ISSN 2063-5346



ANALYTICAL PROFILE OF HERBAL MEDICINE-SEPTILIN, AN ANTI-INFECTIVE TABLET

Mr. Ganesh Nigade¹, Ms. Suneeja Mane¹, Ms. Prajakta Mane¹, Ms. Neha Gambhire¹, Ms. Aditi Patil¹, Mr. Sanket Kunjir¹

Article History: Received: 10.05.2023	Revised: 29.05.2023	Accepted: 09.06.2023

Abstract

Herbal medicine has been used since ancient times for health promotion, prevention, and treatment of various disease conditions. Herbal medicines can affect the immune system in a variety of ways, such as free-radical scavenger activity, cytokine production, conversion of T cells, stimulation of antibodies, and antimicrobial actions. This study investigates the therapeutic efficacy of a herbal medicine-septilin, an anti-infective pill. The main components of Septilin tablets are amla, giloy, guggul, and liquorice. The goal is to discover and characterize possible phytochemicals from Septelin extracts using Ultraviolet-visible, Fourier transform infrared and TLC analysis. Microwave was used to extract septelin tablets by using solvent methanol: water, (70:30). Extract of septelin tablets were evaluated & confirmed the presence of phytochemicals by qualitative phytochemical analysis. The extract of the septelin tablet had 111.15 ± 0.055 milligram GAE/g, 55.20 ± 0.0064 milligram QE/g, and 6.8 ± 0.0015 milligram CE/g of total phenolic, flavonoid, & tannin content, respectively. UV-Vis spectrum of extract of septelin tablet represents the presence of conjugated & aromatic compounds. The existence of the group O-H, aromatic C-H starch, C=C, and C-O groups in the extract from septelin tablets was verified by the Fourier transform infrared spectrum. Thin layer chromatography with just TLC was used to develop a fingerprint for the septilin tablet.

KEYWORDS: Septelin, UV-Vis spectrum, FT-IR, TLC, Phytochemical, JustTLC

¹PDEA's S.G.R.S. College of Pharmacy, Saswad, Pune-412301

DOI:10.48047/ecb/2023.12.9.156

1. INTRODUCTION

Traditional uses of herbal medicine include illness prevention, promotion of good health, and treatment of a variety of ailments¹. Both the lifespan and wellbeing of human have been extended via the use of herbal medicine.² Herbal and alternative medicines have been used since ancient times as primary sources of treatment.³ The World Health Organization estimates that 70% of people worldwide get their healthcare from non-conventional medications, the majority of which come from herbal sources.⁴ There are two types preparations: mono-herbal of herbal preparation, which is prepared from a single herb, and poly-herbal preparation, which is combinations of several different plants.⁵ Nowadays, research focuses on preventing various pathogenic infections by using herbal medicines. A range of infectious diseases are prevented by the immune system.⁶

The treatment of immune-mediated health has always been greatly aided by herbal medicine.^{7,8} In order to strengthen the immunological response, modern medicine has limitation to supports the immune system. Fortunately, herbal medicine can affect the immune system in a variety of ways including antioxidant activity⁹, cytokine production, modulation of the activation and inhibition of T cells, stimulation of antibodies, and antimicrobial actions.¹⁰ Herbal medicines act by altering the humoral and cellular immune function of the immune system.¹¹ Herbal medications modify the intricate immune system in order to avoid infections rather than cure the illnesses.¹²

The therapeutic efficacy of a number of the herbs admired in traditional medicine system has been investigated scientifically, with positive outcomes.¹³ Several phytochemicals with immunomodulatory action have been identified, providing an explanation for and justification of their usage in traditional medicine.¹⁴ Herbal immunomodulation provides an alternative to modern medicine for a number of disorders, particularly in conditions when the host defence mechanism needs to be stimulated despite an impaired immune response.¹⁵ Various phytochemicals extracted from herbs such as alkaloids¹⁶, isoflavonoids, flavones, and anthocyanidins,¹⁷ sapogenins,¹⁸ thiosulfinates,¹⁹ and coumarins²⁰ possess immunomodulatory activity.

Growing interest in the use of herbal medications as immunomodulator leads to focus on its quality. The safety as well as efficacy of herbal medications are directly impacted by their quality control.^{21,22} However, there is no systematic approach to assessing the standard of herbal medicines. Developing an appropriate or accepted research approach for assessing traditional medicines is an important obstacle in the process of scientific assessment.^{23,24}

Here, we explore a herbal medicineseptelin an anti-infective pill. It possesses immunomodulatory, anti-inflammatory, and antioxidant properties that increase the ability to fight off infections and maintain general health. The main components of Septilin tablets are amla, giloy, guggul, and liquorice.

The objective of this study is to perform a preliminary investigation of potential phytochemicals, identify and characterise potential phytochemicals using UV-Vis and FT-IR spectrophotometer, and chromatography (TLC) analysis of the Septelin tablet.

3. Materials and Methodology

3.1. Samples: -

Septilin tablet (Himalaya) was procured from a local pharmacy.

3.2. Equipment: -

UV- Vis spectral data were recorded on Jasco V-630 spectrometer, FTIR spectral data were noted on Shimadzu FTIR 8400S and chromatograph was recorded by Aetron HPTLC system. JustTLC software was used for the scanning of TLC plates.

3.3. Sample Preparation: -

Triturated powder of Septilin tablet (2g) was extracted with 20 mL solvent methanol: water, (70:30) in the microwave at 280 W for 10 minutes. A rotovap was used to concentrate the extract, and the residue has been preserved for later analysis in an airtight container.

3.4. Experimental: -

3.4.1. Organoleptic Evaluation: -

Evaluation of a Septilin tablet was carried using organoleptic criteria, such as colour, odour, taste, and texture.²⁵

3.4.2. Physical parameter evaluation

Physical properties of Septilin tablet was analysed using ayurvedic pharmacopoeial methods.¹⁰ It includes measures of the total ash, acid-insoluble ash, & extractive value, as well as foreign substances.²⁵

3.4.3. Phytochemical Evaluation: -

To find different chemical contents, all of the extract was analysed by preliminary phytochemical screening. Different phytoconstituents were examined for presence or absence using the conventional techniques.¹⁰

3.4.4. Total phenolic content

The Folin Ciocalteu method was used to measure the amount of phenolic compounds in the Septelin tablet extract via a spectrophotometer.^{26,27} Distilled water (2.8 mL) and 1 mL of Septelin tablet extract have been added to a volumetric flask. The volumetric flask was then filled with 0.5 milliliters of a 10x dilution of 2 N the reagent Folin-Ciocalteu in distilled water. After waiting for 3 minutes at 35 °C, 2 milliliters of 20% sodium carbonate was added. The entire mixture was analysed for 765 nm absorbance after being kept in the dark for an hour. The triplicate sample

analyses performed & mean absorbance was calculated. The gallic acid equivalent concentration in the extract of septelin tablet was determined by Gallic acid standard curves. Results were given in milligrams per gramme of gallic acid equivalent.

3.4.5. Total flavonoid content

The Septelin tablet extract was assessed for flavonoid Content by the aluminium method chloride using а spectrophotometer.²⁸ 1 mL of the septelin tablet extract & 100 L of 10% w/v aluminium trichloride were added to the volumetric flask. After 3 minutes of shaking, 100 µL of 1 M potassium acetate was added to the flask. 2.8 milliliter of distilled water was added after letting the flask to one side for 3 min. At room temperature, the mixture had been incubated for 30 minutes. The solution's absorbance was calculated at the wavelength of 430 nm. The tests of the three samples were conducted and the mean absorbance was calculated. The quercetin equivalent concentration in the Septelin tablet extracts were calculated using quercetin standard curves. Results were given in milligrams per gramme of quercetin equivalent.

3.4.6. Total Tannin Content

The extract of septelin tablet was evaluated for total tannin content of by Vanillin method. ²⁹ In volumetric flask, 1 mL of extract of septelin tablet, and 4 % vanillin solution (3 mL) was added. Concentration HCL (1.5mL) was added to a mixture. Using a UV Visible spectrophotometer, the absorption was measured at 500nm after 15 minutes.

3.4.7. Spectroscopy

3.4.7.1 UV Vis Spectrophotometric study

To determine the maximum absorption wavelength, the UV spectra of the extract from the Septelin tablet were recorded on the Jasco UV V-630 between the spectral range of 200 nm and 400 nm.

3.4.7.2 FT-IR study

The Infrared spectra of extract of septelin tablet was recorded on Shimadzu 8400 between the spectral range 4000 cm⁻¹ to 400 cm⁻¹, to detect the vibrational bands of the extract.

3.4.8. TLC Analysis

On previously prewashed, precoated silica gel TLC plates, the extracts of septelin tablet and gallic acid were sprayed using a microlitersyringe ILS (100 μ L) and a regulated stream of nitrogen with a Spraylin (AE-05) sample applicator. Methanol: toluene: ethyl acetate:formic acid were the components of the mobile phase utilised for the development of TLC (1.5: 3.5:1:0.5, v/v/v/v). Prolite (AE-22) carried out the photo documentation, and the bands were scanned at wavelengths of 254 nm and 366 nm.

4. Statistical Analysis

Microsoft Excel was used to calculate all statistical data.

5. Result and Discussion

5.1. Organoleptic evaluation

The Septilin tablet was evaluated for organoleptic characters and results of the same are shown in table 1.

Sr. No.	Particular	Septilin	
1	Appearance	Solid	
2	Colour	Blue (Coat)	
		Brown (core)	
3	Odour	Bitter	
4	Taste	bitter and astringent	

Table 1: Results of organoleptic Evaluation

5.2. Physical evaluation

The triturated powder of Septilin tablet was evaluated for physical parameters and results of the same are shown below (table 2).

Sr. No.	Parameters	Septilin
1	Foreign matter	Nil
2	Moisture content	0.21%
3	Total ash	2.11 %
4	Acid insoluble ash	1.10%
5	Alcohol soluble extractive value Index	21.25 %
6	Water soluble extractive value	12.70 %

Table 2: Results of Physical evaluation

5.3. Qualitative Phytochemical Evaluation

The marketed Septilin tablet was evaluated for qualitative phytochemical evaluation and results of the same are shown in table 3.

Sr. No.	Phytochemicals	Test	Observation	Result
1.	Alkaloids	Picric acid test	Yellow colour	Positive
		Dragendorff 's test	Orange red precipitate	Positive
2.	flavonoids	Alkaline reagent test	Intense yellow colour	Positive
		Mayer's test	Yellowish precipitate	Positive
3.	Tannins & Phenols	FeCl ₃ test	Yellowish orange colour	Positive
		Lead acetate test	Gelatinous precipitate	Positive
4.	Carbohydrate	Benedict's test	Orange colour	Positive
		Fehling's test	Brick-red precipitate	Positive
		Molisch's test	Purple colour	Positive
5.	Glycosides	Borntrager's test	Pink colour	Positive
		Legal's test:	Pink colour	Positive
6.	Steroids	Libermann- Burchard test	No bluish green colour	Negative
7.	Saponins	froth formation Test	No froth formation	Negative

Table 3: Results of Qualitative Phytochemical Evaluation

5.4. Phytochemical evaluation

The extracts of Septilin tablet was evaluated for phytochemicals by UV-Vis spectrophotometer and reported in table 4.

Sr. No.	Parameters	Septilin
1	Total phenolic content	111.15 ±0.055mg GAE/g
2	Total flavonoid content	$55.20 \pm 0.0064 \text{ mg QE/g}$
4.55 3	Total Tannin content	$6.8\pm0.0015~mg~CE/g$

Table No. 4: - Results of Phytochemical evaluation

5.5. UV Vis Spectrophotometric study

The extract of Septilin tablet (10 μ g/mL) was exposed to UV radiation between 200-400 nm. Extract of Septilin tablet showed two absorbance peaks in the UV-Vis spectra. Septilin formulation showed peaks at 273 nm & 340.5nm.

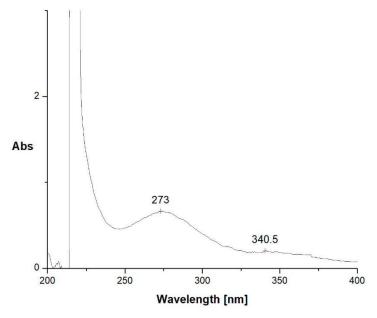


Fig. 1. UV-Vis Spectrum of Septelin tablet Extract

5.6. FT-IR study

The extract of the Septilin tablet's solid forms IR (KBr, cm-1) spectra showed a broad peak of the O-H group between 3500 to 3402 cm⁻¹ and an aromatic (C-H) stretch at 3100-3050 cm⁻¹. It shows peak of aliphatic C-H at 3000 to 2931 cm⁻¹. The C=O group of carboxylate was confirmed by stretching at 2337.80 cm⁻¹. The C=O group of amide was confirmed by stretching at 1700 to 1690 cm⁻¹. The existence of C-H bending was indicated by the vibration's medium appearance between 1450 -1440 cm⁻¹. Stretching between 1040 -1030 cm⁻¹ showed that the C-O group was present. (Fig 2).

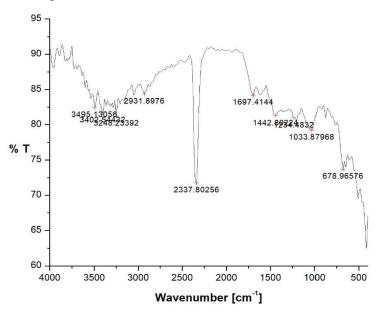


Fig. 2. FTIR Spectrum of Septelin tablet Extract

5.7. TLC analysis

On silica gel TLC plates that were already coated, the extract from the Septilin tablet and gallic acid were spotted. To separate the phytochemicals, methanol: toluene:ethyl acetate:formic acid (1.5: 3.5:1:0.5, v/v/v/v) were utilised as the mobile phase. The phytochemicals of the Septilin tablet showed well-defined spots at Rf values 0.324, 0.49, 0.574, 0.725 & 0.873 under 254 nm & 0.49, 0.574, 0.698, 0.725, 0.77 & 0.873 under 366 nm (Fig.3). Gallic acid is present in the extract of the septelin tablet, as shown by the chromatogram's overlay with gallic acid. (Fig. 4).

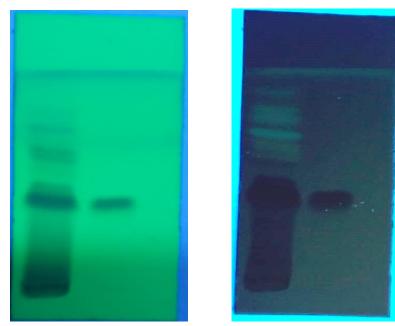


Fig. 3. TLC plate Under 254 nm

TLC plate Under 366 nm

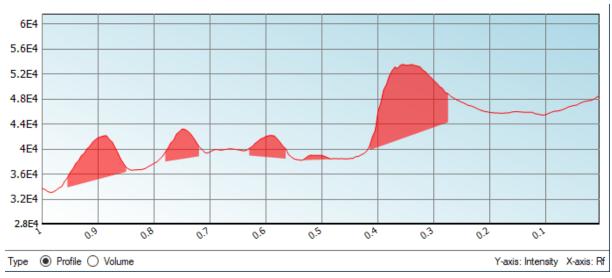


Fig. 4. Chromatoram of Septelin tablet Extract

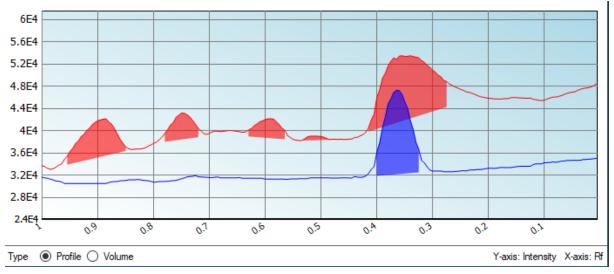


Fig. 5. Overlay of Chromatoram of Septelin tablet Extract & Gallic acid

6. Conclusion

In the routine quality control test of herbal the organoleptic, product. physical. phytochemical, spectral data and TLC fingerprint could be a valuable analytical tool. In organoleptic evaluation shows that, bitter and astringent taste for core tablet. In polyherbal tablet-septelin, the the phytochemicals tests show positive results for alkaloids, flavonoids, tannins, phenolic compounds, carbohydrates and glycosides. The linear regression methods for the quantification of phytochemicals were applied by using а UV-Vis spectrophotometer. The phytochemical evaluation indicates that septelin tablet contain significant amounts of phenolic compounds, flavonoids and tannins. Spectral data of UV-Vis spectrophotometer for the extract of septelin tablet represents the presence of conjugated & aromatic compounds. The presence of numerous functional groups in complex phytochemicals was confirmed by FTIR spectral data. Using only TLC, the Septilin tablets were fingerprinted using thin-layer chromatography.

Reference

- 1. Sissi Wachtel-Galor, Iris F. F. Benzie. Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. In: *Herbal Medicine: Biomolecular and Clinical Aspects.* 2nd ed. Taylor & Francis; 2011.
- Luo Y. Contemporary Neuroscience Meets Traditional Medicine - Towards Understanding Ginkgo Biloba Neuroprotection. 2003;1:49-58.
- 3. Tugume P, Nyakoojo C. Ethnopharmacological survey of herbal remedies used in the treatment of paediatric diseases in Buhunga parish, Rukungiri District. Uganda. **BMC** Complement Altern Med. 2019;19(1):353. doi:10.1186/s12906-019-2763-6
- 4. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014;4,177:1-10. doi:10.3389/fphar.2013.00177
- Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. *Pharmacogn Rev.* 2014;8(16):73-80. doi:10.4103/0973-7847.134229

- 6. Mukhi S, Bose A, Panda P, Rao MM. Pharmacognostic, physicochemical and chromatographic characterization of Samasharkara Churna. J Ayurveda Integr Med. 2016;7(2):88-99. doi:10.1016/j.jaim.2015.11.004
- 7. Khodadadi S. Role of herbal medicine in boosting immune system. *Immunopathol Persa*. 2015;1:(1):1-2.
- Isbill J, Kandiah J, Kružliaková N. Opportunities for Health Promotion: Highlighting Herbs and Spices to Improve Immune Support and Wellbeing. *Integr Med Encinitas Calif.* 2020;19(5):30-42.
- Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Herbal antioxidant in clinical practice: A review. Asian Pac J Trop Biomed. 2014;4(1):78-84. doi:10.1016/S2221-1691(14)60213-6
- 10.Parham S, Kharazi AZ, Bakhsheshi-Rad HR, et al. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. *Antioxidants*. 2020;9(12,1309):1-36. doi:10.3390/antiox9121309
- 11.Mukherjee PK, Nema NK, Venkatesh P, Debnath PK. Changing scenario for promotion and development of Ayurveda – way forward. J Ethnopharmacol. 2012;143(2):424-434. doi:10.1016/j.jep.2012.07.036
- 12.Devasagayam TPA, Sainis KB. Immune system and antioxidants, especially those derived from Indian medicinal plants. *Indian J Exp Biol*. 2002;40(6):639-655.
- 13.Kumar D, Arya V, Kaur R, Bhat ZA, Gupta VK, Kumar V. A review of immunomodulators in the Indian traditional health care system. J Microbiol Immunol Infect. 2012;45(3):165-184. doi:10.1016/j.jmii.2011.09.030
- 14.Alhazmi HA, Najmi A, Javed SA, et al. Medicinal Plants and Isolated Molecules Demonstrating Immunomodulation

Activity as Potential Alternative Therapies for Viral Diseases Including COVID-19. *Front Immunol.* 2021;12:637553. doi:10.3280/fimmu.2021.627552

doi:10.3389/fimmu.2021.637553

- 15.Di Sotto A, Vitalone A, Di Giacomo S. Plant-Derived Nutraceuticals and Immune System Modulation: An Evidence-Based Overview. *Vaccines*. 2020;8(3):468. doi:10.3390/vaccines8030468
- 16.Bafna A. Antioxidant and Immunomodulatory Activity of the Alkaloidal Fraction of Cissampelos pareira Linn. *Sci Pharm*. 2010;78(1):21-31. doi:10.3797/scipharm.0904-16
- 17.Nawrot-Hadzik I. Matkowski A. Kubasiewicz-Ross P, Hadzik J. Proanthocyanidins and Flavan-3-ols in the Prevention and Treatment of Periodontitis-Immunomodulatory Effects, Animal and Clinical Studies. Nutrients. 2021;13(1):239. doi:10.3390/nu13010239
- 18.Khajuria A, Gupta A, Garai S, Wakhloo BP. Immunomodulatory effects of two sapogenins 1 and 2 isolated from Luffa cylindrica in Balb/C mice. *Bioorg Med Chem Lett*. 2007;17(6):1608-1612. doi:10.1016/j.bmcl.2006.12.091
- 19.Moutia M, Habti N, Badou A. In Vitro and In Vivo Immunomodulator Activities of Allium sativum L. *Evid Based Complement Alternat Med.* 2018;2018:1-10. doi:10.1155/2018/4984659
- 20.Stefanova TH, Nikolova NJ, Toshkova RA, Neychev HO. Antitumor and immunomodulatory effect of coumarin and 7-hydroxycoumarin against Sarcoma 180 in mice. *J Exp Ther Oncol.* 2007;6(2):107-115.
- 21.Ernst E, Schmidt K, Wider B. CAM research in Britain: the last 10 years. *Complement Ther Clin Pract.* 2005;11(1):17-20. doi:10.1016/j.ctnm.2004.09.005

- 22.Ribnicky DM, Poulev A, Schmidt B, Cefalu WT, Raskin I. Evaluation of botanicals for improving human health. *Am J Clin Nutr*. 2008;87(2):472S-475S. doi:10.1093/ajcn/87.2.472S
- 23.Kantor M. The Role of Rigorous Scientific Evaluation in the Use and Practice of Complementary and Alternative Medicine. *J Am Coll Radiol*. 2009;6(4):254-262. doi:10.1016/j.jacr.2008.09.012
- 24.General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines. Published online 2001.
- 25.Profiling and determination of phenolic compounds in poly herbal formulations and their comparative evaluation | Elsevier Enhanced Reader. doi:10.1016/j.jtcme.2017.12.001
- 26.Nigade GB, Deodhar MN, Chavan RS. Phytochemical evaluation of the marketed drakshasava formulation by spectroscopic & chromatographic methods. *Int J Health Sci*. Published online October 2, 2022:2969-2981. doi:10.53730/ijhs.v6nS9.13187
- 27.Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: *Methods in Enzymology*. Vol 299. Elsevier; 1999:152-178. doi:10.1016/S0076-6879(99)99017-1
- 28.Pękal A, Pyrzynska K. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. *Food Anal Methods*. 2014;7(9):1776-1782. doi:10.1007/s12161-014-9814-x
- 29.Broadhurst RB, Jones WT. Analysis of condensed tannins using acidified vanillin. J Sci Food Agric. 1978;29(9):788-794. doi:10.1002/jsfa.2740290908