TROPICAL LIFE SCIENCES RESEARCH

Exploring the Antibacterial Activity of *Ipomoea staphylina* Extracts Against *H. pylori*: A Pharmacognostic Investigation of Whole Plant and Matured Stem with Emphasis on Quercetin Isolation

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Highlights

- Research confirms the antibacterial efficacy of *Ipomoea staphylina* extracts against *H. pylori*. Study involves pharmacognostic analysis of the whole plant and matured stem.

- Phytochemical Findings: High presence of phenolics and flavonoids in the extracts. Advanced instrumentation ensures precise identification of quercetin.

- Medicinal Significance & Antimicrobial Efficacy: DME and HLS extracts show exceptional antibacterial activity. Findings support the use of *Ipomoea staphylina's* matured stem latex as a treatment for *H. pylori*-induced stomach ulcers.

Exploring the Antibacterial Activity of *Ipomoea staphylina* Extracts Against *H. pylori*: A Pharmacognostic Investigation of Whole Plant and Matured Stem with Emphasis on Quercetin Isolation

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Abstract. *Ipomoea staphylina Rome & Schult*, entrenched in ethnomedicinal practices, is recognized for its efficacy in treating stomach disorders. Traditionally used in Dharmapuri, Tamil Nadu for stomach ulcers, its matured stem bark latex is therapeutically relevant, especially for *Helicobacter pylori (H. pylori)* infections. This prompts scientific exploration into its antibacterial properties. The research validates the antibacterial efficacy of *Ipomoea staphylina* extracts against *H. pylori*, scrutinizing the whole plant and matured stem through a comparative pharmacognostic analysis. Utilizing herbal standardization techniques, we confirm the heightened purity of the powder. Antimicrobial assessments show exceptional efficacy of DME and HLS extracts. Quercetin isolation by using advanced instrumentation (NMR, HRMS, HPTLC, FTIR) ensures precise compound identification. This methodology guarantees an exhaustive analysis, confirming purity and identifying bioactive components. Standardization underscores the elevated purity of *I. staphylina*, with phytochemical screening revealing a predominant presence of phenolics and flavonoids. Antibacterial investigations highlight significant activity, particularly with DME and HLS extracts. These findings substantiate *Ipomoea staphylina's* medicinal significance,

especially its matured stem latex, as a promising treatment for *H. pylori*-induced stomach ulcers, affirming traditional use by Dharmapuri villagers.

Keywords: Pharmacognostical Evaluations, Phytochemical Screening, Fluorescence Analysis, HPTLC Analysis, Quercetin

INTRODUCTION

Herbal plants have been utilized for centuries in the treatment of various diseases (Balasubramaniam et al., 2020; Süntar, 2020). Medicinal plants and their isolated compounds have (Maqbool et al., 2019)played a significant role in both conventional and alternative medicine for thousands of years (Maqbool et al., 2019; Süntar, 2020). For instance, Cinchona bark has been traditionally used in South America for the treatment of malaria (Tolkushin et al., 2020). Notably, many well-known medications, including Curcumin, Digitoxin, Caffeine, and Atropine, are derived from plants (Maqbool et al., 2019). The World Health Organization (WHO) estimates that around 21,000 plant species have been utilized globally for medicinal purposes (Sundaramoorthy et al., 2021). Herbal medicines form the cornerstone of various alternative treatments that have gained popularity in recent years (Maqbool et al., 2019; Süntar, 2020). Moreover, plant-based medications have a profound impact on drug development and design (Gomathi et al., 2012). The increased utilization of herbal-based products can be attributed to their efficacy, affordability, reduced toxicity, and eco-friendliness (Zhou et al., 2007). In fact, approximately 60% to 75% of cancer and infectious disease medications are derived from natural sources (Newman et al., 2000; Song et al., 2014a). Given their ability to interact with proteins, herbs are considered valuable in drug design, contributing to the creation of novel medications (Newman et al., 2000; Song et al., 2014b).

Ipomoea staphylina Rome & Schult, a plant deeply entrenched in ethnomedicinal practices, boasts a spectrum of biological properties particularly recognized for its efficacy in addressing stomach disorders (Dias et al., 2012; Narra & Kandavara, 2014), the matured stem bark latex of this plant has been traditionally employed by communities in Dharmapuri, Tamil Nadu, for the treatment of stomach ulcers. The therapeutic relevance is noteworthy, especially considering that stomach ulcers are often attributed to *Helicobacter pylori (H. pylori)* bacterial infections (Majumdar & Looi, 2024). This traditional application underscores the potential of *Ipomoea staphylina* as a valuable resource in managing gastric ailments, providing a rationale for scientific exploration into its antibacterial properties against *H. pylori*. Most commonly in human

H. Pylori is the main reason for the stomach ulcer (Youssefi et al., 2021). *Helicobacter pylori* (*H. pylori*) is a gram-positive bacterium that infects the stomach (Kishikawa et al., 2020; Youssefi et al., 2021). *H. pylori* infection typically occurs during childhood (Kishikawa et al., 2020; Lee, 2019; Majumdar & Bebb, 2019). The bacteria can be transmitted through contaminated water, food, or contact with the saliva, vomit, or feces of an infected person. *H. pylori* colonizes the stomach lining, causing inflammation and weakening the protective mucous layer (Alexander et al., 2021). This can lead to the development of peptic ulcers, including gastric ulcers (in the stomach) and duodenal ulcers (in the upper part of the small intestine (Alexander et al., 2021; Kishikawa et al., 2020; Lee, 2019; Majumdar & Bebb, 2019; Youssefi et al., 2021). We can control the stomach ulcer by controlling this bacterial growth. The principal objective of this research is to assess the antibacterial efficacy of *Ipomoea staphylina* against *H. pylori*, thereby substantiating its traditional use for anti-ulcer purposes in the Dharmapuri village. Additionally, the study aims to elucidate the traditional practices associated with *Ipomoea staphylina* in treating stomach ulcers within the Dharmapuri village community.

I. staphylina is an important climber known for its use in the treatment of respiratory problems, as an anthelmintic, purgative, for bronchitis, and for stomach ailments (Narra & Kandavara, 2014; Raghavendra, 2013; Ramesh, Deepa, et al., 2022; Ramesh, Rajeshkumar, et al., 2022). This plant exhibits a range of pharmacological effects, including antimicrobial, anti-inflammatory, antioxidant, anti-diabetic, and anti-mutagenic properties (Raghavendra, 2013). We have done the quantitative phytochemical screening of the plant extract, employing standardized methodologies (Krishnaveni et al., 1984; Soni & Sosa, 2013; Yilmaz, 2020). This analysis provided valuable insights into the composition and concentration of secondary metabolites inherent to the extract. Concurrently, the assessment of antioxidant potential was pursued using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, recognized for its efficacy in gauging radical scavenging activity (Isildak et al., 2022; Sahidin et al., 2022; Sihag et al., 2022). The resulting data showcased a commendable percentage of radical scavenging activity, underscoring the extract's promising antioxidant capabilities. These findings collectively contribute to a deeper understanding of the extract's chemical composition and potential applications in various fields. To confirm the presence of secondary metabolites in the plant extract, crude drugs underwent High Performance Thin Layer Chromatography (HPTLC) analysis with standard drugs (Saxena et al., 2021). HPTLC was employed to identify and authenticate the phytochemicals and markers present in the *I. staphylina* plant extract (Gunjal Sanket & Dighe, 2022; Mir et al., 2020). Through comprehensive antibacterial evaluations, the research endeavors to contribute empirical

evidence supporting the plant's potential therapeutic applications, aligning traditional knowledge with contemporary scientific scrutinyIn traditional medicine.

Ethnopharmacology

I. staphylina, a plant of significant therapeutic importance, has been utilized for the treatment of various disorders, including purgation, stomach disorders, pain, rheumatism, and inflammation (SM & Koneri, n.d.). In the region of Dharmapuri, the matured stem latex of *I. staphylina* has been traditionally employed to address stomach ulcers. Similarly, the residents of Gingee Hills have utilized leaf latex for the treatment of foot cracks (Muralidharan & Narasimhan, 2013)). Notably, the roots of *I. staphylina* have been used as an antidote for snake bites by the Irula and Palliyar tribes (Sarvalingam et al., 2014). Additionally, villagers in Karandamalai have administered a leaf decoction to alleviate stomach problems (Kottaimuthu, 2008). The Chenchus tribes have employed leaf extract as a treatment for piles (T. D. Kumar & Pullaiah, 1999). These traditional uses underscore the diverse therapeutic potential of *I. staphylina* in various cultural settings.

MATERIALS AND METHOD

Chemical and Reagents

Solvents including Methanol, Ethanol, Ethyl acetate, and Petroleum ether were procured from SD Fine Chemicals. Additionally, CHCl3, NaOH, KOH, HCl, H2SO4, HNO3, Na2CO3, Acetic acid, and DPPH were obtained from Avra Chemicals. Reagents required for qualitative phytochemical testing, such as Neutral FeCl3, Alkaline reagent, Mayers solution, Benedict’s reagent, Salkowski reagent, Fehling’s solution, and Folin-Ciocalteu reagent, were sourced from Himedia.

Plant Collection

Plant parts were collected from the Dharmapuri region (Tamil Nadu, Latitudes N 11 47' and 12 33' and Longitudes E 77 02' and 78 40') during the winter season of December 2020. The soil in which the plant was grown is characterized as clay. To ensure accurate identification, the plant specimen has been authenticated by the Botanical Survey of India, Southern Region Coimbatore. The authentication certificate (BSI/SRC/5/23/2022/Tech/429) (Figure 1), which is attached for reference.

Plant Extraction

This research investigation focuses on two specific components of *I. staphylina*, specifically the stem & leaves and matured stem, as targets for study. Fresh plant parts were subjected to extraction using two distinct methods: hot continuous percolation (Soxhlet) and immersion. Additionally, the Soxhlet method was followed for the extraction of bioactive compounds from the dried parts (Dried under sunshade) of *I. staphylina* (Bag & Mumtaz, 2013; López-Bascón & De Castro, 2020). These chosen extraction techniques were carefully selected to ensure optimal recovery of valuable constituents from the plant material, facilitating subsequent analysis and characterization.

Fresh Part Extraction

Fresh plant parts were meticulously washed with distilled water to remove any impurities, followed by pulverization using an electronic blender. The resulting blend was subjected to extraction using two distinct methods: Soxhlet and immersion (Bag & Mumtaz, 2013; López-Bascón & De Castro, 2020). In the Soxhlet method, solvents such as petroleum ether, ethanol, and hydroalcohol (a mixture of 80% methanol and 20% water) were used. Conversely, for the immersion method, distilled water served as the solvent. Following the extraction process, the solvents were evaporated using a Rotavapor R-100 apparatus. The resulting dried crude extract was carefully collected in an airtight container and stored under refrigeration conditions. These standardized procedures ensure the preservation and stability of the extracted compounds for further analysis and experimentation.

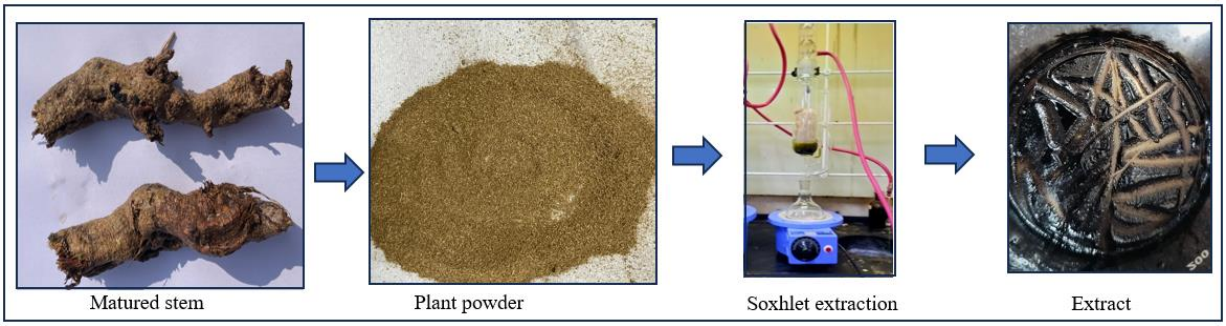

Figure 1: Fresh part extraction matured stem of *I. staphylina.*

Dry Part Extraction

The *staphylina* plant parts (Matured stem and stem & leaf) were carefully dried under shade at room temperature for a duration of 10 days, ensuring the preservation of their chemical constituents. Subsequently, the dried parts were finely pulverized using an electronic blender, resulting in a homogeneous powder. The extraction process was conducted using the Soxhlet method, employing solvents such as petroleum ether, ethanol, and hydroalcohol (a mixture comprising 80% methanol and 20% water) (Bag & Mumtaz, 2013; López-Bascón & De Castro, 2020). This extraction method facilitated the efficient extraction of bioactive compounds from the plant material. Following the extraction, the solvents were evaporated using a Rotavapor R-100 apparatus. The resulting dried crude extract was carefully collected and stored in an airtight container, ensuring protection from moisture and other degrading factors. To maintain the stability and integrity of the extracted compounds, the container was stored in a refrigerator, providing optimal storage conditions (Jeyadevi et al., 2019). These meticulous steps in sample preparation and storage guarantee the quality and usability of the extracted material for further scientific analysis and investigation.

Figure 2: Dried part extraction stem and leaf of *I. staphylina*

Physicochemical Standardization

Before commencing any research work on a specific herb, it is essential to conduct a comprehensive assessment of its physicochemical parameters (Snehalatha & Rasmi, 2021). In order to standardize the herbal powder, various pharmacognostic characteristics were evaluated, including ash value, loss on drying, acid-insoluble ash, water-soluble ash, and fluorescence analysis (Khanal et al., 2018; Khandelwal, 2008; Snehalatha & Rasmi, 2021). These

physicochemical standardizations were performed according to established protocols and guidelines. By examining these pharmacognostic characteristics, it becomes possible to identify potential adulterants and impurities present in the herbal drug, ensuring the purity and quality of the material under investigation. These evaluations serve as crucial preliminary steps in the research process, providing valuable insights into the composition and integrity of the herb being studied.

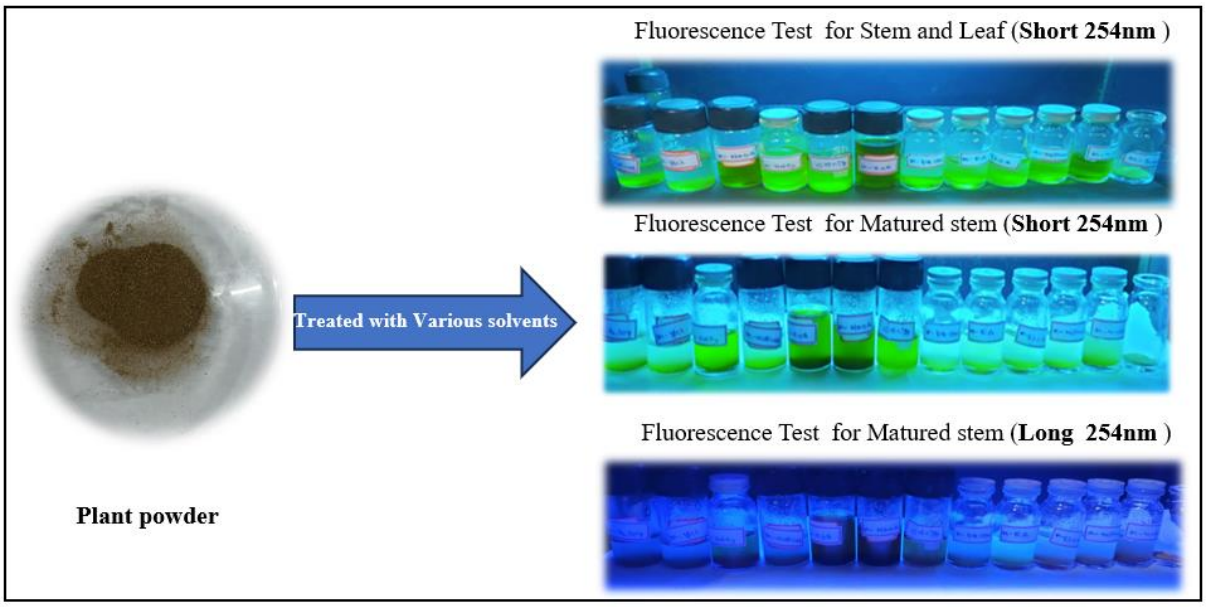

Figure 3: Fluorescence analysis of *I. staphylina*

EXPERIMENTAL SECTION

Pharmacognostic Evaluations

Prior to initiating any research, the standardization of herbal drugs is imperative (Khandelwal, 2008). In the case of *I. staphylina* herbal powder, pharmacognostic evaluations were conducted to ensure its quality and authenticity. These evaluations encompassed essential parameters, including loss on drying, ash value, water-soluble ash, and acid-insoluble ash, the results of which are presented in Table 1 (Khanal et al., 2018; Khandelwal, 2008). These rigorous tests serve the purpose of safeguarding against potential adulterants and maintaining the integrity of the herbal drug. Furthermore, fluorescence analysis was employed as a critical parameter to assess the purity and quality of the powdered drug material. These comprehensive assessments collectively

contribute to the standardization process, guaranteeing the reliability and effectiveness of the herbal drug under investigation.

Fluorescence Analysis

Fluorescence analysis of the powdered drugs was conducted following a standardized procedure (Das et al., 2021; Khanal et al., 2018; Khandelwal, 2008). To perform the analysis, various solvents including water, methanol, ethanol, ethyl acetate, petroleum ether, chloroform, 10% sodium hydroxide, 10% potassium hydroxide, hydrochloric acid (4N), sulphuric acid (4N), nitric acid (4N), and acetic acid (4N) were individually mixed with the desired herb powder. After allowing sufficient time for interaction, the fluorescence of the plant powder was examined under both UV and visible light. The obtained fluorescence patterns provide valuable insights into the unique fluorescent characteristics of the matured stem, stem, and leaf powders, as depicted in Tables 2 and 3, respectively. This fluorescence analysis serves as an important tool for the characterization and assessment of the quality of the herbal drug, contributing to its standardization and reliable utilization in further research endeavors (Amir et al., 2019; Bashir et al., 2019; Jain et al., 2021; Misra et al., 2018; Ved et al., 2022).

Qualitative Phytochemical Screening

The extracted crude samples underwent a qualitative phytochemical investigation to identify the presence of various phytoconstituents, including alkaloids, tannins, flavonoids, phenols, saponins, proteins, sterols, terpenoids, carbohydrates, and fats & oils, utilizing established standard methods (Amir et al., 2019; Banerjee & Firdous, 2020; Bashir et al., 2019; Das et al., 2021; Harborne, 1998; Jain et al., 2021; Khanal et al., 2018; Khandelwal, 2008; Misra et al., 2018; Tiwari & Patel, 2012; Ved et al., 2022). The results of the preliminary phytochemical screening of the freshly dried parts of *I. staphylina* are presented in Table 4 and Table 5.

Quantitative Phytochemical Screening

The quantitative phytochemical screening was done by following standard procedure and using spectrophotometer (Isildak et al., 2022; Krishnaveni et al., 1984; Soni & Sosa, 2013; Yilmaz, 2020).

Estimation of Phenols

Phenols were quantified utilizing the FC technique, slightly modified in accordance with the procedures outlined in the reference. Initially, a 100 µl aliquot was drawn from a 10 mg/ml stock solution, with subsequent adjustment of the volume in each test tube to 3.0 ml through the addition of distilled water. The experimental protocol encompassed the sequential addition of 0.5 mL of Folin-Ciocalteau reagent and 2 mL of 20% Na_2CO_3 solution into the tubes. The resulting mixture was subjected to precise boiling within a water bath for a duration of one minute. After this, the tubes underwent a cooling phase, following which absorbance measurements were taken at a wavelength of 650 nm, employing a spectrophotometer and utilizing a reagent blank as a reference standard. Notably, gallic acid functioned as the standard substance throughout this investigation. To prepare the gallic acid solution, 1 milligram of the compound was dissolved in 1 ml of ethanol. Subsequently, aliquots of this solution, measuring 50 µl, 100 µl, 150 µl, 200 µl, and 250 µl, were meticulously extracted for subsequent analytical procedures.

Estimation of Flavonoids

Total flavonoid content was determined using the aluminium chloride colorimetric assay. A reaction mixture containing 1 mg of extract and 1 ml of distilled water was prepared. Sequential addition of 0.30 ml of 5% sodium nitrite, followed by 0.3 ml of 10% aluminium chloride after 5 minutes, was carried out. After another 5 minutes, 2 ml of 1M sodium hydroxide was added and the solution was diluted to 10 ml with distilled water. Reference standard solutions of Quercetin (20-100 µg/ml) were prepared similarly. Absorbance was measured at 510 nm against a reagent blank using a UV/Visible spectrophotometer. Total flavonoid content was expressed as µg QE/mg of extract.

Estimation of Tannins

The determination of tannin content in the sample was executed utilizing the Folin-Ciocalteu method. This approach relies on colorimetric assessment, wherein the formation of a blue colour arises from the reduction of phosphotungstomolybdic acid by tannin-like compounds under alkaline conditions. To proceed, a solution containing 1 mg of extract or standard tannic acid (ranging from 50 to 250 µl) was prepared, achieving a final volume of 7.5 mL with distilled water. Subsequently, 0.5 mL of Folin-Ciocalteu reagent and 1 mL of 35% sodium carbonate solution

were introduced. Further volume adjustments were made to reach a total of 10 mL using distilled water, following which absorbance measurements were taken at 700 nm. This method facilitated the precise quantification of tannin content in the sample

Estimation of Carbohydrates

Total carbohydrate quantification employed the phenol sulphuric acid method with spectrophotometric analysis (Krishnaveni et al., 1984). Each sample (100 µl) and standard underwent a controlled three-hour incubation in a water bath with 5 mL of 2.5 N HCl, followed by neutralization with solid sodium carbonate. After volume adjustment (100 mL) and centrifugation, a series of test tubes received working standard volumes (50-250 µl), phenol solution (1 mL), and 96% H_2SO_4 (5 mL). Following agitation and a 20-minute water bath incubation, absorbance at 490 nm was measured. Carbohydrate content was quantified using glucose as the standard reference.

Antioxidant Activity

The investigation into the scavenging capacity of free radicals within the crude extracts was executed utilizing a spectrophotometric methodology, with minor adaptations in accordance with the protocol stipulated by the reference (Isildak et al., 2022; Sahidin et al., 2022; Sihag et al., 2022). The samples and the established standard underwent meticulous preparation at varying concentrations of 50, 100, 150, 200, and 250 mg/mL, all dissolved in a methanolic medium. A solution of 0.2 mmol/L^{-1} concentration of DPPH was meticulously diluted in 50 mL of methanol, subsequently subjected to an incubation period of 30 minutes under controlled room temperature conditions, following which the absorbance was quantified at a specific wavelength of 517 nm. For precise quantification, the Microsoft Excel platform was employed to compute the percentage values indicative of the Radical Scavenging Activity (RSA), employing the formula mentioned below. Notably, Rutin was utilized as the reference standard throughout the experimental endeavour.

The RSA% inhibition was mathematically defined by:

$$\% \text{ inhibition} = [(\text{Control Abs}) - (\text{Sample Abs}) / (\text{Control Abs})] \times 100 \quad (1)$$

HPTLC Analysis

Sample preparation

All extracts were weighed at a concentration of 5 mg/ml and dissolved in specific solvents. The resulting solutions were subjected to sonication for 5 minutes and then filtered using Whatman filter paper to remove any particulate matter. Precoated TLC (thin-layer chromatography) aluminum sheets with silica gel 60 F 254 (Merck) were utilized for the analysis. The samples were applied to the TLC plates using a Linomat 5 sample applicator, ensuring a band length of 5 mm and a speed of 150 nl/sec. This meticulous procedure ensures precise and uniform application of the extracts onto the TLC plates, enabling accurate separation and identification of the individual components present in the extracts (A. Kumar et al., 2010; Swaroop et al., 2005; Tiwari & Patel, 2012).

Mobile phase

Following the elution process optimizing with various solvent combinations, a suitable mobile phase was established by employing a mixture of toluene, ethyl acetate, formic acid, and glacial acetic acid in a ratio of (2:6:1:1). For the preparation of samples and standards, 1 mg of the compound was dissolved in 10 ml of the respective solvent. The application of these solutions was carefully performed by marking spots on precoated TLC aluminum sheets coated with silica gel 60 F 254 (Merck). These highly specific and controlled procedures facilitate the precise separation and analysis of the compounds present in the samples, ensuring accurate results and reliable identification of individual components.

Chromatogram and scanning

Chromatogram development was conducted using a twin trough glass chamber for a duration of 20 minutes, with a distance of 80 mm, employing a mobile phase consisting of toluene, ethyl acetate, formic acid, and glacial acetic acid in a ratio of (2:6:1:1). Following the development process, the air-dried TLC plates were subjected to examination under UV light. Subsequently, scanning of the plates was performed using a CAMAG HPTLC Densitometer (Scanner) equipped with Deuterium light and CAMAG winCATS software, using wavelengths of 254 nm and 366 nm.

The obtained results from the HPTLC analysis are presented in Figure 2 and Figure 3, providing valuable insights into the separation and detection of the analyzed compounds.

Column chromatography

To isolate quercetin, the ethanolic extract of *Ipomoea Staphylina* (ESL) was chosen based on the quantity and the results obtained from high-performance thin-layer chromatography (HPTLC). For the precise analysis of 10 g of the plant extract, column chromatography was performed using a stationary phase of silica gel (60–120 mesh) (Davies & Johnson, 2007; Johnston et al., 2013; Reid & Sarker, 2012). The mobile phase used in column chromatography was similar to the one used in HPTLC (composed of a mixture of ethyl acetate, formic acid, glacial acetic acid, and water in a ratio of 20:2:2:1). The yellow-colored spot, corresponding to quercetin, was isolated and verified using thin-layer chromatography (TLC) alongside a standard sample, confirming their identical retention factor (Rf) values. The resulting product was then dried and prepared for further analysis and characterization (Meena & Patni, 2008; Sambandam et al., 2016; Zahoor et al., 2018).

Characterization

FT-IR and NMR spectrum analysis

The isolated compound was subjected to Fourier-transform infrared spectroscopy (FT-IR) analysis to verify the presence of specific functional groups and obtain information about its molecular structure. The FT-IR results provided confirmation of the functional groups present in quercetin (Sambandam et al., 2016).

Additionally, the isolated compounds underwent NMR spectroscopy to determine the precise positioning of the proton and carbon binding sites (Gülşen et al., 2007). The analysis was performed using a Bruker 400Mz instrument, and the obtained results were processed using Topspin software version 3.6.2. The NMR spectra, depicting the chemical shifts and peak patterns, are presented in Figure 5 and Figure 6, allowing for a detailed characterization of the isolated compounds (Gülşen et al., 2007).

HRMS spectrum

High-resolution mass spectrometry (HRMS) was employed to determine the molecular weight of the isolated compound (Parvez et al., 2021). The presence of a molecular ion peak was confirmed based on the data obtained from the HRMS analysis. The molecular weight of the isolated compound was determined using an HRMS Model Name: Waters - Xevo G2- XS - QToF mass spectrometer.

Antibacterial activity

Antibacterial activities of tested sample were evaluated using well diffusion method on *blood Agar*. The bacterial strains *H*. *pylori* (Heliobacter pylori) were used as references for the antibacterial assay (Balouiri et al., 2016; Gonelimali et al., 2018; Wylie et al., 2022). *Blood Agar* plates were inoculated with bacterial strain under aseptic conditions was spread plated by glass L-rod with 100 µl grown culture. Then, squire (10 mm and) compound coated thin film were attached on each plate, after that followed by incubation at 37°C for 24 hours. After the incubation period, the zone of inhibition was measured and reported in millimeters (mm). The MIC was considered as the concentration which inhibited the growth of the respective microorganisms. All assays were performed in triplicate. The extracts were dissolved in distilled water and Chloramphenicol was used as control (Balouiri et al., 2016; Gonelimali et al., 2018; Wylie et al., 2022).

RESULTS AND DISCUSSION

Pharmacognostical Evaluation

The moisture content in mature stems and leaves was found to be 10.32 % and 14.32 % respectively, based on loss on drying. These findings led us to conclude that this particular plant contains some minerals because the matured stem's ash value was 4.4 % and the stem and leaf's ash value was 8.27 %. For the standardization of herbal medication, each of these factors is crucial.

Table 1: Pharmacognostic evaluation *of I. staphylina* powder.

S. No	Parameters	Matured stem	Stem & leaf
1	Loss on drying	10.32%	14.32%
2	Ash value	4.4%	8.27%
3	Water soluble ash	-	2.6%
4	Acid insoluble ash	-	-

Fluorescence studies employ estimations of fluorescence intensity as well as identify any fluorescent phytochemical compounds present in the plant.

Table 2: Fluorescence analysis of matured stem powder of *I.staphylina.*

S.No	Solvents	Visible	Long 365nm	Short 254nm
1	Powder	Brown	Brown	Light green
2	Water	Brown	Brown	Light green
3	Methanol	Brown	Brown	Light green
4	Ethanol	Brown	Brown	Light green
5	Ethyl acetate	Brown	Green	Light green
6	Petroleum ether	Brown	Light green	Light green
7	$CHCl_3$	Brown	Green	Green
8	10%NaOH	Dark brown	Brownish green	Brownish green
9	10%KOH	Dark brown	Brownish green	Brownish green
10	HCl (4N)	Brown	Brown	Light green
11	H_2SO_4 (4N)	Brown	Brown	Light green
12	HNO_3 (4N)	Dark brown	Green	Green
13	Acetic acid(4N)	Brown	Brown	Light green

Table 3: Fluorescence analysis of stem and leaf powders of *I.staphylina.*

S.No	Solvents	Visible	Long 365nm	Short 254nm
1	Powder	Brown	Brown	Light green
2	Water	Brown	Brown	Green
3	Methanol	Green	Dark green	Green
4	Ethanol	Light green	Green	Light green
5	Ethyl acetate	Light green	Green	Light green
6	Petroleum ether	Brown	Light green	Light green
7	$CHCl_3$	Brown	Light green	Green
8	10%NaOH	Dark brown	Light green	Light green
9	10%KOH	Dark brown	Light green	Light green
10	HCl (4N)	Brown	Light green	Light green
11	H_2SO_4 (4N)	Brown	Light green	Light green
12	HNO_3 (4N)	Brown	Green	Light green
13	Acetic acid(4N)	Brown	Light green	Green

Qualitative Phytochemical Screening

According to the phytochemical analysis, flavonoids were found to be abundant in all dry powder extracts and highly abundant in fresh plant extracts. Phenols were present in most fresh plant part extracts, except for water extracts (MS, SL), while they were present in all dry powder extracts. Sterols were predominantly present in extracts from both fresh and dry plant parts, except for HMS extract. Alkaloids were detected in all extracts, both from fresh and dry plant parts, except for MSS and SLS. Furthermore, the phytochemical screening revealed that the extracts of *Ipomoea* plants contain a wide range of secondary metabolites, including tannins, terpenoids, carbohydrates, saponins, and fats & oils. The detailed results can be found in Tables 4 and 5.

Table 4: Fresh plant parts phytochemical screening report.

Secondary metabolites	Name of the test	MS	SL	MSS	SLS	EMS	ESL	HMS	HSL
Phenols	Neutral $FeCl_3$ test	-	-	++	+	+	+	+	+
Flavonoids	Alkaline reagent test	+++	+++	+	+	+++	++	+++	+++
Alkaloids	Mayers test	+	++	-	-	++	+	+	+
Carbohydrates	Benedict's test	-	-	-	-	-	++	-	+
Sterols	Salkowski test	+	+	+	+	+	+	-	+
Proteins	Biuret test	-	-	-	-	-	-	-	-
Saponins	Foam test	+++	+++	-	-	-	-	+	++
Terpenoids		+	+	+	+	+	+	+	+
Tannins		-	-	-	+	-	+	-	-
Fat & Oils		-	-	++	++	++	++	-	-

Table 5: Dried plant parts phytochemical screening report.

Secondary metabolites	Name of the test	DSP	DMP	DSE	DME	DSH	DMH
Phenols	Neutral $FeCl_3$ test	+	+	-	-	+	+
	Gela	++	++	+	+	-	+
Flavonoids	Alkaline reagent test	+	+	+	+	+	+
Alkaloids	Mayers test	++	++	+	++	+	+
Carbohydrates	Benedict's test	-	-	+	++	++	+++
Sterols	Salkowski test	+	+++	++	+++	++	++
Proteins	Biuret test	-	-	-	-	-	-
Saponins	Foam test	-	-	-	+	++	++
Terpenoids	Copper acetate test	+	+	+	+	++	++
Tannins		-	-	+	+	+	+

Fat & Oils	+	+	+	+	-	-

Note: - Negative, + Present, ++ Moderate, +++ Strongly Present

Quantitative Phytochemical Analysis

Estimation of secondary metabolites

Total phenolic content (TPC) of various crude extracts is quantified in terms of gallic acid equivalents (GAE), Flavonoids with various plant extracts quantified in terms of Quercetin equivalents (QE), tannins quantified in terms of Tannic acid equivalents (TE) and carbohydrate quantified in terms of Glucose equivalents (GE). All the quantitative estimation was tabulated in table:

Table 6: Quantitative estimation of plant extracts.

S. No	Sample code	Total Phenolics (mg/g)	Total flavonoids (mg/g)	Total Tannins (mg/g)	Total carbohydrates (mg/g)
1	SLS	113.2	242.3	-	-
2	ESL	226.3	569.4	-	401.9
3	HLS	317.5	256.2	-	325.6
4	MSS	-	196.0	-	-
5	EMS	330.1	128.1	163.0	-
6	HMS	-	43.5	-	-
7	DSP	-	256.6	-	-
8	DSE	-	282.7	-	87.7
9	DSH	110.4	-	-	670.4
10	DMP	28.5	497.6	170.7	-
11	DME	36.6	567.3	-	783.5
12	DMH	-	115.3	-	-

Significant differences were observed among the various stem extracts under investigation. Notably, the ethanolic matured stem extract (EMS) displayed the highest Total Phenolic Content (TPC), registering at 330.1 mg of Gallic Acid Equivalents (GAE) per gram. Meanwhile, HLS extract showcased a slightly lower TPC of 317 mg GAE/g. In terms of Quercetin content, it was found that all extracts, except for the DSH extract, contained minimal amounts of this compound. Notably, the Ethanolic Stem Extract (ESL) stood out by exhibiting the highest Quercetin content among all extracts, measuring at 569.4 mg of Quercetin Equivalents (QE) per gram. It is noteworthy that the presence of tannins was exclusively identified in the EMS and DMP extracts, with tannin concentrations recorded at 163.0 mg and 170 mg of Tannic Acid Equivalents (TE) per gram, respectively. All other extracts lacked tannin content. Furthermore, the DME was

distinguished by its elevated Carbohydrate content, measuring at 783.5 mg of Glucose Equivalents (GE) per gram. These findings underscore the diverse chemical compositions present within the examined extracts.

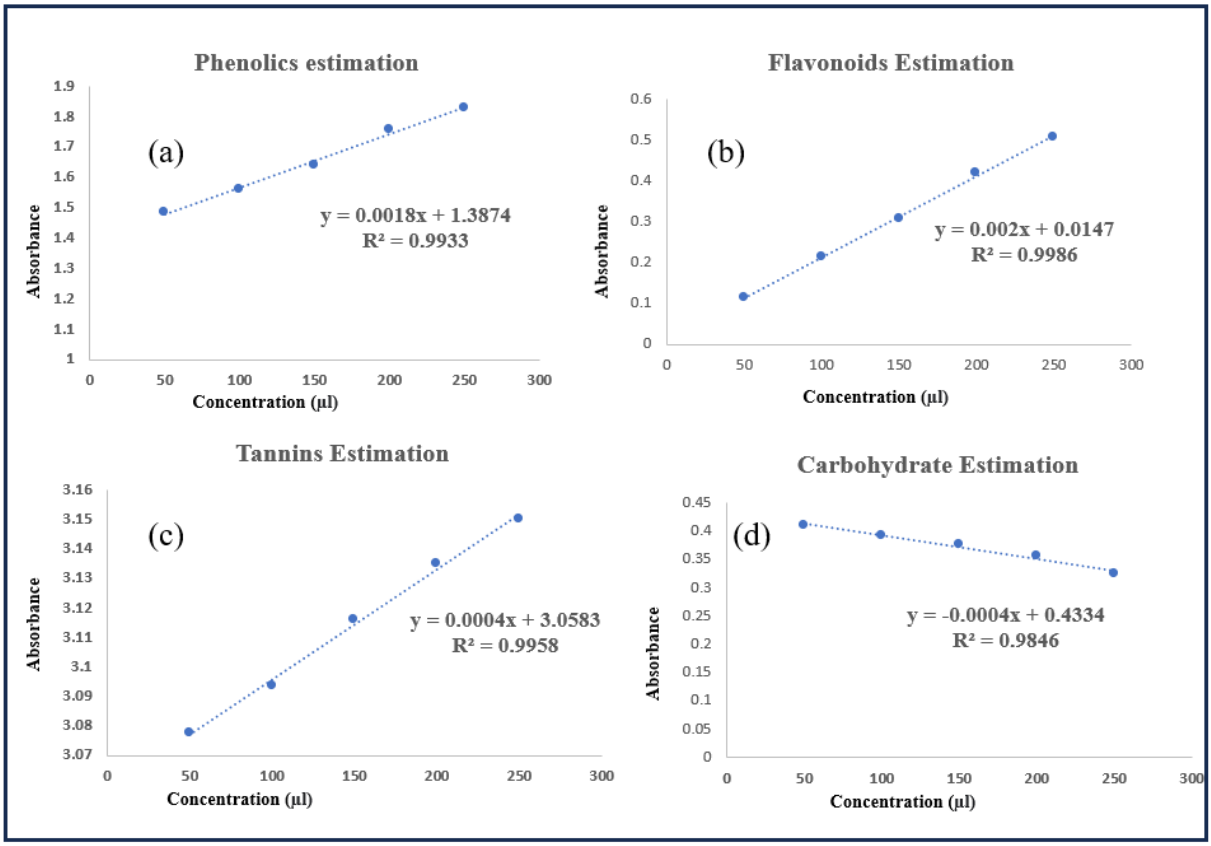

Figure 4: (a) standard correlation graph of phenolics estimations, (b) standard correlation graph of Flavonoids estimations, (c) standard correlation graph of Tannins estimations, (d) standard correlation graph of Carbohydrates estimations.

Antibacterial Activity

Table 7: Antibacterial activity results.

S.No	Sample Name	*H. pylori* (Zone of inhibition(mm) Concentration (µl)			
		25 µl	50 µl	75 µl	100 µl
1.	Chloramphenicol	16	15	20	21
2.	*HLS*	-	12	15	18
3.	*DME*	14	15	16	17

4.	*EMS*	-	-	-	-
5.	*ESL*	-	-	-	-

In contrast to the control represented by chloramphenicol at a concentration of 100 µl, the HLS sample manifests a significant zone of inhibition, measuring 18 mm. Even at a lower concentration of 50 µl, a substantial inhibitory zone of 12 mm is observed. The DME extract, at 25 µl, exhibits a zone of inhibition measuring 14 mm, while at 100 µl, it demonstrates an expanded inhibitory zone of 17 mm. These findings underscore the efficacy of these extracts, particularly against Gram-negative bacteria such as *H. pylori,* hinting at a potential influence on cellular replication mechanisms. This antibacterial activity suggests promising avenues for further investigation and potential applications in combating bacterial infections.

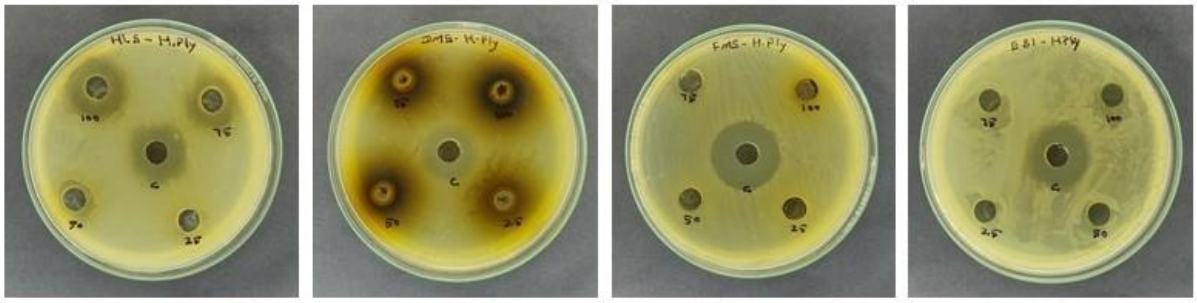

Figure 5: Antibacterial activity of *I. staphylina*

Antioxidant Assay

DPPH stands as a stable free radical commonly employed in evaluating the capacity of extracts to function as radical scavengers or hydrogen donors, thereby assessing their antioxidant ability. The quantified outcomes of DPPH scavenging for extracts are succinctly presented, where Rutin serves as the standard compound, and these results are visually represented in Figure 4&5. The efficacy of various extracts in attenuating the DPPH radical is conveyed through the metric of inhibitory percentage. Noteworthy is the pronounced DPPH radical inhibition showcased by the crude ESL, and EMS extracts, denoting an elevated level of activity. Moreover, the extracts DME and DMH exhibit a commendable yet moderate efficacy in comparison to the standard. All remaining extracts also exhibit favourable scavenging activity against the DPPH radical.

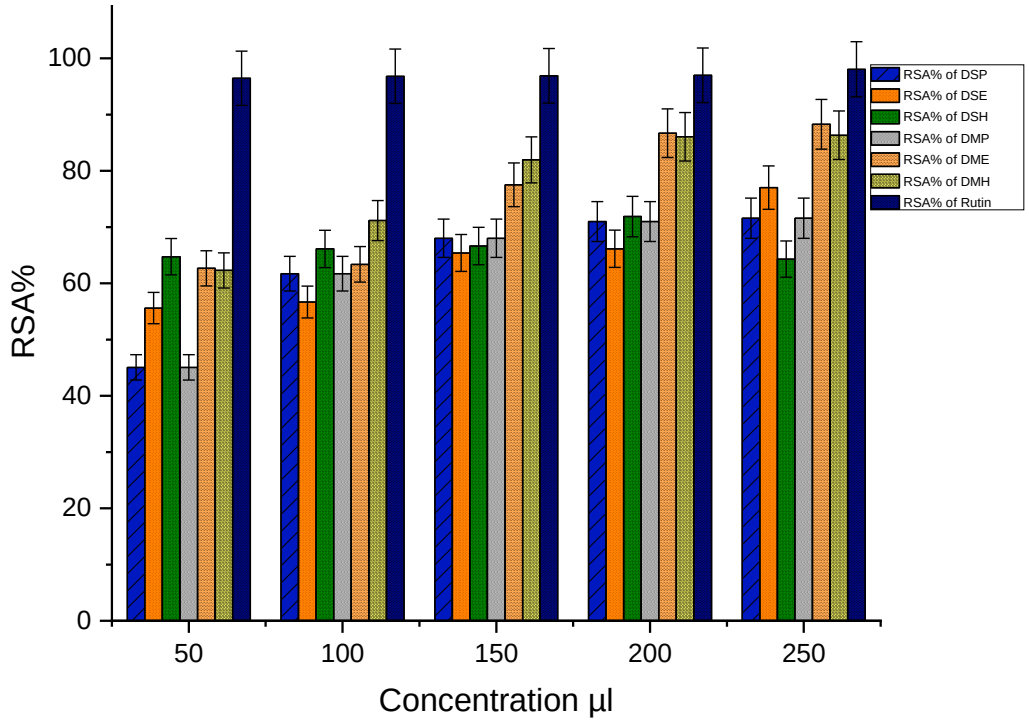

Figure 6: Antioxidant activity of (RSA%) dried extracts

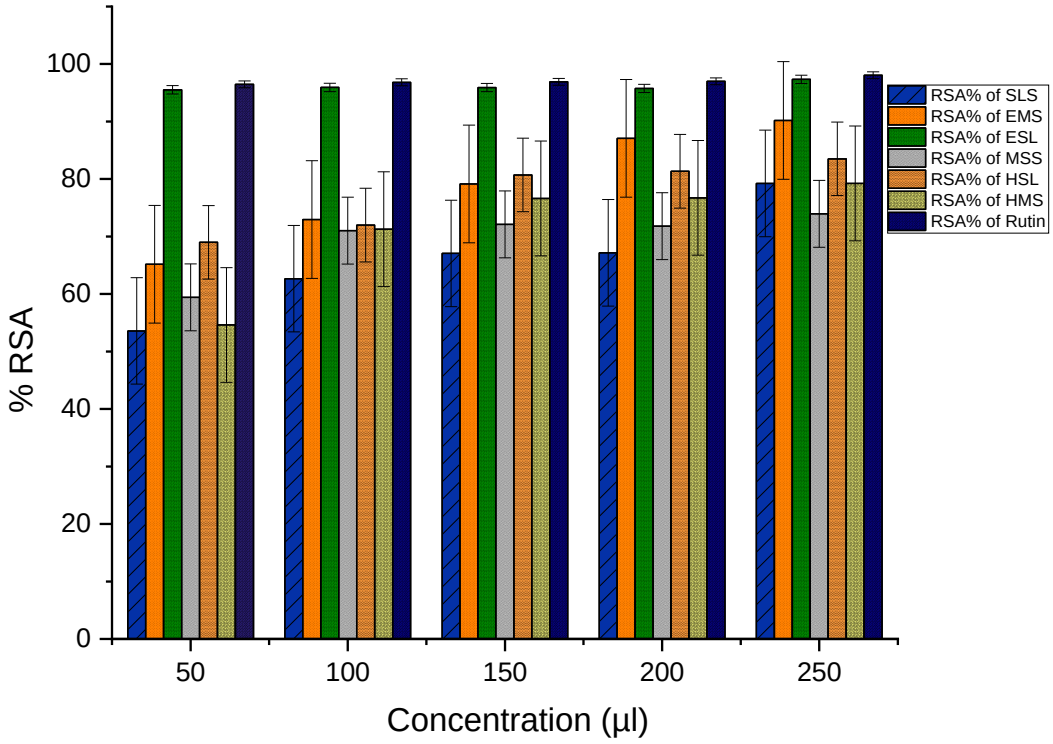

Figure 7: Antioxidant activity of (RSA%) fresh extracts.

HPTLC Analysis

The HPTLC results indicate that among the tested extracts, EMS, HLS, and EMS have the lowest concentration of Quercetin. Additionally, the preliminary phytochemical screening supports the strong presence of flavonoids in EMS, HLS, and EMS. The Rf values of these three extracts match the reference Rf value (as shown in Table 8), confirming the presence of quercetin, a flavonoid. However, the HPTLC results reveal that quercetin is only present in trace amounts in all samples, except for SL and MS.

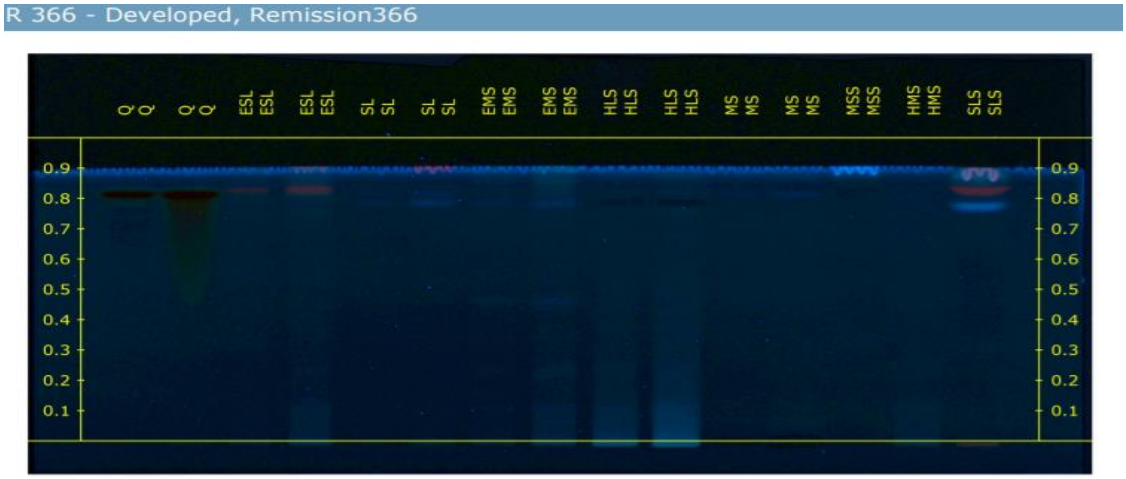

Figure 8: UV remission 366 of *I.staphylina* crude extracts.

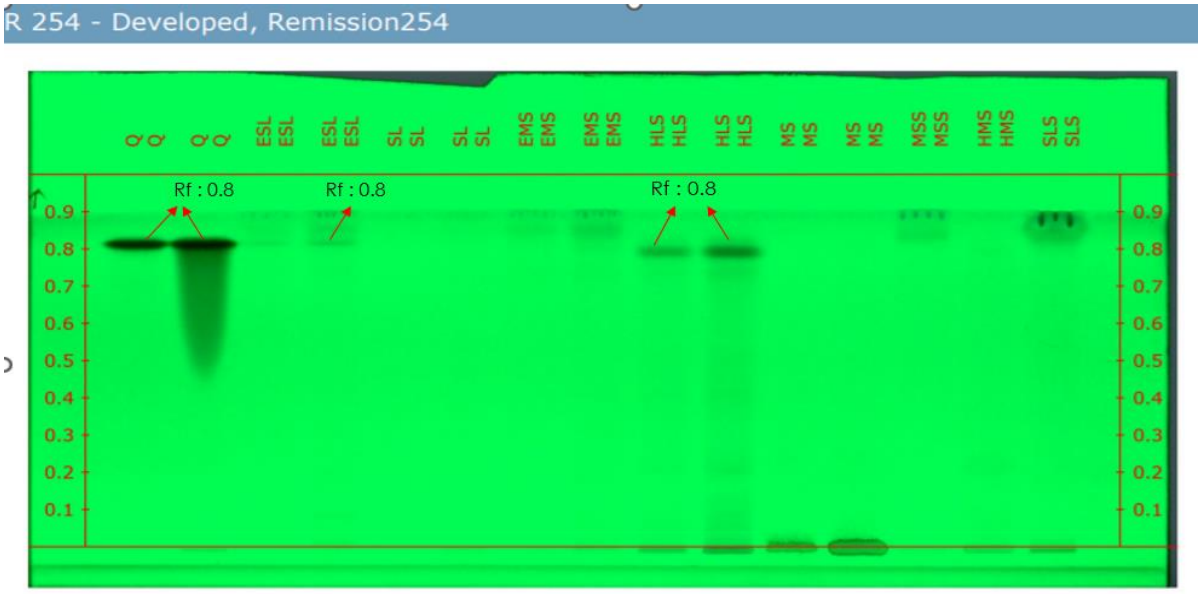

Figure 9: UV Remission 254 nm of *I.staphylina* crude extracts.

Table 8: HPTLC results.

S. No	Compound	No of Peaks	Start R_f	Max R_f	End R_f	Area	Percentage
1	Quercetin 2µl	1	**0.787**	**0.826**	**0.858**	0.02430	100%
2	Quercetin 5µl	1	**0.787**	**0.833**	**0.854**	0.01799	100%
3	ESL 2µl	1	0.804	0.828	**0.854**	0.00101	100%
4	ESL 5µl	1	0.813	0.828	**0.854**		100%
5	SL 2µl	-	-	-	-	-	-
6	SL 5µl	1	0.822	0.836	0.851	0.00022	100%
7	EMS 2µl	-	-	-	-	-	-
8	EMS 5µl	2	0.796	0.813	0.822	0.00015	38.06%
			0.825	0.849	0.857	0.00025	61.94%
9	HLS 2µl	1	**0.787**	0.806	0.833	0.00600	100%
10	HLS 5µl	1	**0.787**	0.807	0.860	0.00858	100%
11	MS 2µl	-	-	-	-	-	-
12	MS 5µl	-	-	-	-	-	-
13	MSS 5µl	1	0.819	0.844	0.861	0.00142	100%
14	HMS 5µl	1	**0.787**	0.806	0.824	0.00052	100%
15	SLS 5µl	1	0.813	0.838	0.851	0.00102	100%

Based on the HPTLC data, the Rf values of the standard quercetin (2µl) were determined to be 0.787, 0.836, and 0.858, while for the 5µl it showed 0.787, 0.826, and 0.858. Most of the samples exhibited very similar Rf values, indicating a comparable composition. Notably, the ESL (5µl) sample displayed an end Rf value that precisely matched the end Rf value of quercetin (5µl), suggesting that the ESL (5µl) contains only a minimal quantity of quercetin. Furthermore, the beginning Rf values of HLS (2µl and 5µl) and HMS (2µl) precisely matched the Rf value of quercetin, indicating that both samples contain only a small amount of quercetin. However, the results of the HPTLC analysis revealed that quercetin is present in trace amounts in all samples, except for SL and MS.

FT-IR Analysis

The FT-IR spectra results detected from the isolated compound (Q-ESL) confirm the OH phenolic stretch and hydroxyl stretch at 3408 cm^{-1}, and 3283 cm^{-1}. Stretching of aryl C=O at 1666 cm^{-1} C-C aromatic band shows at 1610 cm^{-1} and aromatic C=C vibration shows at 1519 cm^{-1}, O-H bands of the phenolic group observe at 1379 cm^{-1}, C-H bond starching observe at 1319 cm^{-1}, C-O stretching shows at 1254 cm^{-1} and 1200 cm^{-1}, aromatic C-H bending appeared at 944, 820, 639, 600, 490 cm^{-1}. The presented results of FT-IR spectra conclude the conformation of the Quercetin functional groups.

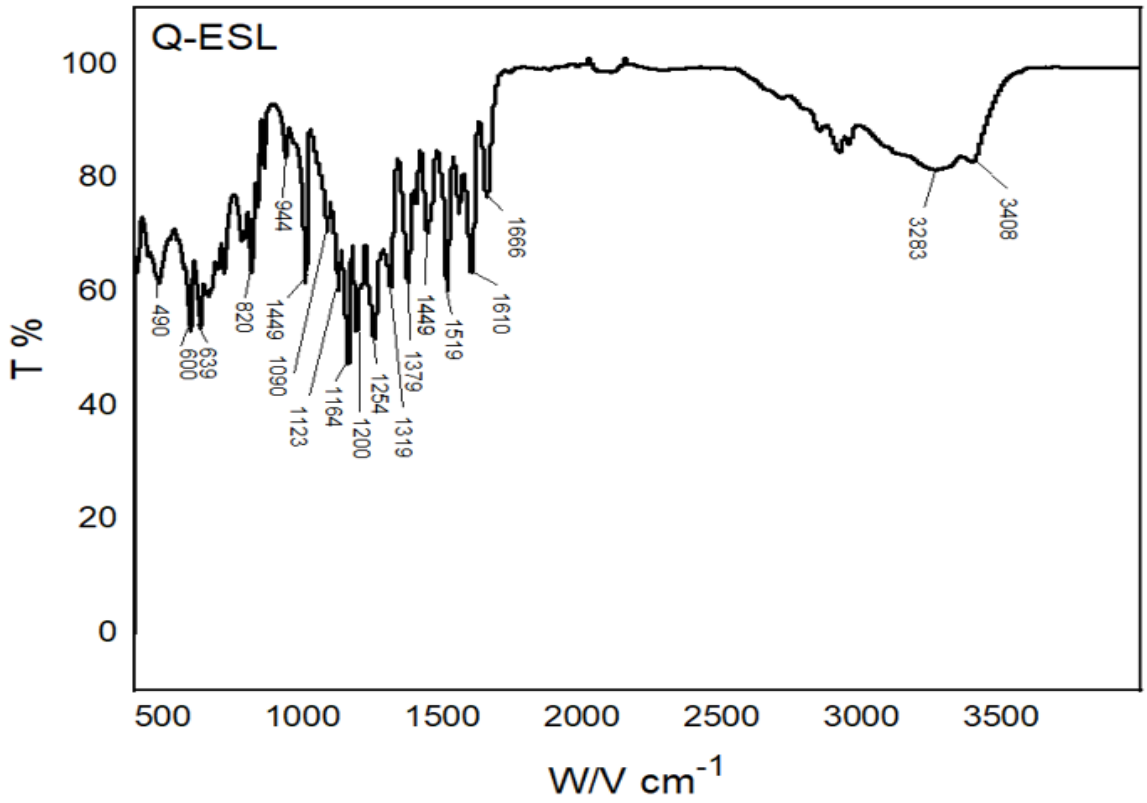

Figure 10: FT-IR spectrum of Q-ESL and HLS.

NMR Spectrum Analysis

Nuclear Magnetic Resonance (NMR) spectroscopy was employed to investigate the proton and carbon bonding in the isolated compound. The NMR analysis provided valuable insights into the structural characteristics of the compound, confirming the presence of quercetin. The obtained NMR results, which offer detailed information about the proton and carbon of Isolated compound is depicted in figs. 9 and 10.

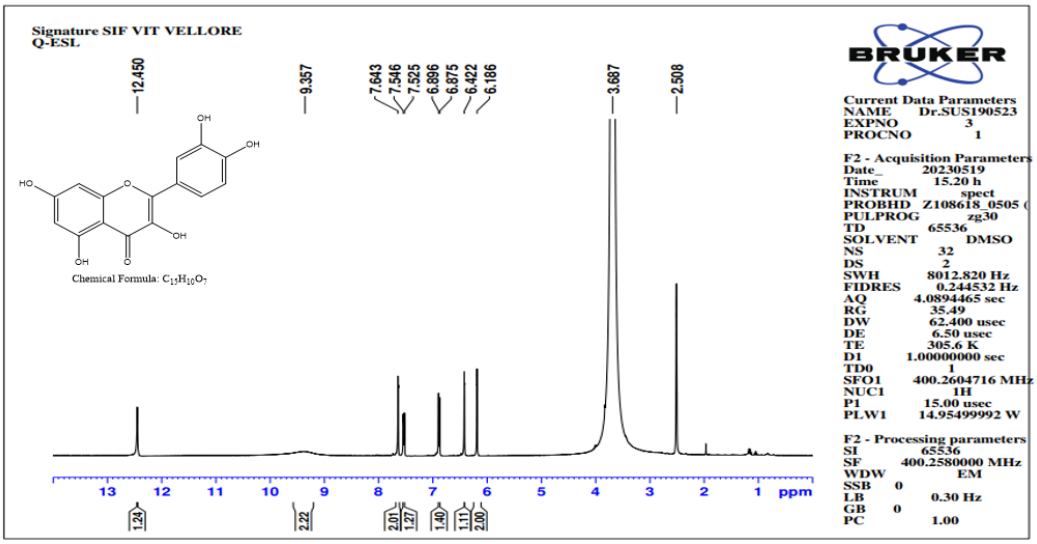

Figure 11: Proton NMR spectra of Q-ESL

The ^{1}H NMR spectrum of **Quercetin (**400MHz, DMSO-d_6): The phenyl OH proton shows at the range of 12.450 ppm (s, H), OH proton at 9.357 ppm (s, 2H). The other two OH protons which have the same environment appeared at the range of 7.643 ppm (s, 2H). Aromatic protons appeared in the range of 7.546ppm (d, *J* =4.2Hz, 1H), 6.896 ppm (d, *J* = 4.2Hz, 1H), 6.422ppm (s, H), 6.186 ppm (s,2H). As per the result aromatic protons appeared in the 6.184-7.643 ppm region and the phenyl OH appeared in the 12.457 and 9.381 ppm which strongly confirms the quercetin structure.

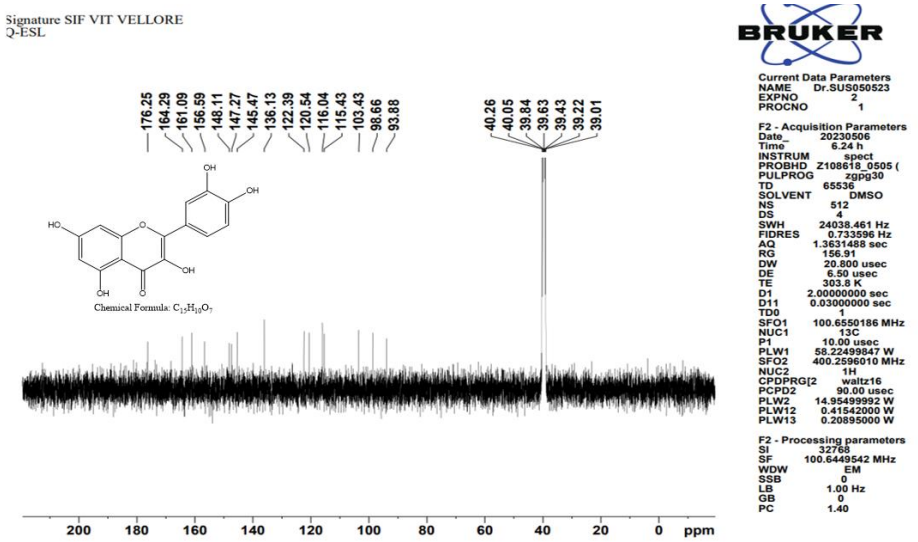

Figure 12: C^{13} Spectra of Q-ESL

The ^{13}C NMR spectrum of Quercetin (100MHz, DMSO-d_6, δ) δ 176.25, 164.29, 161.01, 159.59, 148.11, 147.27, 145.47, 136.13, 122.39, 120.54, 116.04, 115.43, 103.43, 98.66, 93.88. The C^{13} spectra show that 176.25 ppm is a carbonyl carbon peak and the other attached carbons showed 164.29 -156.59 ppm. The respective peaks of 148.11 -122.39 ppm are carbons attached to the OH functional group. All the aromatic carbons appeared at 116.04-93.88 ppm. The advanced NMR spectrum results confirm the structure of Quercetin.

HR-MS Spectroscopy

HR-MS spectroscopy was performed to conclude the molecular weight of the isolated compound. The spectrum confirms the molecular ion peak $[M + H]^+$ at a range of 303.04 which is exactly similar to the Quercetin molecular weight.

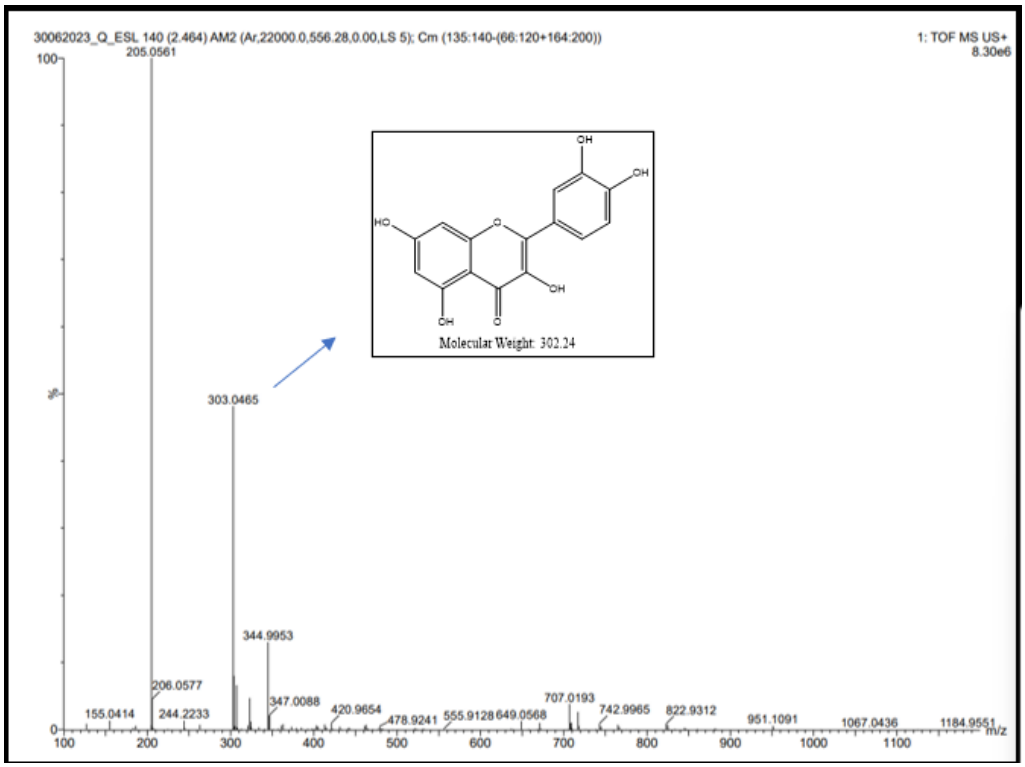

Figure 13: HRMS spectra of Q-ESL

The phytochemical screening of the extract derived from *I. staphylina* yielded compelling findings, highlighting its rich repertoire of secondary metabolites(Banerjee & Firdous, 2020; Bashir et al., 2019; Harborne, 1998). The comprehensive analysis unveiled the presence of various prominent

classes of secondary metabolites, including phenols, alkaloids, carbohydrates, sterols, saponins, terpenoids, tannins, fats and oils, and flavonoids. These extensive phytochemical screenings, coupled with subsequent pharmacognostic investigations, accentuate the profound significance of *I. staphylina* as a valuable source of bioactive compounds.

Employing high-performance thin-layer chromatography (HPTLC), the presence of the flavonoid quercetin was effectively detected, consolidating its status as a constituent within the extract(Jain et al., 2021; Ved et al., 2022). The isolation of the pure bioactive component, quercetin, from the ethanolic extract of *I. staphylina* was accomplished using advanced instrumentation methodologies, facilitating its successful extraction and characterization for further scientific exploration and potential applications.

The main objective of this research is to conduct a comprehensive comparison of crude extracts through pharmacognostical analysis and evaluate the antibacterial activity of matured stem extracts against H. pylori bacteria. Various parts of *I. staphylina* underwent rigorous pharmacognostical studies, leading to successful extraction with different solvents. Tables 1 and 2 present the outcomes of pharmacognostic evaluations and standardization, showcasing the purity of *I. staphylina*. Qualitative and quantitative phytochemical analyses revealed the presence of crucial secondary metabolites, including phenols, alkaloids, and flavonoids. Herbal drug quality was ensured through stringent tests assessing parameters like loss on drying and ash value, along with fluorescence analysis. Additionally, antioxidant potential was assessed using the DPPH assay, yielding promising results. HPTLC confirmed the presence of quercetin, which was isolated via column chromatography and further characterized using advanced techniques such as NMR, FTIR, and HRMS(Davies & Johnson, 2007; Meena & Patni, 2008; Reid & Sarker, 2012). The primary objective of this research is to scrutinize the antibacterial efficacy against *H. pylori,* the causative agent of stomach ulcers. The focus is to substantiate, through scientific means, the traditional utilization of *Staphylina* for addressing stomach disorders and ulcers. Antibacterial assays reveal noteworthy effectiveness in HLS and DME ethanolic extracts derived from matured stems, as evidenced by substantial zones of inhibition against *H. pylori*. Concurrently, these extracts showcase elevated levels of secondary metabolites, with a pivotal bioactive compound isolated from the ESL extract. These secondary metabolites have the ability to disrupt the membrane of the test pathogens thereby leading to the apoptosis of the test pathogens (Baazeem et al., 2021; Odiba et al., 2014). These findings underscore the potential application of *I. Staphylina* in treating stomach ulcers induced by H. pylori. Furthermore, this research provides scientific validation for the traditional use of *I. Staphylina* in Dharmapuri for stomach ulcer management.

CONCLUSION

In conclusion, this study has aimed to evaluate the antibacterial efficacy against *H. pylori*, the causative agent of stomach ulcers, with a specific focus on substantiating the traditional use of *Staphylina* in addressing stomach disorders. The ethanolic extracts from matured stem extract demonstrated significant antibacterial activity, as indicated by substantial zones of inhibition against *H. pylori*. Moreover, these extracts exhibited heightened levels of secondary metabolites, including a crucial bioactive compound isolated from the *I. Staphylina* plant extract. The identified secondary metabolites possess the capacity to disrupt the pathogen's membrane, leading to the apoptosis of *H. pylori*. These findings emphasize the potential therapeutic application of *I. Staphylina* in managing stomach ulcers induced by *H. pylori.* Additionally, the research contributes scientific validation for the traditional use of *I. Staphylina* in Dharmapuri for the management of stomach ulcers, bridging traditional knowledge with empirical evidence.

ABBREVATIONS

HPTLC, high-performance thin layer chromatography; UV, ultraviolet; TLC, thin layer chromatography; MS, fresh matured stem water extract; SL, fresh stem and leaf water extract; SLS, fresh stem and leaf pet-ether extract; MSS, fresh matured stem pet-ether extract; EMS, fresh matured stem ethanol extract; ESL, fresh stem and leaf ethanol extract; HLS, fresh stem and leaf hydroalcoholic extract; HMS, fresh matured stem hydroalcoholic extract; DMP, dried matured stem pet-ether extract; DSP, dried stem and leaf pet-ether extract; DSE, dried stem and leaf ethanol extract; DME, dried matured stem ethanol extract; DSH, dried stem and leaf hydroalcoholic extract; DMH, dried matured stem hydroalcoholic extract.

COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHORS'CONTRIBUTIONS

Lakshmanan Narayanan: conceptualization, investigation, formal analysis, methodology, data curation, writing-original draft, writing.
Suseem S R: formal analysis, investigation, writing-review & editing. All authors approved the final version of the manuscript after they had evaluated the findings.

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