidin was 1.2% (<5%). Delphinidinconcentration in standard solution (SND) was 1 mg/ml, whereas hibiscus flower powder concentration in hydroalcoholic extract (SMP) was 136 mg/mL and Quercetin concentration in standard solution was 1 mg/ml, whereas hibiscus flower powder in hydroalcoholic extract was 187 mg/ml. We used the following calculations to calculate the concentration of Delphinidin and Quercetin in the generated extract:

$$\frac{\text{Peak area of SMP}}{\text{peak area of SND}} \times \frac{\text{concentration of SND}}{\text{concentration of SND}} \times 100 \quad \text{---(1)}$$

$$\frac{\text{Concentration of sample} - \text{peak area of sample}}{\text{Response factor}} \text{-------(2)}$$

Ligand Preparation –

The molecules were initially drawn using a 2D sketching tool to establish their basic structures. These structures were then optimized through geometric minimization with the OPLS 5 force field to improve their accuracy. To further refine the compounds, LigPrep was used, ensuring the molecules were properly prepared for the next stages of analysis[16]. This careful preparation was crucial for improving the reliability of the computational studies and providing an accurate representation of the molecule's properties and interactions.

Ligand Docking with Glide –

Molecular docking study performed with Glide application and the desired docking method: Standard Precision (SP) or Extra Precision (XP) selected. While XP offers greater accuracy, it requires more computational resources. Set up the docking parameters, adjusting options for ligand flexibility, pose sampling and scoring functions as needed. After configured everything, docking process starts[17]. On the completion of docking, examined the resulting score to assess the interactions and binding affinities.

MM-GBSA –

The Prime/MM-GBSA method was used to evaluate the relative binding energies of selected ligands. The pv.maegz file generated from XP docking served as the input. The protein's active site was set up to allow the ligand to move within a 5 Å range, providing a thorough evaluation of the ligand-protein interactions while considering the flexibility of the active site. This approach offered valuable insights into the energetics of ligand binding. Ligands with lower total binding free energies were prioritized for further investigation, as they indicated stronger potential interactions with the target protein[18-19].

ADME Analysis –

The compound was further analysed using the SwissADME server, which is available at http://www.swissadme.ch. At first the compound was converted into their corresponding SMILES Ids using ChemDraw 2D software[20]. These Ids were then submitted to the SwissADME portal for evaluation. The server provided prediction for various physicochemical and pharmacokinetic properties, such as interactions with P-glycoprotein, compliance with Lipinski's Rule of Five (a measure of drug-likeness), the number of hydrogen bond acceptors and donors, potential for blood brain barrier penetration, and gastrointestinal absorption. This thorough analysis offered valuable insights into the drug like characteristics and pharmacological potential of the compounds.

Toxicity Analysis –

The toxicity profiles of the compounds were evaluated using the ProTox-II platform, available at http://tox.charite.de/protox_II. To begin, the SMILES Ids o the compound, generated with ChemDraw 2D software, were uploaded to the server for analysis. The ProTox-II platform then provided comprehensive toxicity predictions, including the LD50 values (median lethal dose), toxicity classifications, and potential organ-specific adverse effects[21-23]. The analysis also covered eye toxicity parameters such as hepatotoxicity, mutagenicity, carcinogenicity and cytotoxicity. This in-depth evaluation helped identify safety concerns and guided the selection of compound with more favourable toxicity profiles for further research.

3. RESULT AND DISCUSSION –

Phytochemical screening -

The result of phytochemical screening provides details on the composition of qualitative and quantitative analysis for ethanolic flower extract. This (Table no-1) outlines the presence of alkaloids, flavonoids, saponins, tannins and phenols. Glycosides, resins, Terpenoids and steroids were also found in the ethanolic extract. The quantitative analysis (Table no.-2) provide data on the concentration or yield of each phytochemical measured using various method. Alkaloids yield was 1.2% w/w, flavonoids yield was 3.5% and the other phytochemicals like saponins, tannins, phenols and steroids are also quantified and the yield ranging from 0.4% w/w to 4.2% w/w.

The qualitative screening of water extract of flower revealed the presence of several bioactive compound. The test (Table no.-3) confirmed the presence of various phytochemicals. Specifically, alkaloids and flavonoids were showed the presence by the formation of orange and yellow precipitates, respectively, while saponins, phenols, tannins, glycosides, resins, carbohydrates and proteins.

Table No.- 1 Phytochemical screening of *Hibiscus rosa-sinensis* flower extract in ethanol

Phytochemical Test	Test Method	Result	Observation
Alkaloids	Dragendorff's Test	+	Cream precipitate
Flavonoids	Lead Acetate Test	+	Yellow precipitate
Saponins	Froth Test	+	Stable froth formation
Tannins	Ferric Chloride Test	+	Green-black color change
Phenols	Ferric Chloride Test	+	black color
Terpenoids	Salkowski Test	+	Reddish-brown precipitate
Steroids	Liebermann-Burchard Test	+	Bluish-green ring at the interface
Glycosides	Keller-Killiani Test	+	Reddish-brown ring
Anthraquinones	Borntrager's Test	+	Pink color in the aqueous layer
Carbohydrates	Molisch's Test	+	Orange-red precipitate
Proteins	Xanthoproteic Test	+	Yellow precipitate
Resins	Acetone-Water Test	+	Turbidity in solution

Table No.2 - Phytochemical screening in terms of % yield in ethanolic extract

Phytochemical	Test/Estimation Method	Concentration (% Yield)	Unit
Alkaloids	Gravimetric Method	1.2	% w/w
Flavonoids	Aluminum Chloride Colorimetric Assay	3.5	% w/w
Saponins	Froth Test (Gravimetric)	2.1	% w/w
Tannins	Folin-Denis Colorimetric Assay	0.8	% w/w
Phenols	Folin-Ciocalteu Method	2.3	mg /g
Terpenoids	Vanillin-Sulfuric Acid Method	0.6	% w/w
Steroids	Liebermann-Burchard Test (Gravimetric)	0.4	% w/w
Glycosides	Keller-Killiani Test (Quantitative)	0.9	% w/w
Anthraquinones	Gravimetric Method	0.7	% w/w
Carbohydrates	Phenol-Sulfuric Acid Method	4.2	% w/w
Proteins	Biuret Assay	1.1	% w/w
Resins	Gravimetric Method	0.5	% w/w

In terms of quantitative analysis (Table no.-4), the percentage yield of various phytochemicals in the water extract was also determined. Flavonoids were the most yield constituent with 2.7% w/w, carbohydrate at 3.8% w/w, alkaloids at 0.8%, saponins at 1.5%, phenols at 1.8% w/w respectively. Other component such as terpenoids, glycosides and anthraquinones at yield of 0.5%, 1% and 0.6% w/w respectively. Proteins (1.3%w/w), steroid (0.3%w/w) and resins (0.4%w/w) were found in smaller amount.

The Hydro-alcoholic flower extract was analyzed for a range of phytochemicals using both qualitative and quantitative methods. Qualitative test (Table no.-5) confirmed the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, steroids, glycosides, anthraquinones, carbohydrates, proteins and resins. The orange precipitate shows the presence of alkaloids, yellow precipitate shows the presence of flavonoids, saponins produced stable froth and tannins, phenols changes color to green-black and deep blue, respectively. Terpenoids,steroids and glycosides were indicated by reddish brown precipitates and a bluish green ring.

For the hydro-alcoholic flower extract quantitative analysis test (Table no.-6) has been performed. The analysis revealed that carbohydrates was at 4.2% w/w yield, flavonoids at 3.5%w/w and saponins at 2.1% w/w. Phenols were found at 2.3% mg, while alkaloids at 1.2%, glycosides at 0.9% and anthraquinones at 0.7% w/w yield. The smaller amount of tannins (0.8% w/w), terpenoids (0.6% w/w),

Table No. – 3 Phytochemical screening of Hibiscus rosa-sinensis flower extract in water

Phytochemical Test	Test Method	Result	Observation
Alkaloids	Dragendorff's Test	Positive	Orange precipitate
Flavonoids	Lead Acetate Test	Positive	Yellow precipitate
Saponins	Froth Test	Positive	Stable froth formation
Tannins	Ferric Chloride Test	Positive	Green-black color change
Phenols	Ferric Chloride Test	Positive	Deep blue
Terpenoids	Salkowski Test	Positive	Reddish-brown precipitate
Steroids	Liebermann-Burchard Test	Positive	Bluish-green ring at the interface
Glycosides	Keller-Killiani Test	Positive	Reddish-brown ring
Anthraquinones	Borntrager's Test	Positive	Pink color in the aqueous layer
Carbohydrates	Molisch's Test	Positive	Violet ring precipitate
Proteins	Biuret Test	Positive	Violet color precipitate
Resins	Acetone-Water Test	Positive	Turbidity in solution

Table No. – 4 Phytochemical screening of flower extract in water in terms of % yield

Phytochemical	Test/Estimation Method	Concentration (% Yield)	Unit
Alkaloids	Gravimetric Method	0.8	% w/w
Flavonoids	Aluminum Chloride Colorimetric Assay	2.7	% w/w
Saponins	Froth Test (Gravimetric)	1.5	% w/w
Tannins	Folin-Denis Colorimetric Assay	0.9	% w/w
Phenols	Folin-Ciocalteu Method	1.8	mg /g
Terpenoids	Vanillin-Sulfuric Acid Method	0.5	% w/w
Steroids	Liebermann-Burchard Test (Gravimetric)	0.3	% w/w
Glycosides	Keller-Killiani Test (Quantitative)	1.0	% w/w
Anthraquinones	Gravimetric Method	0.6	% w/w
Carbohydrates	Phenol-Sulfuric Acid Method	3.8	% w/w
Proteins	Biuret Assay	1.3	% w/w
Resins	Gravimetric Method	0.4	% w/w

Table No. -5Phytochemical screening of flower in hydroalcoholic extract in terms of % yield

Phytochemical	Test Method	Result (Qualitative)	Observation	Concentration (Quantitative)	Unit
Alkaloids	Dragendorff's Test	Positive	Orange precipitate	1.2	% w/w
Flavonoids	Lead Acetate Test	Positive	Yellow precipitate	3.5	% w/w
Saponins	Froth Test	Positive	Stable froth formation	2.1	% w/w
Tannins	Ferric Chloride Test	Positive	Green-black color change	0.8	% w/w
Phenols	Ferric Chloride Test	Positive	Deep blue color	2.3	mg GAE/g
Terpenoids	Salkowski Test	Positive	Reddish-brown precipitate	0.6	% w/w
Steroids	Liebermann-Burchard Test	Positive	Bluish-green ring at the interface	0.4	% w/w
Glycosides	Keller-Killiani Test	Positive	Reddish-brown ring	0.9	% w/w
Anthraquinones	Borntrager's Test	Positive	Red color in the aqueous layer	0.7	% w/w
Carbohydrates	Molisch's Test	Positive	Orange-red precipitate	4.2	% w/w
Proteins	Xanthoproteic Test	Positive	Yellow precipitate	1.1	% w/w
Resins	Acetone-Water Test	Positive	Turbidity in solution	0.5	% w/w

resins (0.5% w/w) and steroids (0.4% w/w). These findings showdiverse phytochemical composition of *Hibiscus rosa-sinensis* and its potential therapeutic application. The phytochemical screening of *Hibiscus rosa-sinensis* flower extracts in ethanol, water and hydro-alcoholic solvents revealed various type of bioactive compounds, which may contribute to its therapeutic potential. The qualitative and quantitative analysis of these extracts demonstrated significant variations in the % yield of various phytochemicals with different solvent used.

The quantitative data presented in Table 2 (ethanol extract), Table 5 (water extract), and Table 6 (hydro-alcoholic extract) demonstrate variations in the yield of phytochemicals. Among the ethanol and hydro-alcoholic extracts, carbohydrates and flavonoids had the highest yields at 4.2% w/w and 3.5% w/w,

respectively, in the ethanol extract, while water and hydro-alcoholic extracts yielded 3.8% w/w and 3.5% w/w for carbohydrates and flavonoids, respectively. Saponins also showed relatively high yields (2.1% w/w in ethanol and 1.5% w/w in water extract), suggesting their abundant presence in these extracts. The ethanol extract exhibited a slightly higher concentration of alkaloids (1.2% w/w) compared to the water extract (0.8% w/w), indicating that ethanol is more efficient for extracting alkaloids from the flowers.

Table No. -6 Comparison of Phytochemicals of flower in hydroalcoholic, ethanolic and waster extract in terms of % yield

Phytochemical	Hydroalcoholic Extract (% w/w)	Ethanolic Extract (% w/w)	Water Extract (% w/w)
Alkaloids	1.2	1.2	0.8
Flavonoids	3.5	3.5	2.7
Saponins	2.1	2.1	1.5
Tannins	0.8	0.8	0.9
Phenols	2.3 mg GAE/g	2.3 mg/g	1.8 mg/g
Terpenoids	0.6	0.6	0.5
Steroids	0.4	0.4	0.3
Glycosides	0.9	0.9	1.0
Anthraquinones	0.7	0.7	0.6
Carbohydrates	4.2	4.2	3.8
Proteins	1.1	1.1	1.3
Resins	0.5	0.5	0.4

Phenolic compounds in both ethanol and hydro-alcoholic extracts were found at 2.3 mg/g and 2.3% w/w, respectively, while water extract showed 1.8% w/w, suggesting that phenols are better extracted in a more polar solvent. Terpenoids, glycosides, anthraquinones, and steroids showed relatively low yields across all extracts, with hydro-alcoholic extract yielding slightly higher amounts of terpenoids (0.6% w/w), while the water extract yielded higher glycosides (1.0% w/w). This indicates that the solvent mixture may optimize the extraction of specific compounds.In comparison, resins and steroids were present in smaller quantities, with ethanol extracts showing higher resins (0.5% w/w) and water extracts showing lower steroid content (0.3% w/w). This demonstrates that certain phytochemicals like resins and steroids are more readily extracted in ethanol compared to water or hydro-alcoholic solutions.

Quercetin (Flavonoid) and Delphinidin (Anthocyanin)analysis –

The hydroacoholic extract was solvent extracted with chloroform in a separating funnel. Exhaustive extraction (EE) uses increasing polarity solvents to extract as many active components as feasible with

the highest biological activity. This caused the extract to be separated into three layers and were separated. The lower organic layer, which had a colored appearance, was allowed to pass through a silica column, producing colored fractions that were collected after a minute interval. The solvents utilized were hexane and dichloromethane (7:3).

Thin Layer Chromatography –

The original 80% hydroalcoholic extract, the upper and lower organic layers acquired by solvent extraction, and six randomly selected colored fractions obtained by column chromatography of the hydroalcoholic extract were all submitted to TLC. TLC was done using hexane and dichloromethane (1:1) as the mobile phase. Figures 1 show the marked spots obtained under the UV lamp. The Rf value of the fractions was discovered to be 0. 3 (Quercetin) and 0.72 (Delphinidin).

HPLC Quantification of Quercetin (flavonoid) and Delphinidin (Anthocyanin) –

HPLC chromatograms for standard Quercetin, and isolated Quercetin from hydroalcoholic Hibiscus flower extract were obtained and are shown in Figures and chromatograms for standard Delphinidine-3-sumbubioside and isolated Delphinidin from hydroacoholic hibiscus flower extract were obtained and are shown in figures.

Quercetin is widely recognized as the main bioactive (flavonoid) component in Hibiscus rosasinensis flower.Previously, reported values of quercetin were 5 µg/mL or less. The HPLC-UV technique detected 3.27 µg/mL of quercetin in hydroalcoholic solvent and 3.219µg/mL in separated quercetin. The obtained HPLC chromatogram of Extract (Figure no.-2) was compared to existing data of standard drug (Figure no.-3). This is due to the care exercised in preparing, storing, and analyzing samples, which were maintained in reagent bottles covered with aluminum foil and refrigerated after each use.

Delphinidin is widely recognized as the main bioactivecomponent'santhocyanin in Hibiscus rosasinensis flower.Previously, reported values of quercetin were 2 µg/mL or less. The HPLC-UV technique detected 1.87 µg/mL of delphinidin in hydroalcoholic solvent and 1.93 µg/mL in separated delphinidin. The obtained HPLC chromatogram of extract (Figure no.-5)was comparedto existing chromatogram (Figure no.-6) of standard drug. This is due to the care exercised in preparing, storing, and analyzing samples, which were maintained in reagent bottles covered with aluminum foil and refrigerated after each use.

Figure No. – 1 TLC of hydroalcoholic extract of hibiscus rosasinensis flower

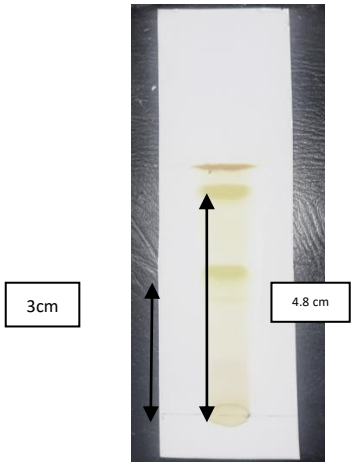


Figure No. – 2 HPLC chromatogram of Quercetin detection in Hydroalcoholic flower extract

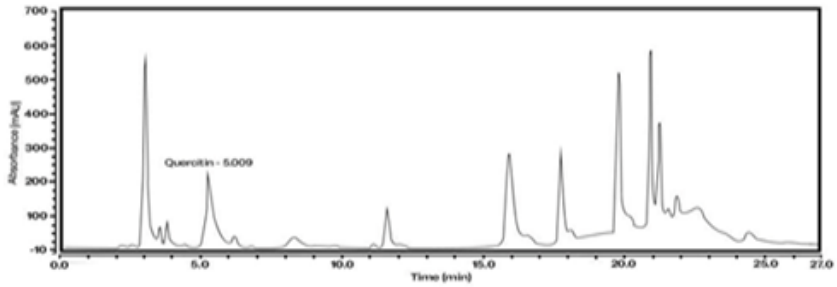


Figure No.- 3 HPLC chromatogram of Quercetin Standard solution

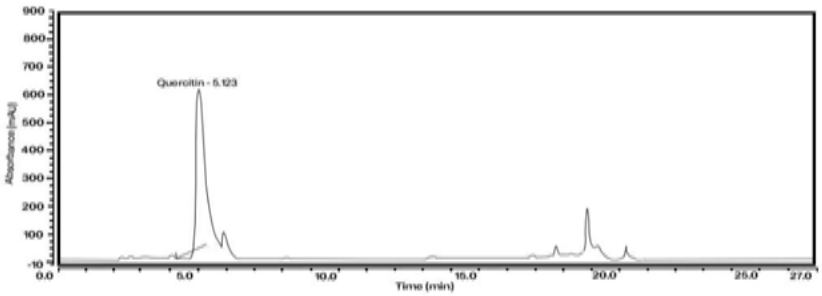


Figure No. – 4 HPLC chromatogram of Delphinidin detection in Hydroalcoholic flower extract

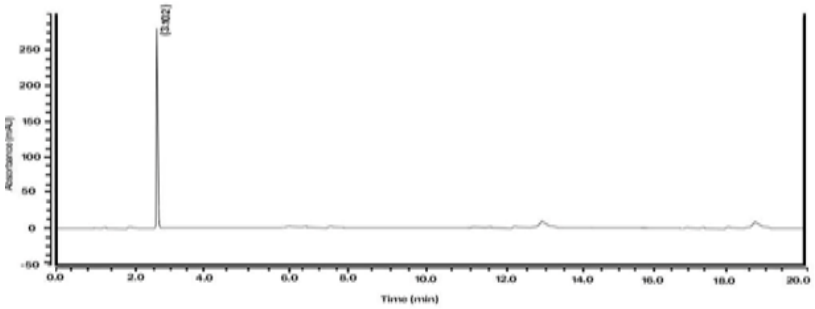
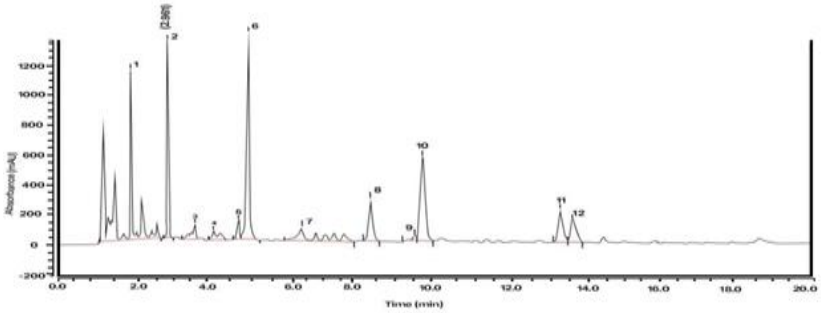


Figure No.- 5 HPLC chromatogram of Delphinidin Standard solution



Docking study –

The Docking study were performed on all the molecules with the PdB IDs (3VXI, 4K7A, 2XFH) and there results are arranged as per the dock score is on the top. Based on their docking scores and binding energies, which are similar to those of the reference molecules, the compounds show potential as Anti-oxidant, Anti-Alopeciaand Anti-fungal activityagents. Their strong binding affinity indicates they may effectively interact with the receptors or pathways involved in hypertension. However, further testing through in vitro and in vivo studies is needed to evaluate their effectiveness, safety, and the specific ways they work to treat healthy cell growth, enhanced hair growth and prevention of microbial growth.

ADME analysis–

The Docking study was performed on the chemical constituents of Hibiscus rosa-sinensis for three biological activity likewise Antioxidant, Anti-Alopecia and Anti-fungal activity. The result of the docking analysis, based on the obtained score (kcal/mol) and the dG binding energies (kcal/mol), along with ADME predictions, are summarized below for each activity.

Anti-oxidant activity (PdB ID : 3VXI) –

The obtain result (Table No.-7) determined scores and binding energies for the anti-oxidant activity. The compound Delphinidin 3-sambuboside exhibited the highest docking scores (-9.462 kcal/mol) and the strongest binding energy (-65.88 kcal/mol), significantly outperforming the standard reference, Ascorbic acid (Table no.-10), which had a docking score of -6.023 kcal/mol and a dG binding of -10.7 kcal/mol. The interactions (Table no.-13) of delphinidin 3–sambubioside with key amino acids (PHE 186, GLY188, GLY188, GLN189, ASP323, GLN316, ASN313) and the formation of several hydrogen bonds (with distances between 1.60 to .34 Å) suggests a strong binding affinity.

ADME prediction (Table no.-16) for Delphinidin 3-sambubioside indicates a high molecular weight (597.50g/mol) and relatively low bioavailability (Score 0.17), with low gastrointestinal absorption (Low). The compound also violates Lipinski's rule (Score 6.34), indicating challenges in druglike properties, despite its strong docking performance.

Anti-Alopecia Activity (PdB ID : 4K7A) –

In the anti-alopecia docking study (Table no.-8), Quercetin-3-diglucoside showed the best docking score (-7.995 kcal/mol) and dG binding energy (-41.32 kcl/mol), indicating a strong interaction with the target protein. The reference drug, minoxidil, had a lower docking score (-4.640 kcal/mol) and dG binding energy (-21.27 kcal/mol), suggesting that quercetin-3-diglucoside may be more promising candidate for treating alopecia (Table n0.-11). This denote that Delphinidin 3-sambubioside may act as an effective anti-oxidant agent by interacting with critical receptors or enzymes involved in oxidative stress pathways.

The binding interactions (Table no.-14) involve hydrogen bonds with residues such as GLU793, ARG786 and LYS861 with distances ranging from 1.76 to 2.94 Å, indicating effective ligand-receptor binding.

For ADME prediction (Table No. 17), Quercetin-3-diglucoside has a molecular weight of 642.52 g/mol, with low bioavailability (0.17) and low GI absorption, indicating a possible challenge for systemic absorption. The compound violates Lipinski's rule (6.56), suggesting the need for modification to improve its drug like properties.

Table No. – 7 Docking score and dG binding of *Hibiscus rosa-sinensis* constituentsfor Anti-oxidant Activity (PdB id:3VXI) -

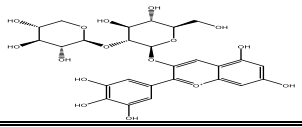
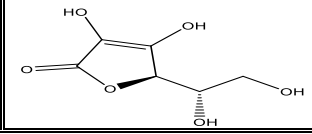
COMPOUND NAME	2D STRUCTURE	DOCK SCORE (kcal/mol)	dG BIND (kcal/mol)
Delphinidin 3-sambubioside		-9.462	-65.88
Standard Reference Ascorbic Acid		-6.023	-10.70

Table No. – 8 Docking score and dG binding of *Hibiscus rosa-sinensis* constituents for Anti-Alopecia Activity (PdB id:4K7A) -

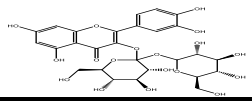
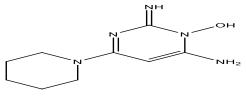
COMPOUND NAME	2D STRUCTURE	DOCK SCORE (kcal/mol)	dG BIND (kcal/mol)
Quercetin-3-diglucoside		-7.995	-41.32
Standard Reference Minoxidil		-4.640	-21.27

Table No.-9 Docking score and dG binding of *Hibiscus rosa-sinensis* constituents for Anti-Fungal Activity (PdB id:2XFH) -

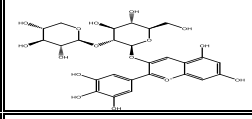
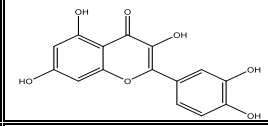
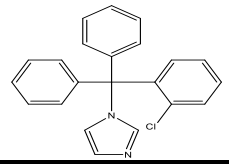
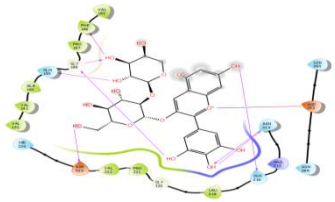
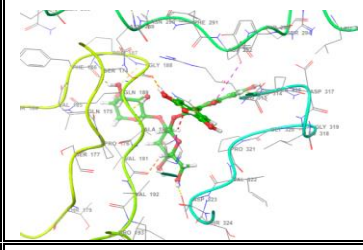
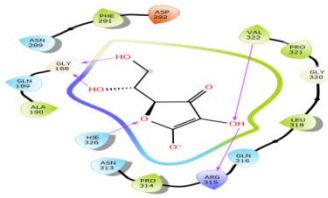
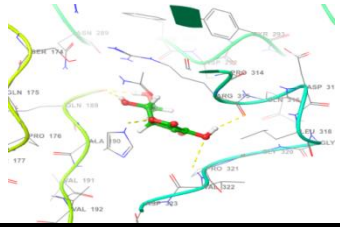
COMPOUND NAME	2D STRUCTURE	DOCK SCORE (kcal/mol)	dG BIND (kcal/mol)
Delphinidin 3-sambubioside		-13.290	-69.38
Quercetin		-7.092	-45.42
Standard Reference Clotrimazole		-6.591	-51.94

Table No – 10 2D and 3D images of Docked complex of *Hibiscus rosa-sinensis* Chemical Constituents for Anti-oxidant activity

Compound name	2D Image	3D Image
Delphinidin 3-sambubioside		
Standard Reference Ascorbic Acid		

Anti-fungal Activity (PdB ID : 2XFH) –

The docking results for anti-fungal activity, summarized (Table No.-9), show that Delphinidin 3-sambubioside had the highest docking score (-13.290 kcal/mol) and the lowest dG binding energy (-69.38 kcal/mol), significantly outperforming the other compounds in this study. Quercetin also

demonstrated a strong binding affinity (-7.092 kcal/mol and -45.42 kcal/mol), suggesting potential anti-fungal properties. The reference drug clotrimazole had a docking score of -6.591 kcal/mol and a dG binding of -51.94 kcal/mol, positioning it as less effective compared to the studied compounds (Table no.-12).Delphinidin 3-sambubioside and Quercetin interacted with amino acid (Table no.-15) residues like ILE392, GLN292, ARG293 and SER345 with distances ranging from 1.72 to 5.13 Å, indicating significant interactions that could lead to inhibition of fungal growth.

Table No – 11 2D and 3D images of Docked complex of *Hibiscus rosa-sinensis* Chemical Constituents for Anti-Alopecia activity

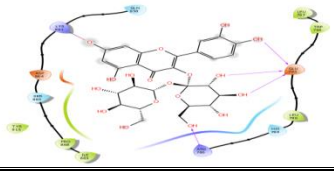
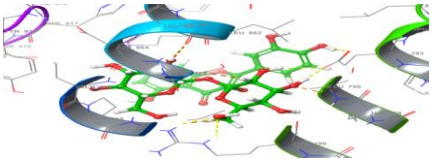
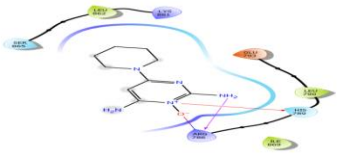
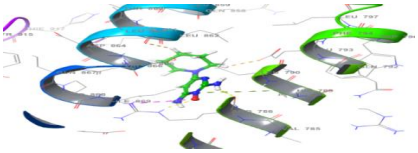
COMPOUND NAME	2D IMAGE	3D IMAGE
Quercetin-3-diglucoside		
Standard Reference Minoxidil		

Table No – 12 2D and 3D images of Docked complex of *Hibiscus rosa-sinensis* Chemical Constituents for Anti-Fungal activity

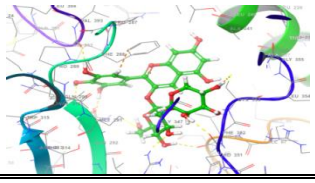
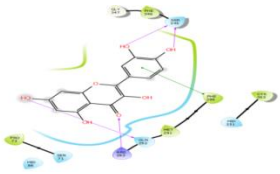
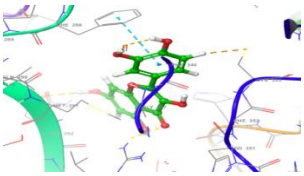
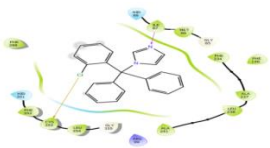
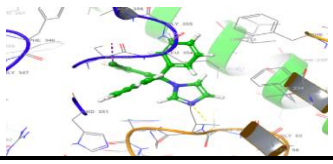
COMPOUND NAME	2D IMAGE	3D IMAGE
Delphinidin 3-sambubioside		
Quercetin		
Standard Reference Clotrimazole		

Table No. – 132D Interactions and Distance from Amino acids used in Docked complex of *Hibiscus rosa-sinensis* Chemical Constituents for Anti-Oxidant activity

Compound name	2D Interaction	Distance(A^0)
Delphinidin 3-sambubioside	H-Bond:PHE186,GLY188,GLY188,GLN189, ASP323,GLN316,ASN313,ASN313 Salt-Bridge:ASP292	1.85,2.34,1.60,1.93, 2.17,2.23,2.14,2.03 4.55
Standard Reference Ascorbic Acid	H-Bond:HIE326,GLY188,GLY188,VAL322, ARG315	2.00,1.91,1.84,2.71, 2.09

Table No. – 142D Interactions and Distance from Amino acids used in Docked complex of *Hibiscus rosa-sinensis*Chemical Constituents for Anti-Alopecia activity

COMPOUND NAME	2D INTERACTION	DISTANCE (A^0)
Quercetin-3-diglucoside	H-bond:GLU793,GLU793,GLU793,ARG786 Salt-Bridge:LYS861	1.99,2.11,1.76,2.28 2.94
Standard Reference Minoxidil	H-bond:ARG786 Salt-Bridge:ARG786 Pi-Cation:HIS789	2.18 3.76 5.00

Table No. –15 2D Interactions and Distance from Amino acids used in Docked complex of *Hibiscus rosa-sinensis*Chemical Constituents for Anti-fungal activity

Compound name	2D INTERACTION	DISTANCE (A^0)
Delphinidin 3-sambubioside	H-bond:ILE392,GLN292,GLN292, ARG293,HID351,CYS353,HID88	1.80,2.22,1.87,1.82, 1.92,2.16,2.00
Quercetin	H-bond:GLN292,ARG293,SER345, SER345 Pi-Pi Stacking:PHE288	1.72,2.11,1.74,2.22 5.13
Standard Reference Clotrimazole	H-bond:ILE87 Halogen Bond:CYS353	2.36 3.35

Table No-16 ADME prediction of all reported molecules for Anti-Oxidant Activity

ADME prediction (http://www.swissadme.ch/index.php)

S. no.	Compound Name	MW	H-bond Acceptor	H-bond Donors	Log P	GI Absorption	BBB Permeant	Bioavailability Score	Lipinskin Violation	Synthetic Accessibility
1	Delphinidin 3-sambubioside	597.50 g/mol	16	11	-4.27	Low	No	0.17	3	6.34
2	Standard Reference Ascorbic Acid	176.12 g/mol	6	4	-0.31	High	No	0.56	0	3.47

Table No-17 ADME prediction of all reported molecules for Anti-Alopecia Activity

ADME prediction (http://www.swissadme.ch/index.php)

S. N.	Compound Name	MW	H-bond Acceptor	H-bond Donors	Log P	GI Absorption	BBB Permeant	Bioavailability Score	Lipinskin Violation	Synthetic Accessibility
1	Quercetin-3-diglucoside	642.52 g/mol	18	12	0.74	Low	No	0.17	3	6.56
2	Standard Reference Minoxidil	209.25 g/mol	3	3	1.23	High	No	0.55	0	2.51

The ADME prediction (Table no.-18) for Delphinidin3-sambubioside shows similar properties to its anti-oxidant activity profile with low bioavailability and poor GI absorption. Quercetin has relatively high bioavailability score (0.55) and high GI absorption, making it a more suitable candidate for systemic absorption despite its docking performance being weaker than Delphinidin 3-sambubioside.

Table No-18 ADME prediction of all reported molecules for Anti-Fungal Activity

ADME prediction (http://www.swissadme.ch/index.php)

S. no.	Compound Name	MW	H-bond Acceptor	H-bond Donors	Log P	GI Absorption	BBB Permeability	Bioavailability Score	Lipinskin Violation	Synthetic Accessibility
1	Delphinidin 3-sambubioside	597.50 g/mol	16	11	-4.27	Low	No	0.17	3	6.34
2	Quercetin	302.24 g/mol	7	5	1.63	High	No	0.55	0	3.23
3	Std (Clotrimazole)	344.84 g/mol	1	0	3.07	High	Yes	0.55	1	2.25

Comparison of Docking scores and binding energies –

Anti-oxidant activity – Delphinidin 3-sambubioside showed the strongest binding affinity, significantly surpassing Ascorbic acid.
Anti-alopecia activity – Quercetin 3-diglucoside outperformed Minoxidil, indicating its potential as a better agent for alopecia treatment.
Anti-fungal activity – Delphinidin 3-sambubiosides again showed the highest docking score, followed by quercetin, both surpassing Clotrimazole in binding affinity.

ADME predictions and druglike properties –

The compound demonstrated strong binding affinities, their ADME predictions reveal some limitations in terms of bioavailability, gastrointestinal absorption and molecular weight. Delphinidin 3-sambubioside and Quercetin-3-diglucoside showed poor bioavailability, which could limit their effectiveness as oral drugs. In contrast, Quercetin exhibited higher bioavailability, making it a better candidate for potential therapeutic use, through its docking score were lower.

4. CONCLUSION –

The comparative analysis of the phytochemical composition of Hibiscus rosa-sinensis flower extracts in ethanol, water, and hydro-alcoholic solvents reveals that the extraction efficiency varies depending on the solvent used. Ethanol and hydro-alcoholic extracts yield higher quantities of alkaloids, flavonoids, and carbohydrates, while water extracts are more efficient in extracting phenolic compounds. The diversity and concentration of these bioactive compounds across different extracts suggest that Hibiscus rosa-sinensis flowers have considerable pharmacological potential, which could be optimized depending

on the solvent used for extraction. Further studies could focus on the synergistic effects of these compounds, which may enhance the therapeutic efficacy of Hibiscus extracts.Based on the docking scores and binding energies, **Delphinidin 3-sambubioside** emerged as the most potent compound for anti-oxidant and anti-fungal activities, while **Quercetin-3-diglucoside** showed promise for anti-alopecia activity. However, the compounds showed some limitations in terms of ADME properties, which could hinder their oral bioavailability. Further studies, including in vitro and in vivo testing, are required to validate the pharmacological potential of these compounds and to address their bioavailability challenges.

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