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Exploring the Therapeutic Potential of Nilavembu Kudineer: Phytochemical Analysis and Formulation Development

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KEYWORDS
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methanolic extract,
HPTLC,
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tablets

Abstract

Nilavembu Kudineer (NVK) is a traditional polyherbal formulation renowned for its potential health benefits. This study is focused on conducting a comprehensive analysis of NVK's phytoconstituents and assessing its suitability for tablet formulation. Phytochemical analysis, which included High-Performance Thin-Layer Chromatography (HPTLC) and revealed the presence of significant flavonoids and terpenoids in NVK extract. Gas Chromatography-Mass Spectrometry (GC-MS) studies identified several crucial compounds, including Andrographolide and gingerol and other compounds, contributing to its therapeutic properties. *In vitro* hepatotoxicity studies employing HepG2 cells demonstrated that NVK extract does not exhibit hepatotoxic effects, affirming its safety profile. Additionally, NVK extract was effectively transformed into tablets, subject to evaluation against various quality control parameters. These tablets successfully met the acceptance criteria, positioning them as a promising dosage form for NVK administration. This research offers valuable insights into the phytochemistry, safety, and formulation potential of Nilavembu Kudineer, underscoring its potential applications in both traditional and modern medicine.

Introduction

Nilavembu Kudineer (NVK) is an integral part of traditional Indian medicine, deeply rooted in the ancient wisdom of Siddha medicine. This herbal formulation is a testament to the profound healing synergy that traditional practices offer. At its core, NVK derives its potency from Nilavembu, aptly known as the "King of Bitters." This remarkable botanical source with a wide range of immune-enhancing properties and serves multiple roles, such as an anti-pyretic, cholagogue, digestive aid, hepatoprotective agent, and anti-inflammatory powerhouse[1]. Its influence transcends various traditional healing systems, including Ayurveda, Siddha, Unani, Homoeopathy, Chinese medicine, and tribal wellness. In Hindi, it is referred to as "Kalmegh" and is recognized as a powerful remedy for intermittent fevers caused by ailments such as malaria, dengue fever, chronic fevers, and chikungunya. It is not merely a symptomatic relief but addresses the root causes of these illnesses, exemplifying holistic healing. As we journey through the crossroads of tradition and modernity, NVK

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stands as a testament to the enduring wisdom passed down through generations. Its significance knows no borders and resonates in the harmonious chorus of various healing systems. In NVK, the legacy of Nilavembu thrives, encapsulating centuries-old wisdom intertwined with an unceasing quest for well-being [2].

Nilavembu Kudineer (NVK) is a remarkable polyherbal formulation that combines the potent qualities of nine distinct herbal constituents in powdered form. These are carefully selected and harmoniously balanced constituents. Nilavembu Kudineer (NVK) comprises a rich tapestry of botanical resources, each contributing its unique attributes to this remarkable polyherbal formulation. Andrographis paniculata, known as Nilavembu, brings its immune-enhancing properties to the mix. Plectranthus vettiveroides, or Vilamichamver, adds its own distinctive attributes. Zingiber officinale, referred to as Sukku, introduces a unique dimension to the formulation. Cyperus rotundus (Koraikizhangu) contributes its distinctive qualities, while Trichosanthes dioica (Pei pudal) offers its botanical essence. Vetiveria zizanioides, commonly known as Vetiver, provides its significance. Piper nigrum (Milagu) lends its distinctive character, and Mollugo cerviana (Parpadagam) further enriches the botanical medley. Finally, the wood of Santalum album, also known as Sandanam, concludes this diverse assortment of botanical treasures, creating a harmonious blend of healing elements in NVK. Each of these elements brings its own unique qualities, contributing to the rich tapestry of botanical resources in NVK[3,4]. Despite the wealth of pharmacological knowledge surrounding these constituent herbs, comprehensive safety evaluations following accepted testing methodologies are essential. This is particularly crucial to ensure the prolonged and sustainable use of NVK while maintaining safety standards[5].

Ensuring the prolonged and sustainable use of Nilavembu Kudineer (NVK) while upholding safety standards is of paramount importance. The primary objective of this research study is to conduct a comprehensive evaluation and standardization of NVK extract. This involves extensive phytochemical analysis through various chemical tests, HPTLC (High-Performance Thin-Layer Chromatography) to identify flavonoids and terpenoids, and GC-MS (Gas Chromatography-Mass Spectrometry) studies to identify the various compounds present in the extract. Additionally, the study includes the formulation and evaluation of NVK extract tablets to assess the suitability of the extract as a solid unit dosage form. Through these rigorous analyses spanning multiple domains, this study aims to provide valuable insights that can inform potential applications of NVK in the field of medicine.

MATERIALS AND METHODS

Nilavembu Kudineer powder was acquired from a government-authorized local store situated in Chennai. All other chemicals used were of laboratory grade.

Preparation of Extracts

The acquired Nilavembu Kudineer powder 1kg was placed within an aspirator bottle, initiating the subsequent extraction process. Employing the cold maceration technique, the powder was subjected to sequential extraction with distinct solvents - acetone, methanol, and water - over a duration of six days for each solvent. At the culmination of each extraction period, the resultant extracts underwent filtration via filter paper to eliminate impurities. The concentrated extracts were subsequently obtained through the utilization of a rotary vacuum evaporator [6].

The color, texture, and extraction efficiency of all extracts were documented.

Percentage yield of NVK Extract

$$\%\text{Yield} = \frac{\text{Wt. of the dry extract}}{\text{Wt. of the dry plant}} \times 100$$

Phytochemical Analysis

The obtained extracts of AP/NVK were subjected to the preliminary phytochemical analysis by various standard methods. By conducting these qualitative chemical tests, we aimed to shed light on the presence of glycosides, volatile oils, alkaloids, tannins, terpenoids flavonoids etc., within the Nilavembu Kudineer extracts. This comprehensive profiling not only contributes to our understanding of the plant's biochemical composition but also serves as a foundation for potential therapeutic applications and further investigations into the extract's potential health benefits[6].

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Determination of crude fibre content

The dried powdered NVK which was stored in three different temperature conditions such as 4, 25 and 40°C for one month period was finely ground and sieved through a No. 20 stainless steel mesh sieve for uniformity. Around 2-3g of the defatted sample was obtained using Soxhlet extraction with petroleum ether to remove fat content. The defatted sample underwent digestion with 1.25% H2SO4 and 1.25% NaOH solutions. Following digestion, it was subjected to drying at 130°C for 2 hours and subsequently incinerated at 600°C for 30 minutes. The resulting decrease in weight upon incineration,

in conjunction with the initial weight prior to the defatting process, was employed to ascertain the percentage of crude fiber content.This analytical process provided insights into the fiber composition of the sample, aiding in nutritional assessment[7].

$$\% \text{ Crude Fibre in Ground Sample} = \frac{\text{Loss in Weight on Ignition}}{\text{Weight of Ground Sample}} \times 100$$

Detemination of Terpenoids and Flavanoids through HPTLC

Thin Layer Chromatography (TLC) was carried out with the methonolic extract samples by employing silica gel 60 F254 HPTLC plates, utilizing a mobile phase solvent blend of methanol/chloroform/hexane (7:2:1, v/v/v). Test solutions, prepared at a concentration of 1 mcg/ml, were meticulously applied onto the plates using a sample injector. Subsequently, the chromatograms were developed at room temperature within glass twin-trough chambers that had been pre-saturated with mobile phase vapor for a duration of 30 minutes. During development, the mobile phase carried the samples a distance of 70 mm. Once development was complete, the plates were allowed to air-dry. The ensuing spots were then subjected to identification under UV light. Notably, chromatograms were observed at both λ = 254 and 366 nm, with images of these chromatograms captured for further analysis. The presence or absence of the compounds under investigation was ascertained based on their Retention Factor (RF) values, a key parameter in thin layer chromatography[8,9]. This analytical approach allowed us to discern the unique composition and distribution of the compounds within the samples, contributing to our understanding of their chemical characteristics and potential applications[10].

Gas Chromatography Mass Spectra Analysis

The GC-MS analysis was carried out methanolic NVK extract samples. The analytical setup utilized an HP5 column from Agilent Technologies, with dimensions of 30m length, 0.25mm internal diameter and a 0.25µm film thickness. This column was integrated with a 6890°N gas chromatography system, coupled with a 5973 N mass spectrometer serving as the detector. For GC-MS detection, an electron ionization system operating at 70eV ionization energy was employed. The carrier gas employed was helium, employed in a split ratio of 5:4. The injector temperature was precisely maintained at 250°C, while the column temperature was set to 280°C. The temperature program was strategically executed in a stepped manner, initiating at 100°C for 2 minutes and gradually ramping up to 280°C at a rate of 10°C per minute. Subsequently, the temperature was held steady at 280°C for an additional 5 minutes, resulting in a total run time of 30 minutes. For sample introduction, a diluted sample of 1µl was manually injected in splitless mode. The mass spectrometer scan range spanned from 35 to 1500 Da, and the peaks were precisely marked with their corresponding retention times within the GC-MS analysis of the methanol extract of NVK. The mass spectra were acquired at an ionization energy of 70 eV, with an output interval of 0.5 seconds, and a mass range spanning from 45 to 450 Da. The complete GC run, incorporating the entire sequence of steps, amounted to approximately 50 minutes[11,12].

***Invitro* toxicity studies**

cytotoxicity study of NVK extract was carried out with HepG2 cells. In log phase it was trypsinized and 1x10^5 cells per well were seeded in 96-well tissue culture plates. They were then treated with freshly prepared NVK extract at concentrations of 25, 50, 100, 200 and 300 µg for 24, 48, and 72 hours at 37°C in a humidified atmosphere containing 5% CO2. Untreated cells served as the control, and Paracetamol was used as the standard reference. After treatment, media was replaced with 100 µl DMEM media, and 20 µl of MTT (5 mg/ml in PBS)

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stock solution was added to each well and incubated for 4 hours. The formazan product that ensued was dissolved in 100 µl of dimethyl sulfoxide (DMSO), yielding a coloured solution. Absorbance at 570 nm was gauged utilizing a microplate reader (Model 680 XR, Bio-Rad Laboratories Inc.)[13,14]. The inhibitory concentration 50 (IC50) value for the HepG2 cell line was calculated following a 24-hour period, signifying the concentration of NVK that inhibits cell growth by 50%. This comprehensive experimental design allows for a detailed assessment of the cytotoxic effects of NVK on HepG2 cells at various concentrations [15,16].

Formulation of *Nilavembu kudineer tablets*

Nilavembu kudineer tablet formulation was prepared by direct compression method. Nine different batches has been formulated with varying the excipients concentrations[17]. All the ingredients as specified in the formulation Table 1 were weighed and mixed one by one with geometric proportion and then passed through the BSS-80 mesh. Then the mixed powders were compressed in to tablets using [10×5mm diameter; standard concave punches] by a Rotary tablet compression machine (Cadmach, Ahmedabad, India). Thus, the powder-mix was examined for bulk-density, angle of repose, drug-content and compressibility-index prior to the compression[18,19].

Table No.1 : Formulation table for Nilavembu kudineer Tablets

S.No.	Ingredients (mg)	NK1	NK2	NK3	NK4	NK5	NK6	NK7	NK8	NK9
1.	Drug	30	30	30	30	30	30	30	30	30
2.	Lactose	34	--	114	34	--	114	34	--	114
3.	Microcrystalline cellulose	80	114	--	80	80	--	80	114	--
4.	Sodium starch glycolate	4	4	4	--	--	--	--	--	--
5.	Cross caramellose	--	--	--	4	4	4	--	--	--
6.	Cross povidone	--	--	--	--	--	--	4	4	4
7.	Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
8.	Talc	1	1	1	1	1	1	1	1	1
9.	Magnesium-stearate	1	1	1	1	1	1	1	1	1

Results and discussion

Preparation of Extracts with Acetone, Water, Methanol

The Nilavembu Kudineer extracts were examined and was characterized by distinct color and consistency attributes. The Acetone extract displayed a faintly yellowish tint and exhibited a sticky mass consistency. In contrast, the Methanol extract presented a brownish hue and possessed a consistency akin to sticky powder. Notably, the Water extract showcased a deep brown color and featured a mucilagenous texture. To quantify the concentration of these extracts, percentages were calculated, revealing values of 1.79%, 4.73%, and 8.02% for Acetone, Methanol, and Water extracts respectively. This comprehensive evaluation offers valuable insights into the physical characteristics and relative abundance of the diverse Nilavembu Kudineer extracts. Table No. 1.

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Table No. 2: The colour, consistency and percentage yield of different extracts with Nilavembu Kudineer

S.NO	Extract	Nilavembu kudineer		
		Colour	Consistency	Yield%
1	Acetone	Slightly Yellow	Sticky mass	1.79%
2	Methanol	Brown	Sticky Powder	4.73%
3	Water	Dark Brown	Mucilagenous	8.02%

Phytochemical Analysis

The provided phytochemical analysis report highlights the presence of a diverse range of compounds within the sample. These compounds encompass a wide spectrum of chemical constituents, each with its distinctive attributes and potential benefits. The presence of these compounds underscores the complexity and richness of the sample's composition, suggesting its potential value for various applications and implications in the field of phytochemistry. Table No.3. The detailed analysis serves as a valuable foundation for further exploration and understanding of the sample's properties and potential contributions.

Table No. 3. Qualitative analysis of Nilavembu kudineer

S.No	Qualitative Tests	Water Extract	Methanol extract	Acetone extract
1	Alkaloids	+	+	-
2	Carbohydrates	+	+	-
3	Falvones and flavanones	+	+	-
4	Fixed oils and fats	-	-	+
5	Gums and Mucilages	+	+	-
6	Proteins and aminoacids	+	+	+
7	Sterols	-	+	+
8	Saponins	+	+	-
9	Tannins- Phenolic compounds	+	+	-
10	Triterpenoids	-	+	-

+ = Presence; - = Absence

Determination of total crude fibre content :

The evaluation of NVK samples stored at various temperatures for a month and the result revealed that the fiber content remained consistent regardless of the temperature changes, indicating that fluctuations in temperature do not significantly impact the fiber content of NVK. Table No.4.

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Table No.4: Total Fibre content of Nilavembu Kudineer at different Temperature

S.No	Temperature	Fibre Content
1	4°C	8.43±0.13
2	25°C	9.58±0.11
3	40°C	8.82±0.29

The crude fibre content of NV signifies the proportion of indigestible components like cellulose and lignin in the substance. This dietary fibre plays a pivotal role in digestive health by aiding regular bowel movements, promoting satiety, regulating blood sugar levels, and supporting heart health. Additionally, it contributes to a healthy colon environment, reduces the risk of chronic diseases, and indirectly aids nutrient absorption. While crude fibre is not fully digestible, its presence in NVK suggests potential health benefits attributed to dietary fibre intake, underscoring its significance for overall well-being.

Determination of Terpenoids and Flavonoids through HPTLC Studies

To find out the presence of terpenoid and flavonoid present in the methanolic NVK various compositions of the mobile phase are evaluated for High-Performance Thin-Layer Chromatography (HPTLC) analysis to obtain high resolutions and reproducible peaks. The desired solvent system for terpenoid has been achieved using n-Hexane-Ethyl acetate (7.2:2.9), for the flavonoid it is Ethyl acetate-Butanone-Formic acid-Water (5:3:1:1).

a) Terpenoid profile of m*ethanol and water extracts of* NVK

The methanolic and water extract of the NVK reflects the presence of nineteen different type of terpenoids with Rf values as reported in Table No.5. Blue, Violet coloured zone observed from the chromatogram after derivatization, confirms the presence of Terpenoid.

Table No: 5. HPTLC profile for Terpenoid with Nilavembu Kudineer extracts.

Track	Peak	Rf	Height	Area	Assigned substance
NVK M	1	0.07	11.4	110.7	Unknown
NVK M	2	0.12	19.1	366.6	Unknown
NVK M	3	0.23	10.4	198.6	Unknown
NVK M	4	0.25	15.2	243.0	Unknown
NVK M	5	0.30	15.6	289.7	Unknown
NVK M	6	0.43	24.1	545.1	Unknown
NVK M	7	0.47	38.2	903.7	Unknown
NVK M	8	0.61	26.6	393.7	Terpenoid 1
NVK M	9	0.64	40.7	1193.7	Terpenoid 2
NVK M	10	0.72	83.2	2634.4	Terpenoid 3

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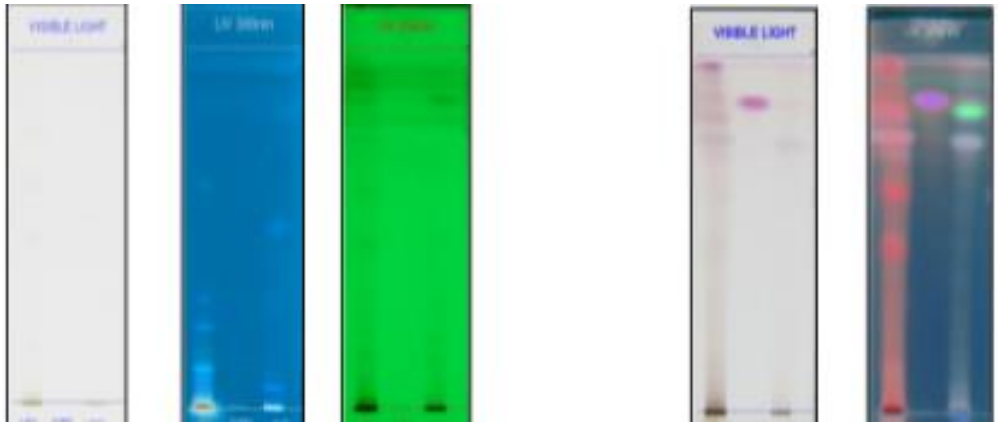
NVK M	11	0.76	156.1	5843.2	Terpenoid 4
NVK M	12	0.82	185.0	6499.4	Terpenoid 5
NVK M	13	0.89	162.2	6434.8	Terpenoid 6
NVK M	14	0.97	339.2	9065.0	Terpenoid 7
STD	1	0.87	390.8	14648.5	Terpenoid standard
NVK W	1	0.22	10.7	292.9	Unknown
NVK W	2	0.74	142.8	5604.6	Terpenoid 1
NVK W	3	0.86	103.9	4609.0	Terpenoid 2
NVK W	4	0.94	58.5	1449.9	Terpenoid 3
NVK W	5	0.97	145.7	2730.7	Terpenoid 4



BEFORE DERIVATIZATION **AFTER DERIVATIZATION**

Figure No.1: Terpenoid chromatogram of Nilavembu kudineer extracts before and after derivatization in 500nm.

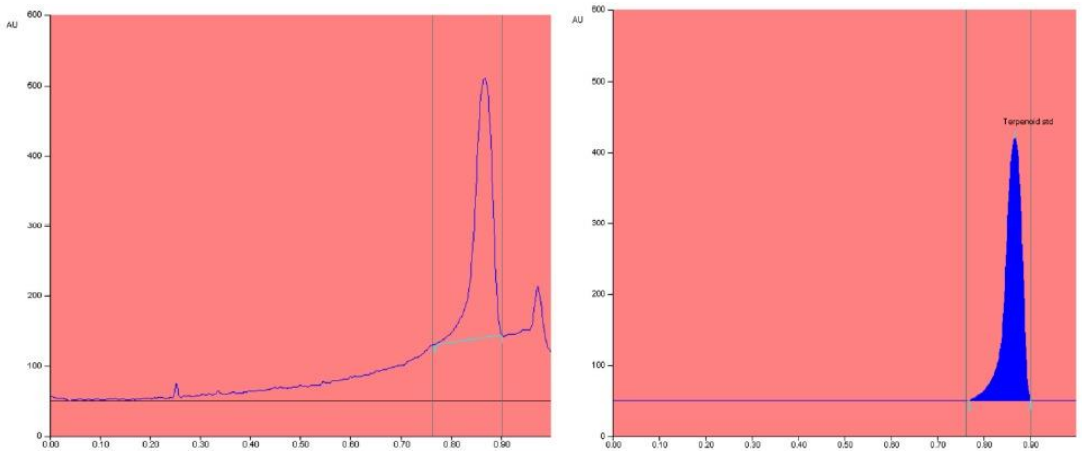


Figure No. 2 : Terpenoid Standard baseline display and Peak densitogram at 500nm

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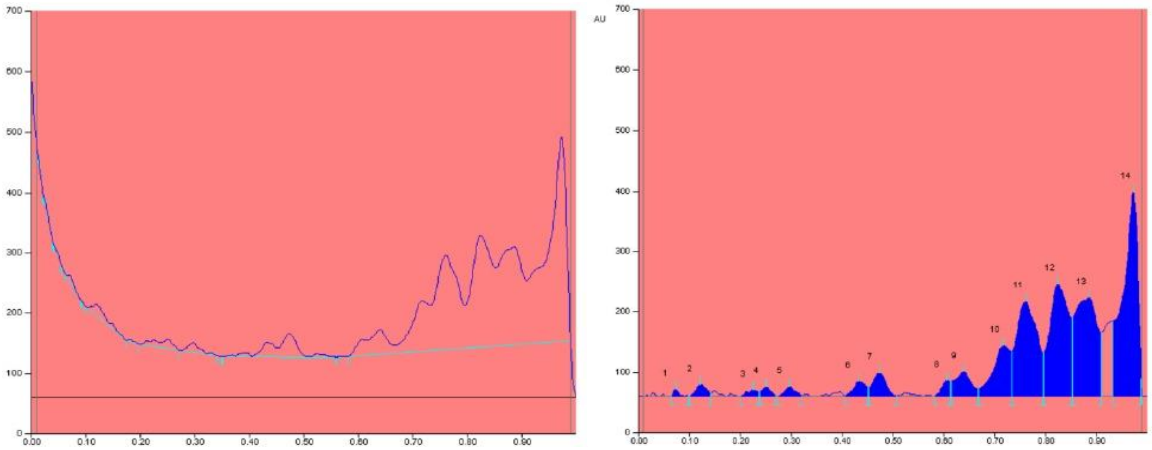

Figure No. 3 : NVK Methanolic extract baseline display and Peak densitogram at 500nm

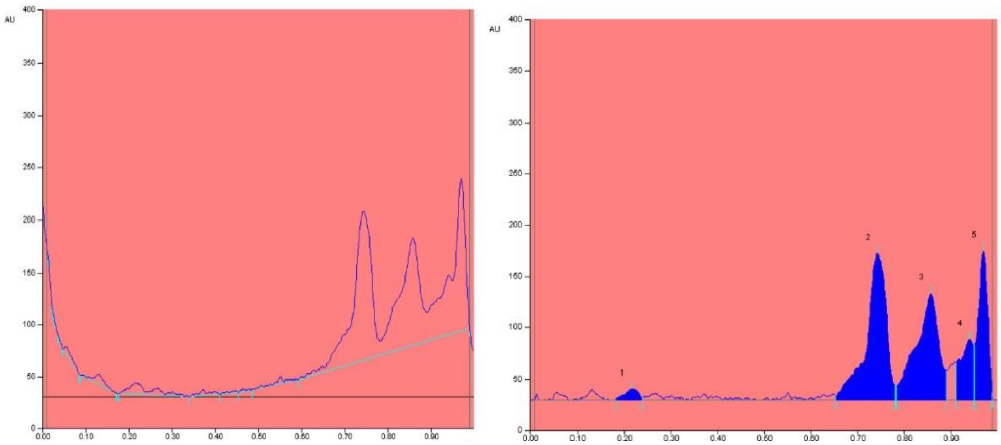

Figure No. 4 : NVK Water extract baseline display and Peak densitogram at 500nm

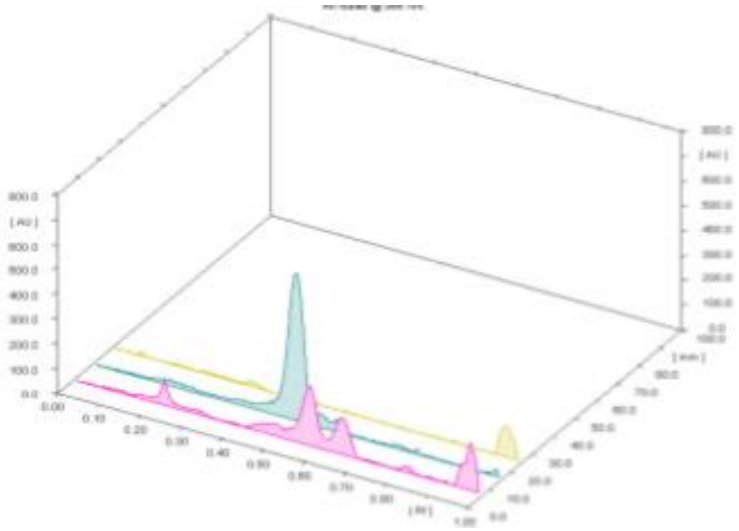

Figure No. 5 : Terpenoids baseline and peak densitogram of methanolic and water extract and 3D display of all the tracks.

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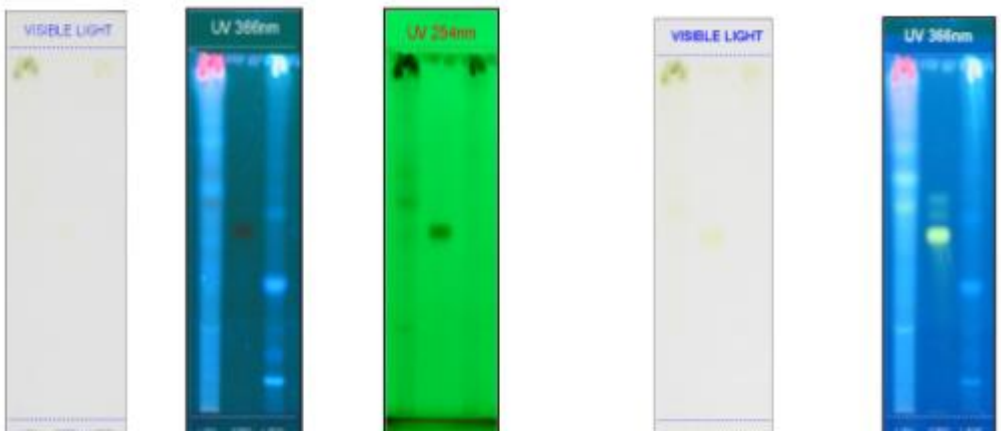
Flavanoid profile of NVK extracts

The methanolic and water extract of NVK showed the presence of eleven different type of flavonoid with Rf values as reported in Table No.8. Yellow, Yellowish blue coloured fluorescent zone after derivatization confirms the presence of Flavonoids.

Table No:6. HPTLC profile for Flavanoids with Nilavembu kudineer extracts

Track	Peak	Rf	Height	Area	Assigned substance
NVK M	1	0.17	17.0	318.8	Unknown
NVK M	2	0.22	97.8	2375.3	Flavonoid 1
NVK M	3	0.30	14.5	420.9	Unknown
NVK M	4	0.49	48.9	2364.6	Flavonoid 2
NVK M	5	0.57	238.0	9191.3	Flavonoid 3
NVK M	6	0.65	147.1	5104.0	Flavonoid 4
NVK M	7	0.81	23.3	525.6	Flavonoid 5
NVK M	8	0.97	187.5	4743.6	Unknown
STD	1	0.49	590.3	22669.0	Flavonoid Standard
NVK W	1	0.07	13.9	188.6	Unknown
NVK W	2	0.35	19.5	600.6	Flavonoid 1
NVK W	3	0.96	126.9	3561.3	Flavonoid 2



BEFORE DERIVATIZATION **AFTER DERIVATIZATION**

Figure No. 6 : Flavonoid chromatogram of Nilavembu kudineer extracts before and after derivatization in 366nm

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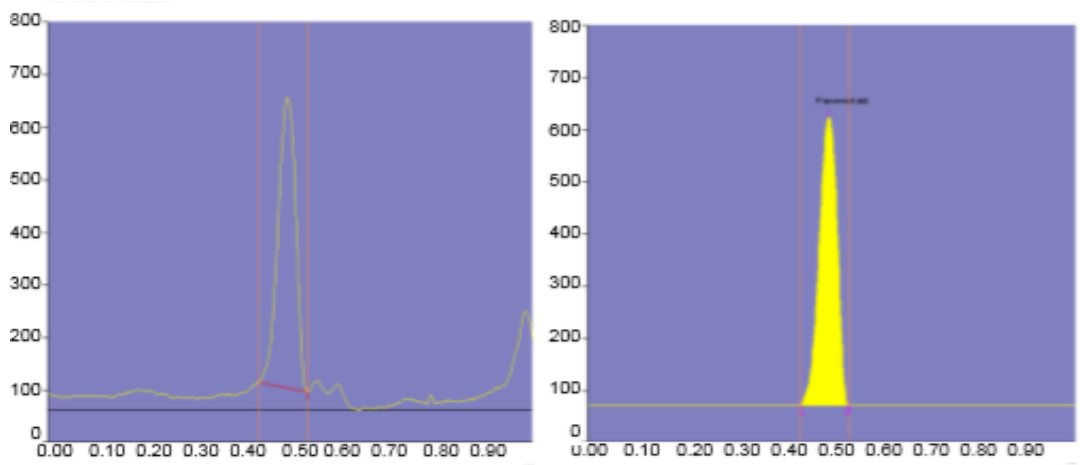

Figure No. 7 : Flavonoid Standard baseline display and Peak densitogram at 366nm

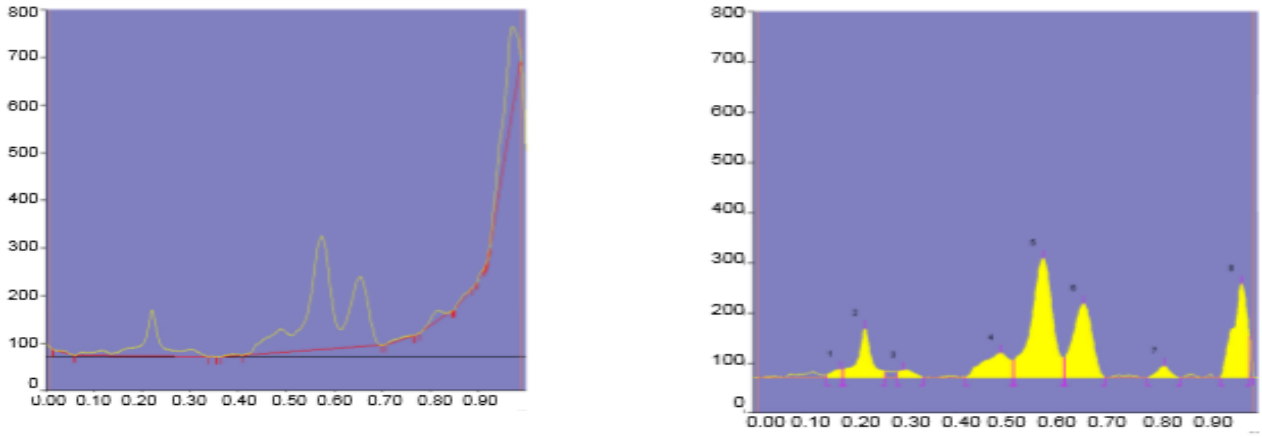

Figure No. 8 : NVK Methanolic extract baseline display and Peak densitogram at 500nm

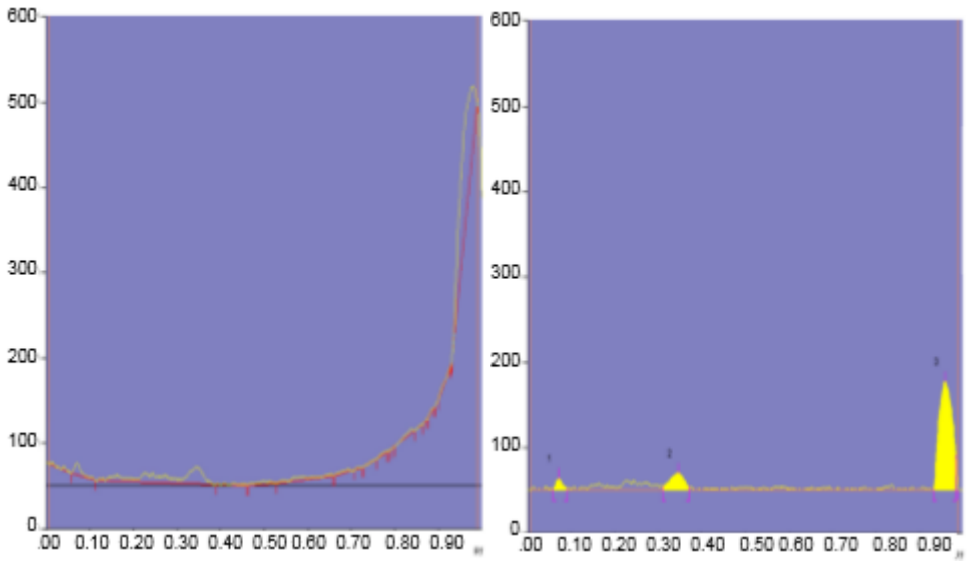

Figure No. 9 : NVK Water extract baseline display and Peak densitogram at 500nm

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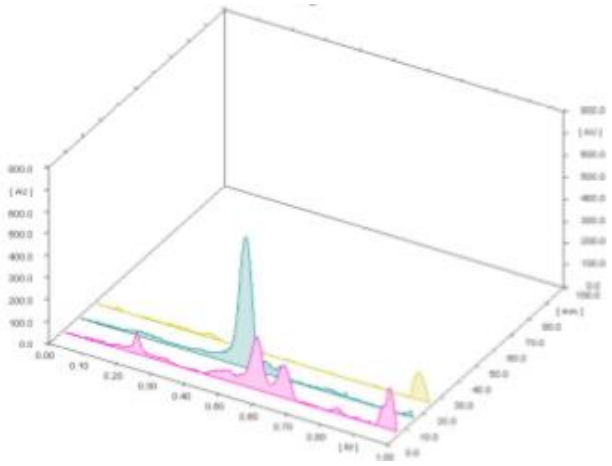

Figure No. 10 : ^{0}Flavonoids baseline and peak densitogram of methanolic and water extract ^{0} and 3D display of all the tracks

GC -MS Analysis

The GC-MS analysis of Nilavembu Kudineer demonstrated a unique fragmentation pattern that aligns with the presence of flavonoids and other compounds, which was clearly observable in the chromatogram. Furthermore, the GC-MS analysis detected the presence of several key compounds in the formulation, including gingerol, santalol, piperine, and andrographolide. These findings strongly indicate the incorporation of specific ingredients such as Andrographis, Santalum album, and Pepper in the formulation. Interestingly, the analysis also unveiled the presence of certain anti-diabetic molecules namely piperine, and andrographolide, underscoring the potential of Nilavembu Kudineer as a formulation with promising anti-diabetic properties.

The analysis of the plant extract using GC-MS revealed the presence of a diverse range of thirty chemical compounds, each possessing distinct phytochemical constituents, thus potentially contributing to the plant's remarkable medicinal properties (Table No.7). The identification of the active principles within the plant extract was methodically confirmed by assessing various factors including retention time, molecular formula and the percentage of peak area. The initial compound identified exhibited a relatively shorter retention time (17.05 min) and was characterized as 1,2-Benzenedicarboxylic Acid, Diethyl Ester (also known as Phthalic acid), while the final compound, LUP-20(29)-EN-3-YL Acetate, presented the longest retention time (45.09 min) before its conclusive identification and other main compouds identified are reported in Table No.7.

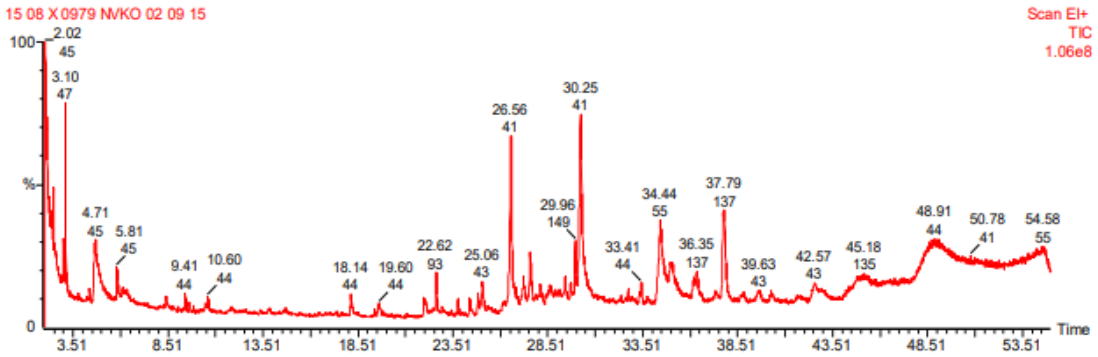

Figure No. 11: GC-MS Chromatogram of methanolic NVK extract

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Table No: 7: List of various chemical compounds determined through GC-MS

S.NO	Retention time	Percentage Peak Area	Name of compound	Formula
1	10.60	0.4362	Homopiperazine	$C_5H_{12}N_2$
2	18.14	1.1663	Piperonal	$C_8H_6O_3$
3	22.62	0.1453	Teresantolol	$C_{10}H_{16}O$
4	25.06	2.2895	Zigerone	$C_{11}H_{14}O_3$
5	26.94	2.8771	Santalol	$C_{15}H_{24}O$
6	26.56	3.2567	Andrographolide	$C_{20}H_{30}O_5$
7	34.4	2.5462	Gingerol	$C_{17}H_{26}O_4$
8	48.91	23.4778	Piperine	$C_{17}H_{19}NO_3$

Invitro Cytotoxicity studies

The results after 24 hours resulted in a slight dose-dependent cytotoxic effects of NVK. Following an MTT assay, the IC50 value in HepG2 cells of NVK was determined to be 123.9 µg/ml (Table No:8). These cumulative findings collectively emphasize the potential of NVK as not a cytotoxic agent specifically with HepG2 cells. Nonetheless, the study acknowledges the necessity for further, in-depth investigations to comprehensively unravel the precise mechanisms of action and to ascertain the potential therapeutic applications of NVK.

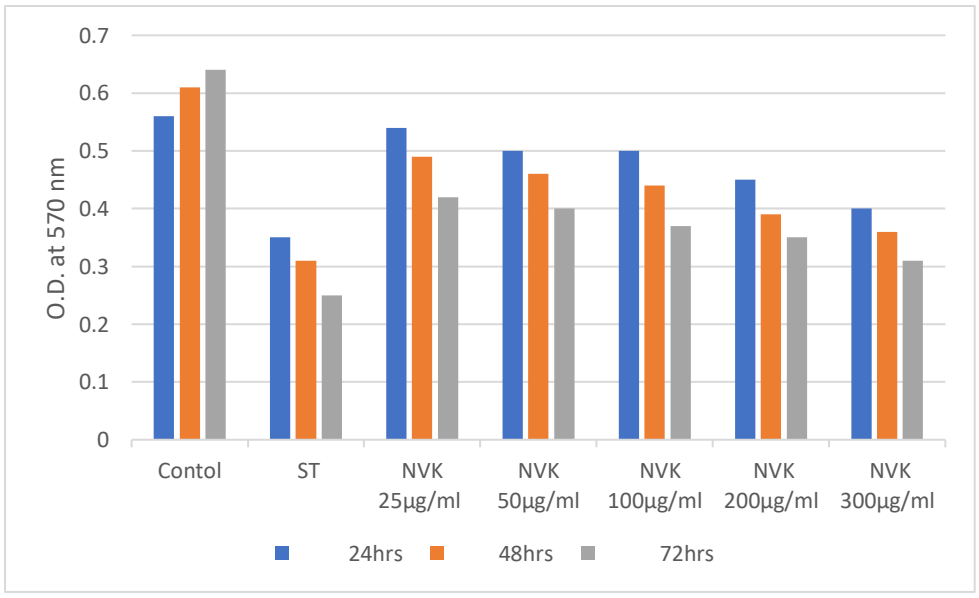

Figure No. 12: Effect of NVK extract on cell cytotoxicity by MTT assay on HepG2 cell lines.

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Formulation of Nilavembu kudineer *Tablets*

Nilavembu kudineer tablets were formulated as nine different batches in various concentrations of the excipients. Here we have used three different disintegrants such as Sodium starch glycolate, Cross caramellose, & Cross povidone. Only the concentrations of Lactose & MCC was varied. All the nine batches were directly compressed with methyl paraben, talc & magnesium stearate as preservatives, glidant & lubricant respectively. The total weight of the tablet was 150 mg with the drug content as 30 mg. The NVK methanolic extract was used for the formulation. Before compression the powder blends were subjected to pre-compression parameters.

The formulated various 9 batches were evaluated for their bulk density, tapped density, compressibility, angle of repose, and Hausner's ratio. The results obtained are reported in the Table No.9. The results show that all the values obtained were good and found to be within the limits.

Table No. 9 : Preformulation characteristics of Nilavembu kudineer tablet with different parameters

S.NO	Parameters	NK1	NK 2	NK3	NK4	NK5	NK6	NK7	NK8	NK9
1.	Angle of repose (°C)	29.61 ±4.7	29.89 ±4.2	28.60 ±3.8	29.54 ±3.2	30.45 ±3.9	29.47 ±2.5	29.83 ±1.8	30.32 ±1.3	30.19 ±1.9
2.	Bulk Density(gm/ml)	0.31 ±0.1	0.30 ±1.0	0.31 ±11.4	0.312 ±1.1	0.3120 ±0.3	0.3097 ±0.7	0.312 ±0.9	0.311 ±1.2	0.316 ±0.8
3.	Tapped Density(gm/ml)	0.352 ±0.5	0.348 ±0.3	0.351 ±0.5	0.3053 ±0.1	0.355 ±0.2	0.347 ±0.5	0.355 ±0.3	0.349 ±0.1	0.354 ±0.4
4.	Compressibility Index (%)	11.22 ±1.9	11.43 ±1.4	10.88 ±1.7	11.61 ±0.9	12.11 ±0.5	10.74 ±1.8	12.08 ±0.1	10.65 ±0.7	10.59 ±0.8
5.	Hausner's Ratio	1.12 ±1.45	1.12 ±0.26	1.12 ±1.78	1.131 ±0.42	1.137 ±0.28	1.120 ±1.25	1.136 ±0.29	1.119 ±1.75	1.118 ±1.22

n = ± 6

Post-Compression Evaluations of *Nilavembu kudineer Tablets*

The tablets of *Nilavembu kudineer* were compressed for all the 9 different batches from F1 to F9 by varying the concentrations of the excipients. The tablets obtained were oval shaped, and the surface was found to be smooth & shiny. The distribution of the plant powder can be seen. No picking, capping or sticking was observed. The compression was found to be satisfactory. Figure No. 18 & 19.

The tablets after compression were evaluated for thickness, hardness, weight variation, friability and disintegration. The results are presented in Table No.10. From the result it was observed that the thickness was about 3.12-3.45mm for the compressed tablets. The weight variation was also optimum within the limits. The hardness was found to be good with the batches NK1, NK4 and NK7. With the disintegration tests the batches NK1, NK4 and NK7 showed lowest disintegration comparatively to other batches. These are batches formulated with both MCC and lactose along with different disintegrating agents such as SSG, C.C, C.P. Table 1. So the batches NK1, NK4 and NK7 were

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selected as optimum batches as their disintegration time was comparatively low and their results were found to be within the limits.

The stability testing of the formulation was performed in two set of conditions. The results are given in Table 10.

Tablets were suitably packed and were kept for stability studies for 6 months at room temperature and refrigerator. The physicochemical changes i.e. change in physical appearance, hardness, weight variation, friability and disintegration time of the dosage form were determined at the end of 6th month. The results showed that there were only slight changes with all the parameters selected above.

Figure No.13 : Tablets of Nilavembu kudineer (NVK) extract

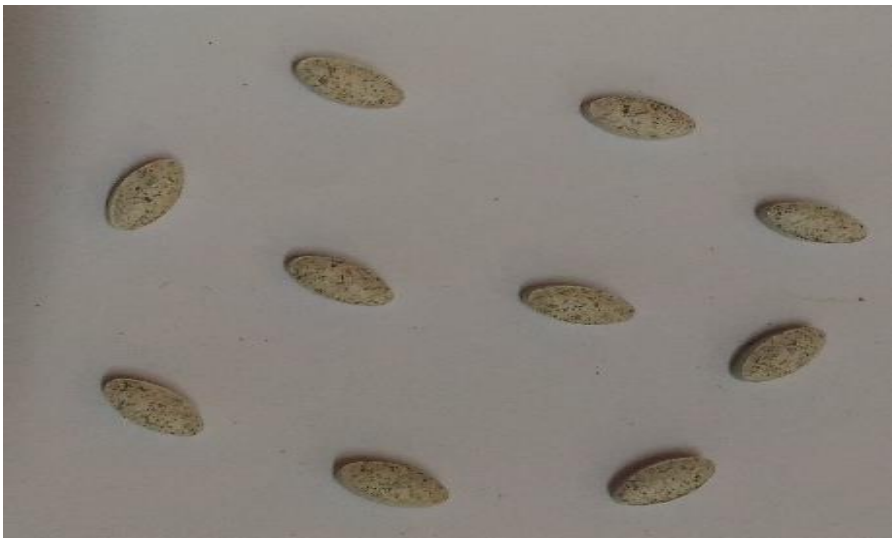

Table No. 10 : Post Compression parameters of Nilavembu kudineer tablets.

S.NO	Parameter	NK1	NK2	NK3	NK4	NK5	NK6	NK7	NK8	NK9
1	Thickness (mm)	3.22±0.2	3.45±0.14	3.26±0.13	3.53±0.08	3.12±0.17	3.18±0.09	3.29±0.11	3.42±0.16	3.36±0.07
2	Weight Variation (mg)	152± 1.51	149± 0.72	151± 0.77	155± 1.46	148± 1.35	150± 0.81	150± 0.99	156± 1.47	153± 1.43
3	Hardness (kg/cm^2)	4.42±1.23	4.12±0.67	4.02±0.72	4.82±0.79	4.25±1.15	3.28±1.12	4.24±0.82	4.02±0.99	3.81±0.77
4	Friability (%)	0.98 ± 0.25	1± 0.12	0.90 ±0.25	0.82 ±0.24	1± 0.18	0.92±0.20	1± 0.12	0.94±0.22	0.95±0.15
5.	Disintegration(min)	1.14±0.35	1.84±0.24	1.78±0.45	1.11±0.28	1.25±0.32	1.42±0.20	1.00±0.35	1.22±0.31	1.31±0.31

n =± 3

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Table No.11 : Stability studies of *Nilavembu kudineer* tablets

S.No	Parameters	NK 1	NK 2	NK 3	NK 4	NK 5	NK 6	NK 7	NK 8	NK 9
1	Physical appearance	**	**	**	**	**	**	**	**	**
2	Hardness **(kg/cm^2)**	4.82 ±0.84	4.43 ±0.45	4.51 ± 0.72	4.98 ± 0.38	4.36 ± 0.67	3.82 ± 0.87	4.59 ±0.83	4.35 ±0.95	4.08 ± 0.84
3	Weight variation **(mg)**	167 ±2.67	158 ±1.43	164 ± 2.57	173 ± 3.98	169 ± 1.56	172 ± 3.28	168 ± 3.78	175 ±2.56	171 ± 1.56
4	Friability **(%)**	0.85 ±0.23	0.96 ±0.35	0.80 ± 0.23	0.79 ± 0.69	0.98 ± 0.58	0.82 ±0.75	0.95 ±0.48	0.90 ±0.38	0.75 ± 0.65
5	Disintegration **(min)**	1.30 ±0.33	1.97 ±0.24	1.88 ± 0.47	1.30 ± 0.83	1.40 ± 0.46	1.54 ± 0.27	1.05 ±0.17	1.37 ±0.27	1.48 ± 0.36

** = no significant change

Conclusion

In conclusion, the comprehensive analysis of Nilavembu Kudineer (NVK) in this study yielded a highly promising results, highlighting its suitability for tablet formulation and potential applications in both traditional and modern medicine. The phytochemical analysis, including HPTLC and GC-MS studies, revealed the presence of significant flavonoids and terpenoids, as well as crucial compounds which contribute to its therapeutic properties. The *invitro* hepatotoxicity studies conducted with HepG2 cells demonstrated that NVK extract is safe and does not exhibit any hepatotoxic effects. Furthermore, the successful transformation of NVK extract into tablets, coupled with meeting the acceptance criteria for various quality control parameters, positions these tablets as a promising dosage form for NVK administration. This not only ensures the preservation of its phytoconstituents but also enhances its convenience and accessibility as a potential medicinal product. Overall, this research provides valuable insights into the phytochemistry, safety, and formulation potential of Nilavembu Kudineer, making a strong case for its integration into both traditional and modern medicinal practices, thereby expanding its scope and benefit for a wider population. Further studies and clinical trials may be warranted to confirm its efficacy and safety in practical clinical applications.

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