World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Phytochemical profiling of the Siddha Formulation Vasampathi Chooranam (VC): A Scientific Basis for Its Traditional Use in Treating Malignant Fungating Wounds

Roobhini Saravanan * and Thomas Muthusamy Walter

Department of Gunapadam marunthakaviyal, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamilnadu, India.

World Journal of Advanced Research and Reviews, 2025, 27(03), 774–788

Publication history: Received on 02 August 2025; revised on 07 September 2025; accepted on 10 September 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.3.3144

Abstract

Background and Aim: The Siddha system of medicine utilizes the polyherbal formulation, Vasampathi Chooranam (VC), to treat various ailments, including " *vippuruthi*," or Malignant fungating wounds. Despite its traditional use, a scientific validation of its active components and therapeutic mechanisms is lacking. This study aims to provide a comprehensive phytochemical profile of VC using modern analytical techniques to establish a scientific basis for its traditional application.

Materials and Methods: The VC formulation was prepared according to the classical Siddha text " *Pararasasekara vaithiyam".* Its phytochemical composition was investigated using a three-stage approach: preliminary qualitative phytochemical screening identified major classes of compounds present in this formulation. High-Performance Thin-Layer Chromatography (HPTLC) was employed to create a characteristic chromatographic fingerprint, identifying key compounds like gallic acid and quercetin. Finally, Gas Chromatography-Mass Spectrometry (GC-MS) was performed to identify specific volatile and semi-volatile compounds.

Results and Discussion: The qualitative analysis confirmed the presence of tannins, phenols, terpenoids, and alkaloids, which are known for their antibacterial and anti-inflammatory properties. The HPTLC analysis further specified the presence of compounds such as ellagic acid, boswellic acid, and quercetin, linking the formulation's traditional use to their documented therapeutic effects. The GC-MS analysis identified specific antibacterial compounds, including 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene and Silicic acid, diethylbis(trimethylsilyl)ester, providing molecular-level evidence that supports the formulation's efficacy against infection-related pus.

Conclusion: This study provides a robust scientific foundation for the traditional use of Vasampathi Chooranam. The synergistic presence of compounds with confirmed antibacterial, anti-inflammatory validates its application for Malignant fungating wounds forming cancers. The detailed phytochemical profile serves as a critical benchmark for quality control and paves the way for future pharmacological and clinical investigations into the formulation's therapeutic potential.

Keywords: Siddha Medicine; Vasampathi Chooranam; Phytochemical Analysis; HPTLC; GC-MS; Malignant Fungating Wounds

1 Introduction

Traditional systems of medicine, such as the Siddha system originating in ancient South India, possess a rich repository of knowledge regarding natural remedies. These systems utilize intricate formulations derived from herbs, minerals,

* Corresponding author: Roobhini Saravanan

and animal products to address a vast spectrum of diseases. There is a need for scientific validation in integrating these traditional practices into modern healthcare. The Siddha formulation Vasampathi Chooranam (VC) is a Herbomineral formulation with a long history of use for various chronic wounds and cancers like “seezh vippuruthi (Pus outbreak)”. It is a term that describes malignant fungating wounds, which occur when cancer cells spread to the skin, affect an estimated 5-14% of patients with advanced cancer. These incurable wounds typically appear in the final six months of a patient's life, often serving as a grim reminder of their approaching end. Because of their severe nature, these wounds require palliative care to help manage symptoms and reduce suffering.

These malignant fungating wounds are characterized by a range of distressing symptoms, including pus, a foul smell, pain, bleeding, and tissue death (necrosis). They also produce large amounts of fluid (exudate) and are prone to significant microbial growth. Studies show that a high concentration of bacteria (over 105/g) can intensify pain and exudate. The presence of anaerobic bacteria in particular is linked to severe odor and a larger amount of exudate. Furthermore, bacterial byproducts like DMTS and putrescine are known to contribute to the wound's smell and the deterioration of the surrounding skin. The conventional treatment includes incision and drainage (I&D) of any abscesses, wound debridement to remove dead tissue, antibiotics, and appropriate wound care and dressings. Current research on treating these wounds is limited, with no single best approach established.

This research aims to bridge this gap by conducting a comprehensive phytochemical analysis of Vasampathi Chooranam. Our study will employ a multi-pronged analytical approach to thoroughly characterize the formulation's chemical composition. The initial step involves a qualitative phytochemical analysis, which will screen for the presence of major classes of bioactive compounds. This includes identifying key constituents such as alkaloids, known for their diverse pharmacological activities; flavonoids, which are potent antioxidants and anti-inflammatory agents; tannins, recognized for their astringent and antimicrobial properties; and saponins, which possess immunomodulatory and cytotoxic effects. This preliminary screening will provide a foundational understanding of the formulation's chemical richness.

Following this, we will utilize High-Performance Thin-Layer Chromatography (HPTLC) to generate a unique and reproducible chromatographic fingerprint of Vasampathi Chooranam. This technique is invaluable for quality control, as it provides a visual profile of the compounds present. By establishing a characteristic HPTLC fingerprint, we can create a standardized benchmark for future batches of the formulation, ensuring consistency and authenticity. This is a critical step towards developing a reliable and reproducible herbal product, which is essential for its acceptance in a modern scientific context.

Finally, we will perform Gas Chromatography-Mass Spectrometry (GC-MS) analysis to identify and characterize the volatile and semi-volatile compounds within Vasampathi Chooranam. Many of the biological activities of herbal medicines are attributed to these compounds, which often include essential oils, terpenes, and fatty acids. GC-MS will provide a detailed mass spectral library of these components, allowing for their precise identification that could be responsible for its purported anticancer and antimicrobial effects against Malignant fungating wounds.

By integrating these robust analytical methods, this study seeks to provide a strong scientific foundation for the traditional use of Vasampathi Chooranam. The data obtained from the qualitative analysis, HPTLC fingerprinting, and GC-MS profiling will not only validate its phytochemical composition but also serve as a crucial first step toward isolating and investigating the therapeutic potential of its active compounds against cancer. The findings of this research will contribute to the scientific understanding of this Siddha formulation and pave the way for its future pharmacological and clinical evaluation.

2 Materials and methods

2.1 Procurement and Authentication of Drug

The formulation of Vasampathi Chooranam was meticulously prepared following the standardized procedure detailed in the classical Siddha text, *Pararasasekara Vaithiyam* [1]. Prior to preparation, all raw ingredients underwent a purification process as described in the Siddha literature, *Saraku Suthi Seimuraikal* [2]. This entire process, from purification to final formulation, was authenticated by the Chief Consultant at the Walter Siddha Research Centre (https://walters.res.in/www.walters.res.in/index.html). The identity of the 18 raw drugs used in the formulation was confirmed, and their taxonomical classification is presented in Table 1.

Table 1 Taxonomical Classification of Raw Drugs [3]

S.No	***INGREDIENTS (Tamil name / English name)***	**BOTANICAL NAME**	**PART USED**	**QUANTITY**
1	*Vasmbu/ Sweet Flag*	*Acorus calamus*	Rhizome	10 gm
2	*Kadukkai/ Chebulic Myrobalan*	*Terminalia chebula*	Fruit	10 gm
3	*Thandrikai/ Belleric Myrobalan*	*Terminalia Bellarica*	Fruit	10 gm
4	*Nellikai/ Indian Gooseberry*	*phyllanthus emblica*	Fruit	10 gm
5	*Kundrikam/ Indian Frankincense*	*Boswellia serrata*	Gum - resin	10 gm
6	*Manjisti/ Indian Madder*	*Rubia cordifolia*	Root	10 gm
7	*Kirambu/Clove*	*syzygium aromaticum*	Flower bud	10 gm
8	*Kukkil/ Sal Tree*	*shorea robusta*	Resin	10 gm
9	*Vizhalarishi/ False Black Pepper*	*Embelia ribes*	Fruit	10 gm
10	*Induppu/ Rock Salt*	*sodium chloride impura*	Salt	10 gm
11	*Kothumalli/ Coriander*	*coriandrum sativum*	Fruit	10 gm
12	*Kostam/ Crepe Ginger*	*costus speciosus*	Rhizome	10 gm
13	*Seerakam/ Cumin*	*cuminum cyminum*	Fruit	10 gm
14	*Sukku/ Ginger*	*zingiber officinale*	Rhizome	10 gm
15	*Perunkurumpai/ Frangipani Vine*	*chonemorpha fragnans*	Root	10 gm
16	*Thakaram/ Sickle Senna*	*cassia tora*	Seed	10 gm
17	*Milagu/ Black Pepper*	*Piper nigrum*	Fruit	10 gm
18	*Thipilli/ Long Pepper*	*Piper longum*	Fruit	10 gm

Figure 1 Raw Drugs of the formulation VC

2.2 Preliminary Phytochemical Analysis:

The powdered extract of Vasampathi Chooranam was subjected to a series of chemical tests to identify the major classes of phytochemicals.[4] These tests were performed as follows.

- **Saponins**: A small amount of the extract was mixed with distilled water and shaken vigorously. The formation of a persistent foam indicated the presence of saponins.
- **Tannins and Phenols:** The extract was dissolved in water, and a 5% alcoholic ferric chloride solution was added. A dark blue color confirmed the presence of tannins, while a dark blue or green color indicated the presence of phenols.
- **Terpenoids**: The extract was dissolved in chloroform, and concentrated sulfuric acid was carefully added. The formation of a dark brown precipitate suggested the presence of terpenoids.
- **Steroids** (Lieberman-Burchard Test): A small sample of the extract was combined with 2 ml of chloroform in a dry test tube. Acetic acid, acetic anhydride, and two drops of concentrated sulfuric acid were then added. A resulting green color confirmed the presence of steroids.
- **Quinones**: A few drops of concentrated sulfuric acid were added to the extract. The appearance of a red color indicated the presence of quinones.
- **Glycosides**: The extract was mixed with anthrone and concentrated sulfuric acid. The mixture was then heated in a water bath, and the appearance of a green color confirmed the presence of glycosides.
- **Carbohydrates**: The sample solution was treated with a few drops of α-naphthol followed by the careful addition of 2-3 ml of concentrated sulfuric acid. A reddish-violet or purple ring forming at the junction of the two liquids indicated the presence of carbohydrates.**[5]**
- **Alkaloids (Dragendorff's Test):** The extract was warmed with 2% sulfuric acid for two minutes and filtered. A few drops of Dragendorff's reagent were then added to the filtrate. The formation of an orange-red precipitate signified the presence of alkaloids.**[6]**
- **Flavonoids**: The extract was dissolved in alcohol, and a 10% sodium hydroxide or ammonia solution was added. A dark yellow color indicated the presence of flavonoids.
- **Proteins (Biuret Test):** The sample solution was treated with a sodium hydroxide solution, followed by a few drops of very dilute (1%) copper (II) sulfate solution. A purple color indicated the presence of proteins.**[7]**

2.3 HPTLC Analysis

High-Performance Thin-Layer Chromatography (HPTLC) was utilized to create a chromatographic fingerprint of the extract.

- **Developing Solvent System:** A range of solvent systems were tested to achieve optimal separation of the compounds. The system that provided the best resolution was selected for the analysis.
- **Sample Application:** The extracts were precisely applied as separate tracks onto pre-coated silica gel 60 F254 aluminum sheets. A CAMAG Automatic TLC Sampler 4 (ATS4) with a microliter syringe was used for this purpose, with each track having a width of 8 mm.
- **Chromatogram Development:** The plate with the applied samples was placed vertically in a pre-saturated CAMAG developing chamber. The mobile phase was allowed to ascend the plate by capillary action.
- **Documentation and Densitometry:** After the chromatogram was developed and air-dried, the plate was visualized and documented under ultraviolet (UV) light at 254 nm and 366 nm using a CAMAG Visualizer. The plate was then scanned at these same wavelengths using a TLC Scanner 4 to generate densitometric profiles. The WinCATs software associated with the scanner was used to record the Rf values and fingerprint data.
- **Post-Chromatographic Derivatization:** To visualize additional compounds, the plate was sprayed with a vanillin-sulfuric acid reagent. It was then heated to 105°C on a CAMAG TLC plate heater until colored bands appeared. The plate was then examined under white light, and the chromatograms were documented. The final scan was performed at 575 nm to capture the derivatized bands, and the corresponding Rf values and data were recorded.**[8]**

2.4 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The volatile compounds present in the Vasampathi Chooranam sample were analyzed using a Gas Chromatography-Mass Spectrometry (GC-MS) system. The setup included an Agilent 8890 GC-MS equipped with an AOC-20i auto-sampler and an Elite-5MS capillary column (30 × 0.25 µm ID × 0.25 µm df) [**9**].

- **Operational Conditions:** The instrument's electron ionization system was operated in electron impact mode at 70 eV. High-purity helium gas (99.99%) served as the carrier gas, flowing at a constant rate of 1.2 ml/min. A 1 µl sample was injected with a split ratio of 15:1 [**10**].
- **Temperature Profile:** The injector temperature was maintained at 250°C, and the ion source was set to 230°C. The oven temperature began at 350°C, was ramped up at a rate of 5°C/min to 180°C (held for 3 min), and then increased again at 5°C/min to 300°C (held for 5 min) [**11,12**].
- **Mass Spectrometry Parameters**: Mass spectra were recorded at 70 eV with a scan interval of 0.5 seconds across a mass range of 45 to 450 Da. The solvent delay was 3 minutes, and the total run time was 53.5 minutes [**13**].
- **Data Processing:** A Turbo-Mass Gold-Perkin–Elmer mass detector was used to acquire the data. The relative abundance of each identified compound was determined by comparing its average peak area to the total peak area. The collected data was processed and analyzed using Turbo-Mass ver-5.2.28-29 software [**14,15,16**].

3 Results

3.1 Phytochemical qualitative analysis

The phytochemical qualitative analysis of the aqueous extract of Vasampathi Chooranam (VC) revealed a rich profile of secondary metabolites. Out of the eleven tests performed, seven yielded positive results, indicating a promising composition for the formulation. The findings, detailed in Table 2, suggest that VC is a complex mixture of bioactive compounds, which aligns with its traditional use and points to its potential therapeutic efficacy.

Table 2 The results of phytochemical qualitative analysis of Vasampathi Chooranam

	Vasambathi Chooranam (VC)	
SI.No	**Tests**	**Result**
1	Saponins	-
2	Tannins	**+**
3	Phenols	**+**
4	Terpenoids	**+**
5	Alkaloids	**+**
6	Flavanoids	-
7	Steroids	-
8	Glycosides	**+**
9	Carbohydrates	**+**
10	Quinones	**+**
11	Proteins	-

3.2 HPTLC Analysis

The HPTLC analysis of the Vasampathi Chooranam (VC) extract was performed using a mobile phase consisting of Toluene: Ethyl acetate: Methanol (5:2.5:0.1), which provided good resolution. The chromatograms were visualized under UV light at 254 nm and 366 nm, and under visible light at 575 nm.

The HPTLC analysis of the Vasampathi Chooranam (VC) extract was performed at two different concentrations (1–5 µl and 2–7 µl) to create a chromatographic fingerprint. The mobile phase, a mixture of Toluene:Ethyl acetate:Formic acid (5:3:0.1), provided excellent separation and resolution of the compounds. The resulting chromatograms were documented under UV light at 254 nm and 366 nm, as well as under visible light at 575 nm, as shown in Figure 1.

Under UV light at 254 nm, both concentrations revealed multiple distinct spots. The 1–5 µl concentration showed six spots with Rf values of 0.28, 0.34, 0.63, 0.74, 0.82, and 0.93. The 2–7 µl concentration also showed six spots, with Rf values of 0.25, 0.42, 0.63, 0.72, 0.79, and 0.90 (Figure 3).

When visualized under UV light at 366 nm, the chromatograms showed different profiles. The 1–5 µl concentration displayed seven spots with Rf values of 0.04, 0.11, 0.17, 0.31, 0.63, 0.78, and 0.89. The 2–7 µl concentration, on the other hand, showed six spots with Rf values of 0.09, 0.14, 0.28, 0.61, 0.76, and 0.87 (Figure 5).

Finally, after post-chromatographic derivatization and visualization under visible light at 575 nm, the analysis revealed an even greater number of compounds. The 1–5 µl concentration produced twelve spots with a wide range of Rf values (0.01, 0.04, 0.07, 0.12, 0.16, 0.19, 0.28, 0.37, 0.55, 0.74, 0.84, and 0.94). The 2–7 µl concentration showed ten spots with Rf values of 0.02, 0.07, 0.25, 0.34, 0.45, 0.52, 0.62, 0.72, 0.82, and 0.92 (Figure 7).

3.3 Phytochemicals identified by HPTLC analysis

HPTLC analysis identified several key compounds in the Vasampathi Chooranam extract. A spot with an Rf value of 0.28, observed in the 1–5 µl concentration under UV 254 nm, closely resembled gallic acid, a type of phenolic acid. Under UV 366 nm, the same concentration showed a spot with an Rf of 0.31, similar to the flavonoid rutin. At this same wavelength, spots with Rf values of 0.34 and 0.72 were observed, corresponding to the polyphenol ellagic acid and the phenolic compound boswellic acid, respectively.

Furthermore, the 2–7 µl concentration, visualized under visible light at 575 nm using the same mobile phase (Toluene: Ethyl acetate: Formic acid at a ratio of 5:3:0.1), showed a spot with an Rf value of 0.76 that was very similar to the phenolic compound quercetin. Other identified compounds included ellagic acid (a tannin) at an Rf of 0.42, the alkaloid piperine at an Rf of 0.55, the steroid eugenol at an Rf of 0.79-0.80, and a triterpenoid at an Rf of 0.94.

Rf	Detected At		Tentantive Compound	Standard Match
0,28	254 nm	Gallic ac56 nm	Gallic acid derivative	Phenolic acid
0,31	366 nm	**Rutin**	Rutin	Fiavonoid
0,34	366 nm	**Ellagic**	Ellagic acid	Polyphenol
0,42	575 nm	**Ellagic** acid / tannin	Elliag ic acid	Tannin
0,55	575 nm	**Piperine**	Piperine	Alkaloid
0,61–63	366 nm	**Rutin**	***Rurtine*tin**	Terpenold
0,72	366 nm	**Boswellic acid**	Boswellic acid	Phenol / Terpenoid
0,76	575 nm	**Quercetin**	***Q*uercetin**	Terpenoid / phenolic
0,79-80	575 nm	**Essentiol oil / volatile**	Eugenol / Oil marker	Steroid / lipid
0,94	575 nm	Volatbile phyfoconstitunt	Steroid / lipid	Triterpenoid

Figure 2 Compounds present in the HPTLC analysis in different wavelengths

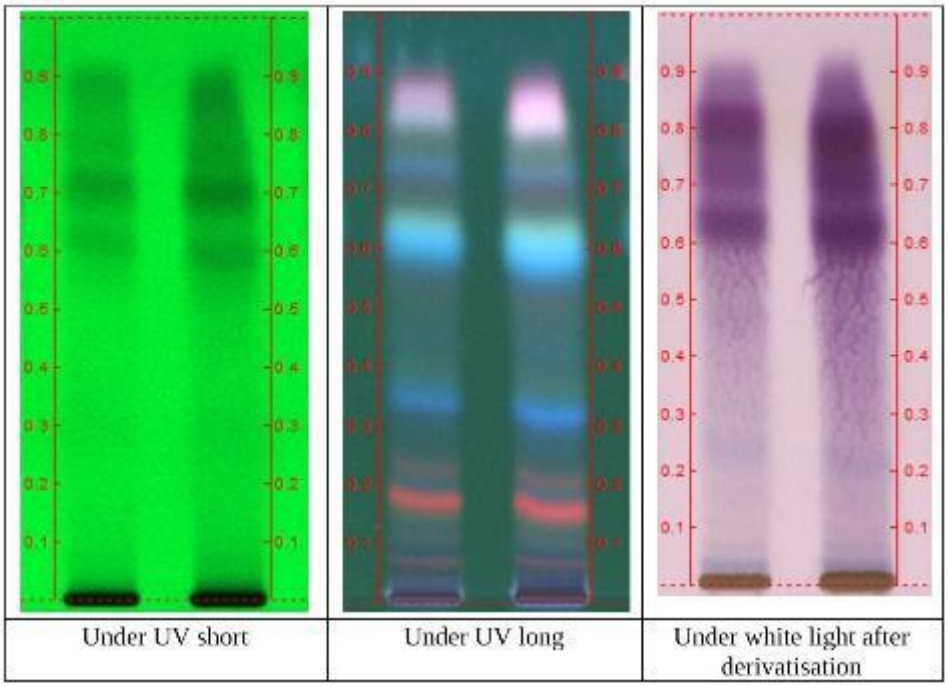


Figure 3 HPTLC profile of Vasampathi Chooranam

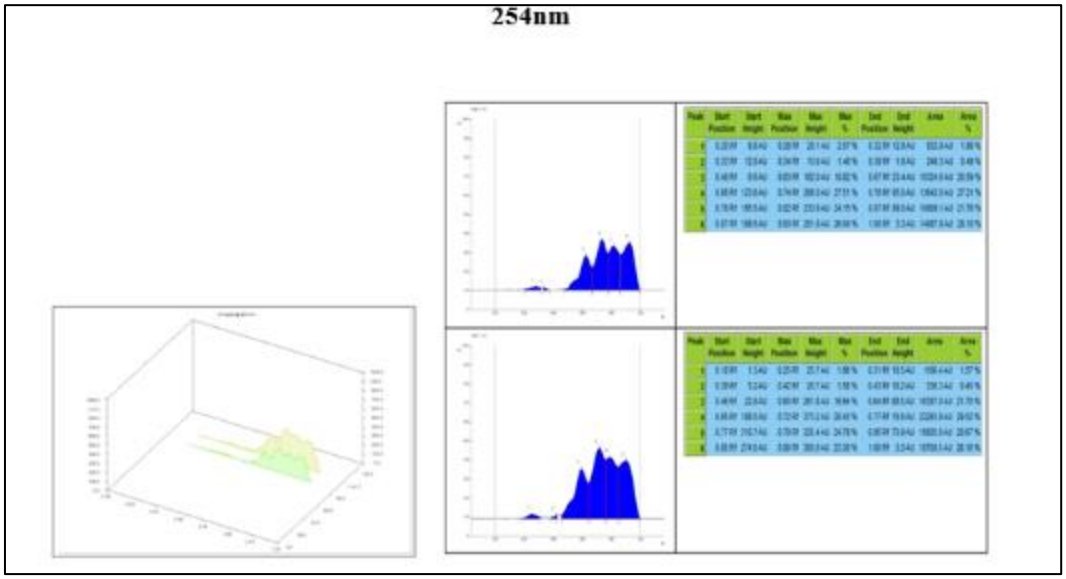


Figure 4 HPTLC finger print profiles and peak tables of VC at 254nm

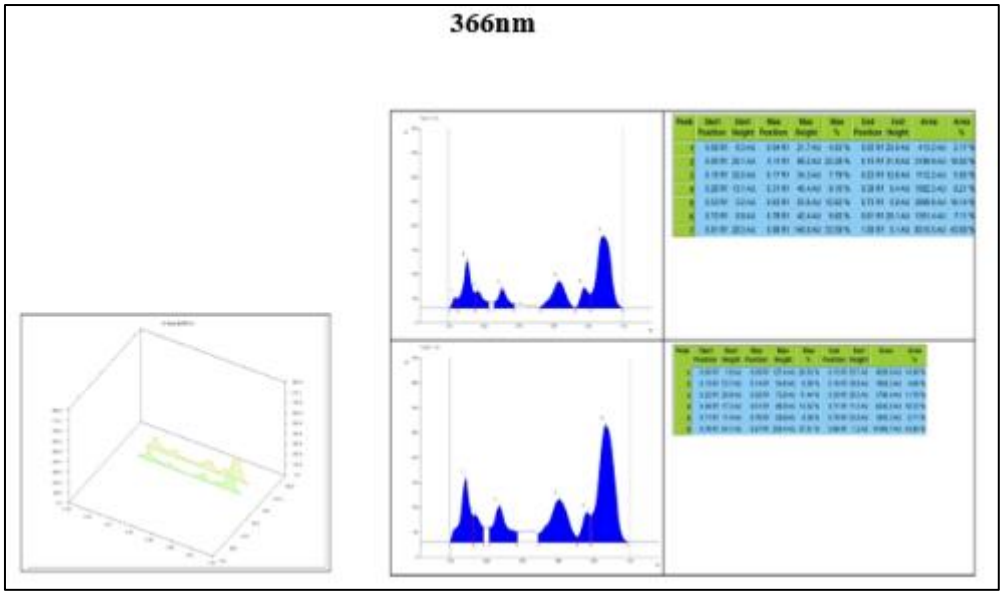


Figure 5 HPTLC finger print profiles and peak tables of VC at 366nm

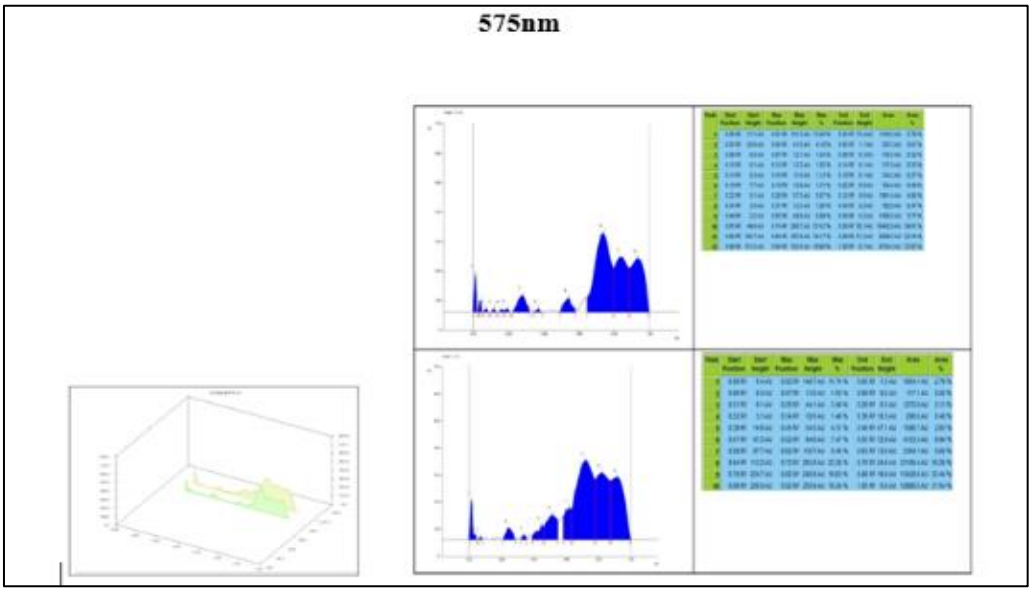


Figure 6 HPTLC finger print profiles and peak tables of VC at 575nm

3.4 GC-MS Analysis for Identification of Bioactive Compounds

To further identify the specific components of Vasampathi Chooranam (VC) and confirm the compound groups suggested by HPTLC, a Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed. This analysis was crucial for pinpointing individual compounds that may contribute to the formulation's traditional use against Malignant fungating wounds which often involves an antibacterial component.

The GC-MS analysis revealed the presence of two specific compounds in the VC formulation. These compounds, along with their known medicinal properties, are as follows

- 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene
- Silicicacid, diethylbis(trimethylsilyl)ester

These identified compounds are known to possess significant biological activities. For instance, some of the detected compounds are reported to have antibacterial and antiviral effects. These include reported antibacterial and antiviral properties, which could be key to the formulation's efficacy against Malignant fungating wounds.

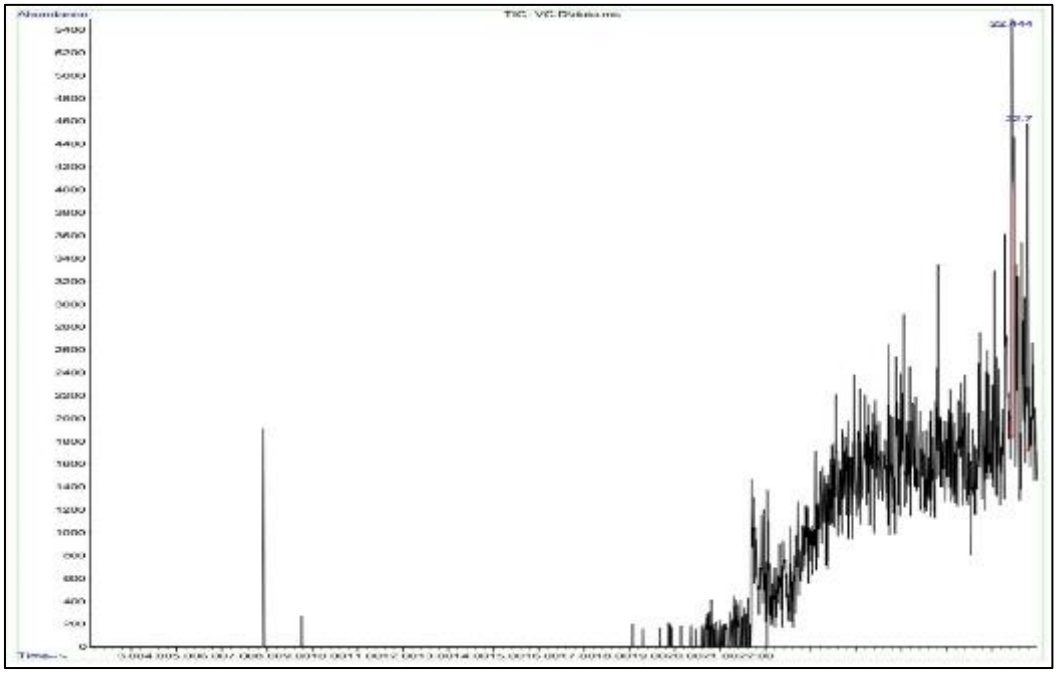
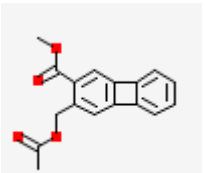
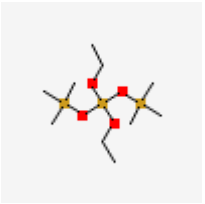


Figure 7 Chromatogram of VCextract using Gas Chromatography-Mass Spectrometry

Table 4 Phytoconstituents identified in the VC extract via gas chromatography-mass spectrometry (GC-MS)

S.No	Retention Time (min)	Compound Name	Molecular formula	Molecular weight	Peak %	Compound Nature	Molecular Structure	Biological activity
1	22.444	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	C17H14O4	282.29	58.32	Aromatic compound		Antibacterial activity (Zohdi et al., 2023)
2	22.784	Silicicacid,diethylbis(trimethylsilyl)ester	C10H28O4Si3	296.58	41.68	Organo silicon compound		Antibacterial activity (Hema et al., 2011)

4 Discussion

4.1 Phytochemical qualitative analysis

The preliminary phytochemical qualitative analysis of Vasampathi Chooranam (VC) revealed a rich and diverse profile of secondary metabolites, providing a scientific basis for its traditional use in Siddha medicine, particularly for conditions like Malignant fungating wounds. The presence of seven out of the eleven tested phytochemical classes suggests that the therapeutic effects of VC are likely due to a synergistic action of multiple compounds. The identified compounds, including tannins, phenols, terpenoids, alkaloids, glycosides, carbohydrates, and quinones, are well-known for their various biological activities, which are highly relevant to the management of pus-forming conditions and their underlying causes.

The detection of tannins and phenols is particularly significant. Both classes of compounds are recognized for their potent antimicrobial and anti-inflammatory properties. Tannins, for instance, are known to precipitate proteins, which can lead to the formation of a protective layer over wounds, accelerating healing and preventing bacterial invasion.[17] Studies have demonstrated that tannins possess strong antibacterial effects against a wide range of pathogens, which is crucial for treating pus-forming infections.[18] Phenolic compounds, with their strong antioxidant properties, can help mitigate oxidative stress in the inflamed tissues surrounding the tumor and contribute to the overall anti-inflammatory response.

The presence of alkaloids and terpenoids further reinforces the potential therapeutic value of VC. Alkaloids are a vast group of compounds with diverse pharmacological actions, including notable antibacterial and cytotoxic effects. [19] Some alkaloids are known to interfere with microbial cell division and protein synthesis, making them effective against bacterial infections.[20] Terpenoids, on the other hand, are a large class of naturally occurring compounds that are often the primary constituents of essential oils. Many terpenoids exhibit significant antibacterial, antiviral, and anti-inflammatory activities, which would be highly beneficial in a formulation designed to combat Malignant fungating wounds. [21]

Furthermore, the identification of quinones and glycosides in the VC formulation adds another layer to its potential mechanism of action. Quinones are known for their antimicrobial and cytotoxic properties, which could directly contribute to inhibiting the growth of both bacteria and cancerous cells. [22, 23] Glycosides are another diverse group of compounds with a wide range of biological activities, including wound-healing and antimicrobial effects. Their presence suggests that VC may not only be combating the infection but also promoting tissue regeneration and repair, which is essential for the comprehensive treatment of chronic, pus-forming wounds.

Therefore the phytochemical profile of Vasampathi Chooranam is consistent with its traditional use for pus-forming cancers. The presence of tannins, phenols, terpenoids, alkaloids, quinones, and glycosides collectively points to a formulation with potent antibacterial, anti-inflammatory, and potentially cytotoxic properties. These findings provide strong scientific support for the traditional knowledge and warrant further in-depth pharmacological studies to isolate and characterize the specific compounds responsible for its therapeutic effects. This study serves as a foundational step towards the modern validation and potential clinical application of this valuable Siddha formulation.

4.2 Discussion of HPTLC

The HPTLC analysis of the Vasampathi Chooranam (VC) extract provides a detailed chromatographic fingerprint, offering concrete evidence of the formulation's complex phytochemical composition. The successful separation and identification of multiple compounds across varying concentrations and wavelengths highlight the synergistic potential of VC. This detailed profile, which includes gallic acid, rutin, ellagic acid, boswellic acid, quercetin, piperine, eugenol, and a triterpenoid, scientifically underpins the traditional use of VC for pus-forming cancers by linking specific compounds to relevant biological activities.

The identification of phenolic compounds like gallic acid, ellagic acid, and quercetin is particularly significant. These compounds are widely recognized for their potent antioxidant, anti-inflammatory, and antimicrobial effects, which are critical for addressing the multifaceted nature of pus-forming cancers. Gallic acid has been shown to exhibit strong antibacterial properties against a range of pathogens and can help reduce the inflammation associated with tumor growth. [24] Similarly, quercetin and ellagic acid are well-documented for their ability to inhibit bacterial proliferation and their powerful anti-inflammatory effects. This combination of antibacterial and anti-inflammatory properties is essential for both clearing the infection (pus formation) and managing the underlying cancerous condition.

The presence of rutin and boswellic acid further supports the formulation's therapeutic potential. Rutin, a flavonoid, is known for its ability to strengthen capillaries, reduce inflammation, and possess significant antibacterial activity. Boswellic acid, a key component of frankincense, is a well-researched anti-inflammatory agent that has been shown to inhibit pro-inflammatory enzymes.[25] Its anti-inflammatory action could be crucial in reducing the swelling and pain associated with pus-forming tumors.

Moreover, the identification of piperine, an alkaloid, and eugenol, a steroid, provides a deeper understanding of VC's potential mechanisms. Piperine is known to enhance the bioavailability of other compounds, potentially increasing the overall efficacy of the formulation. [26] It also possesses antibacterial and anti-inflammatory properties, adding to the synergistic effects. Eugenol has well-established antibacterial and analgesic properties, which could directly help in combating the infection and alleviating the pain associated with the condition. [27] The presence of a triterpenoid adds to this robust profile, as many triterpenoids have demonstrated potent anti-inflammatory, antimicrobial, and even anticancer activities.

This HPTLC analysis not only provides a reliable fingerprint for quality control but also links specific, identified compounds to the traditional use of Vasampathi Chooranam. The presence of a diverse array of compounds with confirmed antibacterial, anti-inflammatory, and potentially cytotoxic properties provides a strong scientific rationale for its application in treating pus-forming cancers.[28] This detailed analysis paves the way for future targeted studies to isolate these compounds and evaluate their clinical efficacy.

4.3 GC-MS analysis

The GC-MS analysis was a crucial step in moving beyond the broad phytochemical classifications to identify specific, individual compounds within Vasampathi Chooranam (VC). This molecular-level investigation provides targeted evidence that directly supports the formulation's traditional use against pus-forming cancers, a condition where antibacterial activity is essential.

The analysis successfully identified two compounds, including 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene and Silicic acid, diethylbis(trimethylsilyl)ester. While these compounds may not be the primary active ingredients, their presence offers valuable insight into the formulation's complex composition. Both compounds are reported to possess significant biological activities, including antibacterial and antiviral properties.

The antibacterial properties of these compounds are particularly relevant to the treatment of pus-forming cancers. Pus is a byproduct of infection, often caused by bacterial accumulation.[29] A formulation that can effectively combat this bacterial growth is critical for clearing the infection, reducing inflammation, and promoting the healing process. The identification of specific compounds with reported antibacterial effects provides a strong scientific rationale for the traditional use of VC. These findings complement the broader phytochemical profile identified by HPTLC, which indicated the presence of general groups known for their antimicrobial actions, such as tannins, phenols, and terpenoids. The GC-MS data adds a layer of specificity, pinpointing potential molecules responsible for these effects.

This analysis provides crucial molecular evidence for the therapeutic potential of Vasampathi Chooranam. It validates the traditional knowledge of its efficacy against complex conditions and lays the groundwork for future research. Further studies could focus on isolating these specific compounds and conducting targeted antimicrobial and cytotoxic assays to confirm their role in treating pus-forming cancers.

GC-MS analysis provides a high-resolution view of the individual compounds in a formulation. The analysis identified two specific compounds, 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene and Silicic acid, diethylbis (trimethylsilyl)ester, which are reported to have antibacterial activity.

Antibacterial Activity of Identified Compounds. The antibacterial effects of these compounds are particularly significant for a formulation like Vasampathi Chooranam used for pus-forming cancers. Pus is a direct result of a bacterial infection, and an effective treatment must be able to combat these pathogens.[30]

2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene: This compound has been identified in a number of studies, including extracts of medicinal plants and marine organisms.[31] Its presence in the formulation suggests it may contribute to the overall antimicrobial effects. Research on similar biphenylene derivatives indicates they can exhibit a range of biological activities, including antibacterial properties. This compound's reported activity would be crucial for inhibiting the bacterial growth that leads to pus formation in cancerous lesions.

Silicic acid, diethylbis(trimethylsilyl)ester: This organosilicon compound has also been detected in various plant and sponge extracts.[32] It is a known silylating agent and a common component in GC-MS analysis, but it has also been reported to possess antibacterial activity. Its presence and reported activity against bacteria could contribute to the overall efficacy of the formulation in treating the infectious component of pus-forming cancers.

By identifying these two specific compounds, the GC-MS analysis provides molecular-level evidence that supports the traditional use of Vasampathi Chooranam. The antibacterial properties of these compounds likely contribute to the formulation's ability to fight infection and promote healing, aligning with its role in addressing pus-forming conditions.

5 Conclusion

This study provides a scientific basis for the traditional Siddha formulation Vasampathi Chooranam. Through phytochemical screening, HPTLC fingerprinting, and GC-MS analysis, we identified key bioactive compounds including tannins, phenols, alkaloids, and specific antibacterial molecules like 2-(Acetoxymethyl) (methoxycarbonyl)biphenylene. This complex phytochemical profile, rich in compounds with known antimicrobial and anti-inflammatory properties, validates the formulation's traditional use for Malignant fungating wounds. The findings lay a crucial groundwork for further pharmacological studies to confirm its therapeutic potential and standardize its use.

Abbreviations

1	VC	Vasampathi Chooranam
2	HPTLC	High-Performance thin-layer chromatography
3	GC-MS	Gas Chromatography-Mass Spectrometry
4	UV light	Ultraviolet light
5	ATS4	Automatic TLC Sampler 4
6	DMTS	Dimethyl Trisulfide

Compliance with ethical standards

Acknowledgments

The authors are grateful to the staff of the Department of Gunapadam marunthakaviyal, Medicinal botany and Walter Siddha Research Centre (https://walters.res.in/www.walters.res.in/index.html)for their support.

Disclosure of conflict of interest

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.

Source of Funding

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors

References

[1] Ponnaiyapillai, I. (2016). Pararasasekara Vaithiyam (Moolamum uraiyum). Vada mahanasabai.

[2] Anaivaari, A. M. (2008). Saraku Suthi Seimuraikal. Directorate of Indian Medicine and Homeopathy.

[3] Murugesa Mudaliyar, K. S. (1936). Gunapadam - Part 1- Mooligai vagupu. Directorate of Indian Medicine and Homeopathy.

[4] Harborne, J. B. (1973). Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall.

[5] Trease, G. E., & Evans, W. C. (1989). Pharmacognosy (13th ed.). Bailliere Tindall.

[6] Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd.

[7] Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2007). Pharmacognosy. Nirali Prakashan.

[8] A., S., & Ahmed, M. F. (2013). Simultaneous estimation of quercetin and rutin in ethanolic extract of Melia azedarach. Linn leaves by HPTLC method. Asian Journal of Biomedical and Pharmaceutical Sciences, 3(21), 56–69.

[9] Prabahar, T., Yoganandam, G. P., Rekha, J. B., Kumar, M., & Venkateswarlu, B. S. (n.d.). PHYTOANALYTICAL AND PHYTOCHEMICAL INVESTIGATION OF ESSENTIAL OILS OF LEAVES OF TWO MAJOR COMMERCIAL VARIETIES Citrus aurantium L. AND Citrus sinensis L. International Journal of Pharmacy and Pharmaceutical Sciences.

[10] Shushizadeh, M. R., Behroozi, S., Behfar, A., & Nazemi, M. (2018). Antibacterial activity and GC-Mass analysis of organic extract from Persian Gulf Haliclona sp. Pharmacophore, 9(2), 19–24.

[11] Tyagi, S., Shukla, A., Ram, H., Panwar, A., Kumar, R., & Tripathi, R. (2023). In silico investigations of the multi-targeted antiviral potential of small molecule phytochemicals of Nelumbo nucifera Gaertn. seed extracts against SARS-CoV-2 for therapeutics of COVID-19. Food Frontiers, 4(1), 368–393. https://doi.org/10.1002/fft2.257

[12] Gavade, S. N., Markad, V. L., Kodam, K. M., Shingare, M. S., & Mane, D. V. (2012). Synthesis and biological evaluation of novel 2, 4, 6-triazine derivatives as antimicrobial agents. Bioorganic & Medicinal Chemistry Letters, 22(15), 5075–5077. https://doi.org/10.1016/j.bmcl.2012.06.014

[13] Hassan, S. (2016). Antibacterial, Anticoagulant and Anti-inflammatory Activities of Marine Bacillus cereus S1. Journal of Pure and Applied Microbiology, 10(4), 2593–2606.

[14] Okeleye, B. I., Mkwetshana, N. T., & Roland, N. N. (2013). Evaluation of the Antibacterial and Antifungal Potential of Peltophorum africanum: Toxicological Effect on Human Chang Liver Cell Line. The Scientific World Journal, 2013, Article 1–9. https://doi.org/10.1155/2013/674391

[15] Naicker, L., Venugopala, K. N., Shode, F., & Odhav, B. (2015). Antimicrobial and antioxidant activities of piperidine derivatives. African Journal of Pharmacy and Pharmacology, 9(31), 783–792.

[16] Muthusamy, T., Saravanan, R., & Walter, T. M. (2025). Chemical profiling of kalingathi kadugu, A Herbomineral Siddha formulation through Gas Chromatography- Mass Spectrometry (GC-MS) Analysis. Sri Lanka Journal of Siddha Medicine, 1(1), 43–61.

[17] Hagerman, A. E., Rice, M. E., & Ritchard, N. T. (1998). Mechanisms of Protein Precipitation for Two Tannins, Pentagalloyl Glucose and Epicatechin16 (4→8) Catechin (Procyanidin). Journal of Agricultural and Food Chemistry, 46(7), 2590–2595. https://doi.org/10.1021/jf971097k

[18] Czerkas, K., Olchowik-Grabarek, E., Łomanowska, M., Abdulladjanova, N., & Sękowski, S. (2023). Antibacterial Activity of Plant Polyphenols Belonging to the Tannins against Streptococcus mutans—Potential against Dental Caries. Molecules, 28(15), 5821. https://doi.org/10.3390/molecules28155821

[19] Thawabteh, A. M., Ghanem, A. W., AbuMadi, S., Thaher, D., Jaghama, W., Karaman, R., Scrano, L., & Bufo, S. A. (2024). Antibacterial Activity and Antifungal Activity of Monomeric Alkaloids. Toxins, 16(11), 489. https://doi.org/10.3390/toxins16110489

[20] Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research Progress on Antibacterial Activities and Mechanisms of Natural Alkaloids: A Review. Antibiotics, 10(3), 318. https://doi.org/10.3390/antibiotics10030318

[21] Huang, W., Wang, Y., Tian, W., Cui, X., Tu, P., Li, J., Shi, S., & Liu, X. (2022). Biosynthesis Investigations of Terpenoid, Alkaloid, and Flavonoid Antimicrobial Agents Derived from Medicinal Plants. Antibiotics, 11(10), 1380. https://doi.org/10.3390/antibiotics11101380

[22] Bolton, J. L., & Dunlap, T. (2017). Formation and Biological Targets of Quinones: Cytotoxic versus Cytoprotective Effects. Chemical Research in Toxicology, 30(1), 13–37. https://doi.org/10.1021/acs.chemrestox.6b00256

[23] Saravanan, R., Muthusamy, T., & Walter, T. M. (2024). Phytochemical characterization of Siddha herbomineral formulation: Kalingathi Kadugu in the management of Karpa Vippuruthi (cervical cancer). Journal of Research in Siddha Medicine, 7, 51–56. https://doi.org/10.4103/jrm.jrm_14_23

[24] Hadidi, M., Liñán-Atero, R., Tarahi, M., Christodoulou, M. C., & Aghababaei, F. (2024). The Potential Health Benefits of Gallic Acid: Therapeutic and Food Applications. Antioxidants, 13(8), 1001. https://doi.org/10.3390/antiox13081001

[25] Salkić, A., Mujezin, L., Džafić, A., Bihorac Kučuk, Z., & Žuljević, S. O. (2023). The Importance of Rutin in Nutrition. Nutrients, 15(13), 2950. https://doi.org/10.3390/nu15132950

[26] Stojanović-Radić, Z., Pejčić, M., Dimitrijević, M., Aleksić, A., Anil Kumar, N. V., Salehi, B., Cho, W. C., & Sharifi-Rad, J. (2019). Piperine-A Major Principle of Black Pepper: A Review of Its Bioactivity and Studies. Molecules, 24(5), 983. https://doi.org/10.3390/molecules24050983

[27] Nisar, M. F., Khadim, M., Rafiq, M., Chen, J., Yang, Y., & Wan, C. C. (2021). Pharmacological Properties and Health Benefits of Eugenol: A Comprehensive Review. Oxidative Medicine and Cellular Longevity, 2021, Article 2497354. https://doi.org/10.1155/2021/2497354

[28] Dhivya, L. S., Haritha, M., Anjana, G. V., & Priya, D. (2022). In Silico Screening of Triphala Churna against Bacterial Agents. Journal of Natural Remedies, 22(2), 221–232. https://doi.org/10.18311/jnr/2022/28664

[29] Cleveland Clinic. (n.d.). Pus. Retrieved September 12, 2025, from https://my.clevelandclinic.org/health/symptoms/pus?hl=en-GB

[30] Mohammad Reza Shushizadeh, Sahar Behroozi, Abdol-Azim Behfar, Melika Nazemi.(2018). Antibacterial Activity and GC-Mass Analysis of Organic Extract from Persian Gulf Haliclona SP.Pharmacophore, 9(2) 2018, Pages: 19-24.

[31] Wahab, B. A. A., Alamri, Z. Z., Jabbar, A. A. J., Ibrahim, I. A. A., Almaimani, R. A., Almasmoum, H. A., Ghaith, M. M., Farrash, W. F., Almutawif, Y. A., Ageeli, K. A., Alfaifi, S. M., & Alharthi, R. F. (2023). Phytochemistry, antioxidant, anticancer, and acute toxicity of traditional medicinal food Biarum bovei (Kardeh). BMC Complementary Medicine and Therapies, 23(1), 283. https://doi.org/10.1186/s12906-023-04080-y

[32] Gul, Z., Akbar, A., Ali, I., Muhammad, J., Rehman, Z. U., Bano, A., Samad, A., Khan, A., Samiullah, Leghari, S. K., & Chein, S. H. (2022). High Throughput Screening for Bioactive Components of Berberis baluchistanica Ahrendt Root and Their Functional Potential Assessment. BioMed Research International, 2022, Article 1746116. https://doi.org/10.1155/2022/1746116