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RESEARCH ARTICLE

Standardisation of *Perunkaya Vaippu Sarakku* Using Organoleptic, Physicochemical, HPTLC Fingerprinting, GC-MS, and *In Vitro* Pharmacological Studies

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Abstract

Background: Asafoetida (*Perunkayam*), an oleo-gum resin derived from the roots of plants in the Ferula genus, is renowned for its efficacy as gastroprotective. It has been scientifically validated for its antioxidant, antispasmodic, neuroprotective, combating inflammation and hepatotoxicity, and various anticancer activities. However, asafoetida is not indigenous to India, and its scarcity has led to a significant price increase. *Siddha*, one of the oldest systems of medicine, utilises metals, herbs, and minerals to create synthetic formulations, a process known as *Vaippumuraigal*, to serve as alternatives to natural drugs. **Aim:** To standardize *Perunkaya Vaippu Sarakku* (PVS) using modern scientific methods. **Methods:** Synthetic *Perunkayam* was prepared and standardized through rigorous scientific procedures. **Results:** The prepared PVS was brownish powder, pH-4.7 and 5.73%w/w moisture. Phytochemical screening revealed the presence of alkaloids, carbohydrates, phytosterols, phenols, flavonoids, terpenoids and fixed oils. HTPLC yielded 8 fractions, Rf values ranging from 0.09-0.93. GC-MS revealed the presence of pentadecane, 2,4-di-tert-butylphenol, hexadecane, hexadecanoic and cis-vaccenic acid. PVS showed antioxidant activity compared to BHT (EC_{50}-235.9 and 6.597µg/mL respectively). The EC_{50} of acetylcholine in the presence of PVS (4.782µg/ml) was higher than Acetylcholine alone (3.678µg/ml) indicating effective antispasmodic activity. Experiments on goat renal artery undeluded endothelium (en^+) showed significant vasodilatory activity with an EC_{50} of 79.31µg/ml which was significantly ($p<0.001$) lower than denuded endothelium (352.4µg/ml). PVS showed significant cytotoxicity on colon-cancer (HCT-116) cell lines (IC_{50}-116µg/ml) and low cytotoxicity on HepG2 and Vero-cell lines (IC_{50}-538.5 and 1458µg/ml). PVS significantly ($p<0.001$) prevented the ethanol-induced damage in HepG-2 cell lines at 10µg/ml, inhibiting viral growth with a selectivity index of 11.26 and IC_{50} of 129.4µg/ml. **Conclusion:** PVS potently exhibited all the activities of natural asafoetida, advocated the claim of using PVS as an alternative to natural-asafoetida and standardization of the formulation confirmed the quality of PVS as per pharmacopeial standards in *Siddha* medicine.

Major Findings: Synthetic asafoetida potently exhibited all the activities of natural asafoetida (*Perunkayam*) and showed the remarkable similarity and also uncovering novel compounds in synthetic alternatives.

Keywords: Asafoetida, *In Vitro* Activities, *Perunkayam*, *Vaipu Sarakku*, Phytochemical Analysis

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1. Introduction

Oleo gum resin known as asafoetida is made from the rhizome and root of *Ferula foetida/narthex* and other plants in the *Ferula* genus of the *Umbelliferae* family. This spice is used in food, pickles, and as a digestive aid. Despite its unpleasant smell, asafoetida is effective at treating various conditions, including yeast infections, painful gas, flatulence, anxiety, and stomach aches. Asafoetida exhibits a diverse range of therapeutic and pharmacological properties, that includes sedation, neuroprotection, memory improvement, antioxidant, antispasmodic, hypotension, hepatoprotection, anticarcinogenisis, anthelmintic, antibacterial, anticancer, anti-obesity, anti-cytotoxicity, and providing antagonistic effects[1,2].

Siddha is the oldest system of medical system of Indian origin which is based on incorporating natural products for therapeutic values[3]. *Siddha* medicine is well known for preparing herbs-mineral preparations via a wide range of processing techniques such as purification using herbal juice resulting in reduced toxicity of the metals[4]. On the other hand, certain herbs were available and confined to a specific season or location. The non-availability of certain herbs at a particular season may not allow for the preparation of the particular formulation every time. To overcome this problem, *Siddhars* has described *Vaippumuraigal,* which means the preparation of substitute drugs. They can be prepared by mixing a few different herbs and following certain procedures to prepare similar natural preparations[5].

Vaippu Sarakku (VS) is a preparation of a special synthetic or semi-synthetic drug that is similar to the original drug used as a substitute for many rare natural products in *Siddha* medicine. Various references showcasing different methods of preparation of VS including asafoetida were available through literature[6]. PVS can be prepared by mixing a few herbs and following certain special procedures and the resultant product claims to possess similar physical, chemical, and pharmacological properties as the natural asafoetida[7].

Although there is an enormous impact of modern medicine with the great use of synthetic drugs, the use of traditional/herbal cures is supported and proven through various scientific publications as stated by WHO[8]. Though numerous *Siddha* formulations are affordable and potent without any notable adverse effects, the traditional evidence of therapeutic potency is not universal and popular[9]. Thus, standardization of *Siddha* medicines is an important step using scientific methods to prove the chemical composition, quality, and safety of the formulation to build trust and precision of the therapeutic use[10]. So, this research aims to prepare PVS and to standardize the formulation in terms of its physico-chemical, phytochemical, and pharmacological properties.

2. Materials and Methods

2.1 Chemicals

All the chemicals and culture media that are used in the research were procured from Sigma Aldrich, India, and were of analytical grade.

2.2 Cell Lines

HepG2 (human hepatoma cell lines), HCT-116 (human colon cancer cell lines), and Vero cell lines were procured from NCCS, Pune. Dulbecco's Modified Eagle Medium (DMEM) (pH-7.2) (Sigma-Aldrich, India; Code-D9443) supplemented with 10% FBS, Antibiotics (Penicillin+Streptomycin+Amphotericin-B Solution, 0.5 mL (Final concentration Penicillin: 10Units/mL, Streptomycin: 10µg/mL, Amphotericin-B: 25ng/mL) in a dynamic incubation system at 37°C in a 5% CO_2. After the 3rd to 5th succession culture, the cells were used for further analysis.

2.3 Preparation of *Perunkayam Vaippu Sarakku* (PVS)

All the ingredients in Table 1 were collected and authenticated by R. Aruna, Assistant Professor of Botany, Thiagarajar College, Madurai. The plant parts were shade-dried for 5-7 days based on the hardness of the drug. After all the ingredients were ready, 35 grams of cleaned 1, 2, 3, 4, 5, 6, and 7 drugs were ground into small pieces were placed in a small mud pot. 35 grams of mustard oil and the latex of *Sathurakkalli* were added to the ground pieces and mixed well. The pot was sealed using clay and the seal was allowed to dry. A 1.5m deep ditch was made and the pot was placed with straw pieces as a base. The ditch was covered with clay sand and the markings were made. The surface of the soil

was covered with a waterproof sheet and was left alone for 90 days. The pot was carefully taken out and after collection of contents of the pot they were powdered finely. This was stored in an air-tight container till use/ further analysis.

2.4 Evaluation of the Prepared Formulation

2.4.1 Identification and Organoleptic Characteristics

The identification test of PVS was done[11] and organoleptic properties like colour, odour, taste, and appearance were performed using standard procedures[12].

2.4.2 Physicochemical Evaluation

The physicochemical characteristics like the pH, ash values, moisture content, and extractive values were determined using standard procedures[13].

2.4.3 Phytochemical Analysis

The phytochemical analysis of PVS was performed by extracting it with distilled water using maceration for 3 days at 37°C in a beaker with occasional stirring. The extract (0.51g) was reconstituted with 50m distilled water and used to perform a phytochemical assay using standard procedures[14]. The extract was tested for various chemical constituents like carbohydrates, proteins, glycosides, alkaloids, phenols, steroids, flavonoids, tannins, volatile oils, fats, saponins etc[15].

2.4.4 HPTLC Fingerprinting Analysis

In a beaker about 2.5g of PVS was placed and ethanol was used for maceration for 24 hrs. The solution was filtered and concentrated. The dried extract was redissolved in ethanol and used for TLC analysis[16]. Pre-coated silica Gel 60 F_{254} (stationary phase) and Toluene: Ethyl acetate (7:3) (mobile phase) were used for the analysis. 5 µl of sample solution was spotted (0.2mm) on an HPTLC plate (Merck) using a Linomat-5 sample applicator. The mobile phase was run to 8cm distance and the visualization was done by scanning at 254 and 366nm using a CAMAG Reprostar[3].

2.4.5 GC-MS Study

The GC-MS study of the methanol extract of *P.hydropiper* was performed using the Thermo-GC-Trace Ultra(5) and Thermo-MS-DSQ(II) equipment. The analysis utilized a non-polar DB (35) MS column with Capillary Standard, measuring 30mmx0.25mmx0.25µm film. A flow rate of helium was set at 1ml/min. The determination of constituents was dependent on compound libraries by Willey and NIST, incorporating retention indices integrated into the GC-MS instrument for comparison[17,18].

2.4.6 In Vitro Pharmacological Screening

50gm of drug powder was packed and extracted with methanol (99.5% pure; Fisher Scientific, Mumbai) using a Soxhlet apparatus till clear solvent was run in the side tube. The solution in the RBF was collected and filtered using Whatman filter paper #1. The filtered extract was evaporated using a rotary evaporator to dryness and the thick paste of extract (MES) was stored in an air-tight container away from direct sunlight at 4°C till further use. In experiments involving organs, the methanol extract of PVS was reconstituted to the required dilutions using Tyrode's Solution hence named as MTS.

Table 1. Ingredients used to prepare *Perunkayam Vaippu Sarakku*

S. No.	Tamil name	Botanical name	Used part
1	*Chukku*	*Zingiber officinale* Roscoe	Dry rhizome
2	*Milagu*	*Piper nigrum* (L.)	Dry fruit
3	*Kadugu*	*Brassica juncea* (L.)	Seed of white variety
4	*Ulundumavu*	*Vigna mungo* (L.) Hepper	Flour of seed
5	*Veppampattai*	*Azadirachta indica* A. Juss.	Bark
6	*Murungapattai*	*Moringa oleifera* Lam.	Bark
7	*Omum*	*Carum copticum* L.	Seed
8	*Kadukuennai*	*Brassica juncea* (L.)	Seed oil
9	*Sathurakkallipaal*	*Euphorbia antiquorum* (L.)	Latex

2.4.6.1 Investigation of Antioxidant Activity

In a test tube, 100µl of MES of each concentration (50, 100, 150, 200, and 250 µg/ml) was mixed with 3ml of DPPH solution (24mg in 100ml methanol) and incubated in a dark place for 30min. The solution was filtered and the absorbance in UV was measured at 517nm using a double-beam UV spectrophotometer. The DPPH solution along with 100µl of methanol without MES was considered as the control solution. BHT (2, 4, 6, 8 and 10µg/ml). The percentage of antioxidant activity was calculated based on the absorbance of the control[19,20].

2.4.6.2 Investigation of Antispasmodic Activity

The chicken intestine tissue near the ileum and duodenum was collected from the local slaughterhouse. This was washed with distilled water to remove the attached contents and immediately placed in ice-cold Tyrode's Solution (TS) (Sigma Aldrich). Aeration was provided to the tissue in the laboratory. A 2cm section of ileum was isolated carefully and transferred into the TS immediately. An organ bath was filled with TS and maintained at 37±1 ºC and aerated using an organ tube at 1 or 2 bubbles/sec.

The S-shaped aerator tube and the frontal writing lever were attached to both ends of the tissue. The tissue was allowed to calibrate itself under a constant weight of 500mg for 15min and the tissue was washed at regular intervals of 3mins to wash out the metabolites accumulating in the tissue. Concentration dose-response curves (CDC) were drawn on the kymograph paper using Sherrington's recording drum at a constant rotation speed of 0.25mm/sec. Acetylcholine (ACh) (Sigma Aldrich) (1µg/ml) dose-response curves (CDC) were drawn at various concentrations (0.1, 0.2, 0.4, 0.8, and 1.6ml) with a contact time of 60sec and a baseline of 30 sec. Now the tissue is washed thrice with plain TS at 30 sec intervals. The reservoir was replaced with MTS (1µg/ml) and the CDCs were drawn with ACh at previous concentrations. In both cases, the height of the response curve on the kymograph was measured in cm and a graph was drawn accordingly[21,22].

Dose ratio = EC_{50} of Ach + MES/EC_{50} of ACh

2.4.6.3 Investigation of Vasodilatory Activity

After a goat was slaughtered in the local slaughterhouse, the renal artery was identified and approximately 3cm portion was isolated. Immediately the artery was transferred into a physiological saline solution (0.9%w/v NaCl)). This portion of the artery was trimmed off the attached fat and connective tissue and blood remnants were washed off using a saline solution. 10 rings were cut out of the artery with 2-3mm length and preserved in TS saline solution. To denude the endothelium, a wooden stick was soaked in TS for 30 min, and after ensuring complete soaking of the solution into the stick, it was rubbed into the lumen of the artery. The lumen was rubbed gently for 30sec duration and after visible tissue was seen on the surface of the stick the rubbing was stopped.

The vertical chamber of the organ bath was filled with TS and maintained at 37±1 ºC and aerated using an organ tube *at* 1 or 2 bubbles/sec. Two ends of the ring were inserted into the lumen without damaging the endothelium and a force transducer coupled with a bridge amplifier conditioned with a polygraph was attached at one end and the other to the organ bath. The resting weight of 1g was used to stabilize the tissue for 2 hours before the experiment by washing it multiple times at 15-minute intervals with the TS. The rings were exposed to the solution of phenylephrine (PE) (10 µM) (Sigma Aldrich Ltd. India) and the maximum contraction plateau was noted by determining the tension of the tissue. The solution was added with Acetylcholine (ACh) (10µM) (Sigma Aldrich Ltd. India) and the tension was noted. More than 80% of the relaxation indicated the integrity of the endothelium. The artery with denuded endothelium was also tested for the relaxation effect of the ACh at the same concentration which showed no effect on the contractions induced by PE. Thus, denudation of the endothelium was confirmed. After this, both tissues were washed with TS multiple times. The tissue was administered with KCl solution (60mM) and the concentration dose-response curve (CDC) was drawn till the tissue reached the plateau of contraction. During this phase, various concentrations of MTS (1, 5, 10, 20, 50, 100, 250, and 500 µg/ml) were added to the bath solution exposed for 15mins intervals. The responses were recorded by taking the plateau of the contraction as a control to determine the percentage relaxation[23-25].

The percentage relaxation was calculated using the formula:

$$\%\text{relaxation} = \frac{(Tk - Te)}{Tk} x100$$

Where,

Tk = tension due to KCl

Te = tension due to extract

2.4.6.4 Investigation of Cytotoxicity Assay

The 75-80 % confluent cells (HCT-116, Vero, and HepG2) were detached from the media bed using a detaching solution (0.2%-trypsin, 0.02%-EDTA, 0.05%-glucose in Phosphate Buffered Saline (PBS)). These cultures were grown in 15mL DMEM on 75cm^2 culture plates and reseeded in a 96-well plate after dilutions. Cell lines were seeded in a 96-well plate at 2×10^4 cells/ml/well. After incubating for 12 hours at 37°C in 5% CO_2, 100µl containing various doses of test samples at varying concentrations (1-1000µg/ml) was used to replace the medium. The cells were allowed to incubate for 48 hours. Cell viability was calculated using the MTT assay. The cells were washed using a sterile PBS twice and 20 µl MTT along with 5mg/mL PBS was added to each well, followed by incubation at 37°C in 5% CO_2 for 2–4 hours. The resultant cells were washed with excess PBS and mixed with 150µL DMSO per well with shaking for 10 minutes at 120rpm[26,27]. Absorbance was read at 570nm using a Microplate Reader using untreated cells as control. The values were taken as triplicate readings. The concentration displaying 50% cytotoxicity was determined as IC_{50}.

Cell proliferation was calculated as per the following formula:

% Cell Viability = (Ab1/Ab0) x100

Where Ab1 = absorbance of the test;

Ab0 = absorbance of the control

2.4.6.5 Investigation of Hepatoprotective Activity Against Ethanol-induced Damage

80% confluent HepG2 cell lines (6×10^4/well) were incubated in the 96-well tissue culture plates for 12 hours. The DMEM medium was replaced with 100µl containing various doses of test samples at varying concentrations and 24 hrs. The cells were exposed to 700mM of ethanol and incubated for 3 hrs[28,29]. Cell viability was calculated in a similar procedure as the cytotoxicity assay.

2.4.6.6 Investigation of Antiviral Activity

Isolation of Viral strain: SARS-CoV-2 was isolated from COVID-19 patients using a diagnostic nasal swab sample by the Microbiology Department of Govt Hospital. The viral isolates on the swab were applied to the monolayer of the Vero cells. The virus culture supernatant was collected after observing the cytopathic effect and the culture is stored at -10°C till further use.

2.4.6.6.1 Assay

80% confluent Vero cell lines (2.4×10^4) were incubated in the 96-well tissue culture plates for 12 hours. The cells were washed using PBS and exposed to the viral strain culture for 1hr at 32°C. The DMEM medium was replaced with 100µl containing various doses of test samples at varying concentrations. The cells were fixed using 100µl of 4% paraformaldehyde solution for 20min. 1%w/v solution of crystal violet in distilled water was used to stain the cells for 15min and the cells were incubated at 37°C in 5% CO_2 for 72 hours. 100µl of methanol was added to each well[30-33]. Absorbance was read at 570nm using a Microplate Reader using untreated cells with viral load as control.

cell proliferation was calculated as per the following formula:

% Viral Viability = (Ab1/Ab0) x100

Where Ab1 = absorbance of the test;

Ab0 = absorbance of the control

The concentration displaying 50% cytotoxicity on the vero cell lines was determined as CC_{50}. The concentration showing 50% viral inhibition was determined as IC_{50}

The selectivity index (SI)=CC_{50}/IC_{50}.

3. Results

PVS was prepared using the traditional method (Figure 1) and the obtained formulation was powdered and the organoleptic properties were determined. The appearance of the formulation is in the form of pale brown amorphous powder with an aromatic odour. The powder tasted acrid and dry to the touch.

3.1 Physicochemical Evaluation

The physicochemical evaluation of the prepared formulation, *Perunkayam Vaippu Sarakku* was performed and the results were under acceptable limits as per Indian Pharmacopeia and *Siddha* Pharmacopeia of India Part I, Vol I as shown in Table 2.

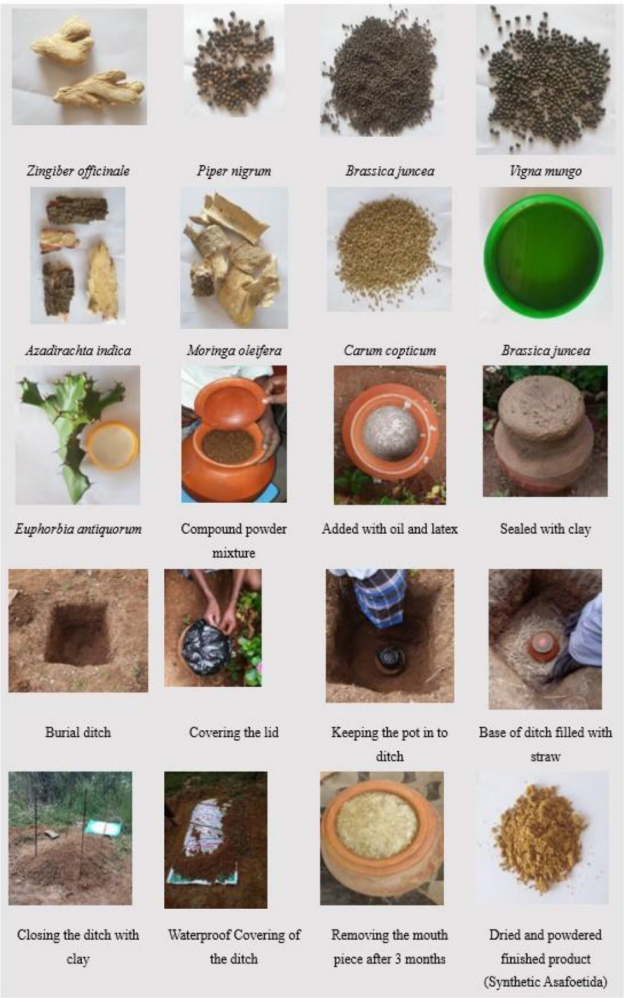


Figure 1. Various stages of preparation of *Perunkayam Vaippu Sarakku* (PVS).

3.2 Preliminary Phytochemical Screening

The identification and phytochemical screening of the prepared formulation was performed and the results indicate the presence of alkaloids, carbohydrates, phytosterols, phenols, flavonoids, proteins, terpenoids, and fixed oils as in Table 3.

3.3 HPTLC Fingerprinting Profile

The HPTLC analysis of *Perunkayam Vaippu Sarakku* was performed and the chromatogram indicates the presence of 8 peaks corresponding to different compounds with R_f values of 0.09, 0.24, 0.32, 0.42, 0.67, 0.80, 0.86, 0.93. as shown in Table 4 and Figure 2.

3.4 GCMS Profile

The GCMS analysis of the prepared formulation was performed and the results were tabulated in Table 5 and Figure 3. The corresponding similarities with existing molecules were recorded.

Table 2. Physiochemical parameters of the prepared *Perunkayam Vaippu Sarkku*

S. No.	Parameters	Value
1	Appearance and Description	Brown coloured powder
2	pH (1% w/v solution with distilled water)	4.77
3	Total ash	3.16% w/w
4	Acid insoluble ash	0.09% w/w
5	Loss on drying at 105°C	5.73% w/w
6	Water Soluble Extractive (WSE)	20.5% w/w
7	Alcohol Soluble Extractive (ASE)	20.2% w/w
8	Alcohol insoluble fraction	2.69% w/w

Table 3. Phytochemical screening of the prepared *Perunkayam Vaippu Sarakku*

S. No.	Parameters	Standard test	Result
1	Alkaloids	Mayer's test	+
2	Carbohydrates	Molish's Test	+
3	Glycosides	Modified Borntrager's Test	-
4	Cardiac glycosides	Keller Killani's test	-
5	Saponins	Froth's Test	-
6	Phytosterols	Salkowski's Test	+
7	Phenols	FerricchlorideTest	+
8	Tannins	GelatinTest	+
9	Flavonoids	AlkalineReagentTest	+
10	Protein and amino acid	NinhydrinTest	+
11	Gum	Extract+Alcohol Test	+
12	Mucilage	Extract+Alcohol Test	+
13	Fixed oil	Spot Test	+
14	Terpenoids	Salkowski test	+
15	Starch	Iodine Starch test	-

3.5 Antioxidant Activity of *Perunkayam Vaippu Markku*

The antioxidant activity of the Methanol Extract of *Perunkayam Vaippu Sarakku* (MES) was evaluated using the DPPH assay and compared with Butylated Hydroxytoluene (BHT) (Table 6 and Figure 4). MES

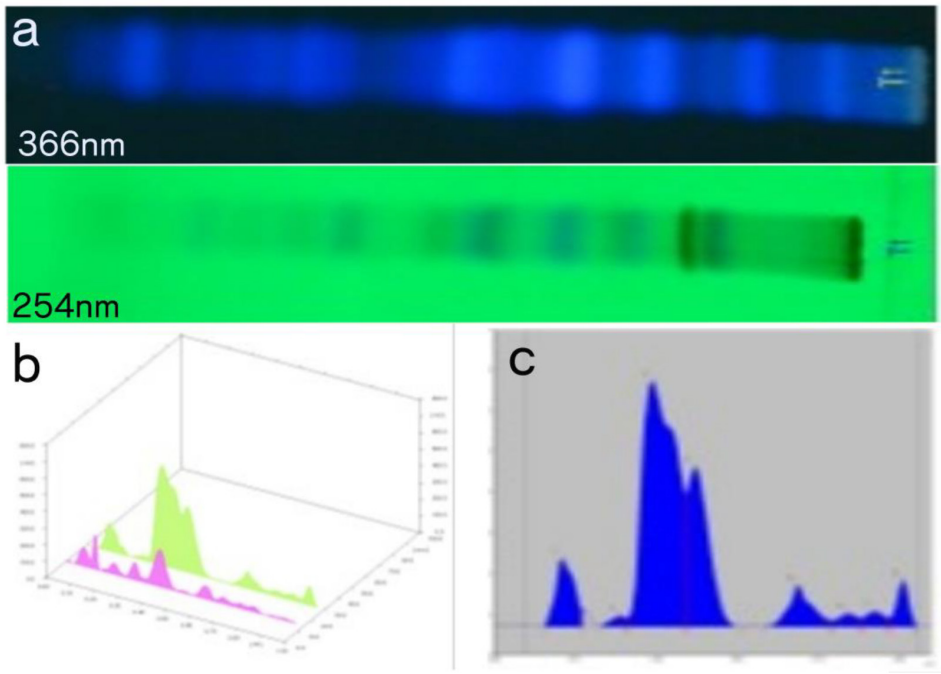


Figure 2. HPTLC Fingerprinting Profile of *Perunkayam Vaippu Sarakku*; **(a).** HTPLC plates visualized in UV; **(b).** Overlap chromatograms; **(c).** HPLC chromatogram.

Table 4. R_f values under UV 366nm for *Perunkayam Vaippu Sarakku* in HPTLC

Peak	Start R_f	Start Height	Max R_f	Max Height	Height %	End R_f	End Height	Area	Area %	Assigned Substance
1	0.05	0.3	0.09	165.9	11.6	0.14	44.5	5651.8	8.32	Unknown
2	0.18	0.2	0.24	25.1	1.72	0.25	21.8	674.7	0.99	Unknown
3	0.25	21.9	0.32	599.9	41.06	0.40	325.4	37474.6	55.18	Unknown
4	0.40	325.7	0.42	390.0	26.69	0.53	0.0	15022.3	22.12	Unknown
5	0.59	0.1	0.67	100.3	6.86	0.76	15.1	4198.6	6.18	Unknown
6	0.76	15.1	0.80	31.6	2.17	0.83	20.3	1112.1	1.64	Unknown
7	0.83	20.7	0.86	36.4	2.49	0.90	21.4	1279.0	1.88	Unknown
8	0.90	21.7	0.93	111.8	7.65	0.96	3.1	2502.0	3.68	Unknown

showed a dose-dependent increase in antioxidant activity, starting from 20.541 ± 1.749% at 50 µg/mL and reaching 54.252 ± 1.547% at 250 µg/mL. BHT used as a standard, displayed higher antioxidant activities at lower concentrations, with 32.201 ± 2.232% at 2 µg/mL and 60.356 ± 1.030% at 10 µg/mL. The EC50 value for MES was 235.9 µg/mL, higher comparable to BHT of 6.597 µg/mL.

3.6 Antispasmodic Activity of *Perunkayam Vaippu Sarakku*

At a dose of 0.1 mL, acetylcholine alone induced a response height of 6.416 ± 1.010 mm, which was reduced to 3.416 ± 1.127 mm in the presence of MES. As the dose increased, the contractile response for acetylcholine alone rose from 10.416 ± 0.877 mm at 0.2 mL to 25.083 ± 0.629 mm at 1.6 mL. The combination of acetylcholine and MES consistently resulted in a lower response height across all doses, ranging from 7.754 ± 0.661 mm at 0.2 mL to 20.833 ± 0.803 mm at 1.6 mL. The EC50 values of Acetylcholine alone were 3.678 mL and 4.782 mL in the presence of MES and the dose ratio was 1.300 as in Table 7 and Figure 5. These findings suggest that MES exhibits significant antispasmodic activity by reducing the contractile response induced by acetylcholine in the chicken ileum.

Table 5. Peak report of *Perunkayam Vaippu Sarakku* in GC-MS

Peak#	R.Time (R_t)	Area	Area%	A/H	Similarity	Peak Report TIC
1	7.120	45021	0.38	1.59	87	Phenol
2	8.929	35036	0.30	2.84	78	3-Ethyl-3-methyl heptane
3	9.033	59516	0.50	6.79	44	Isopropyl palmitate
4	10.036	34229	0.29	1.88	87	Decane, 2,3,5,8-tetramethyl-
5	11.937	35808	0.30	2.14	81	Benzene, 1,3,5-trichloro-
6	12.484	217169	1.83	2.05	96	Tridecane
7	14.253	59959	0.51	1.93	84	Decane, 2,3,7-trimethyl-
8	14.618	80010	0.67	2.53	84	Thymol
9	16.170	16723	0.14	1.57	86	Benzaldehyde, 3-hydroxy-
10	17.067	410734	3.46	2.00	97	Pentadecane
11	17.957	24199	0.20	2.87	68	Quinoline, 1,2-dihydro-2,2,4-trimethyl-
12	18.413	39474	0.33	2.18	85	2-Bromo dodecane
13	18.935	68434	0.58	2.49	84	Nonane, 3-methyl-5-propyl-
14	19.309	67476	0.57	1.83	89	2,4-Di-tert-butyl-phenol
15	20.369	590943	4.98	2.77	96	Dodecanoic acid
16	20.605	84543	0.71	4.92	54	4,8,12-trimethyltridecanoic acid, 2,2,3,3,4,4,4-heptafluorobutyl ester
17	21.183	262866	2.22	2.23	97	Hexadecane
18	22.513	104480	0.88	3.02	87	2(3H)- Benzothiazolone
19	22.983	6062	0.05	1.14	70	Methoxyacetic acid, 4-hexadecyl ester
20	24.883	45324	0.38	2.02	86	Heptadecane
21	25.163	48871	0.41	2.12	90	Pentadecanal-
22	26.263	57331	0.48	2.58	62	1-Heptyloxymethyl-4,5-dimethoxy-2-nitrobenzene
23	26.990	2275760	19.18	2.17	97	Hexadecanoic acid, methyl ester
24	27.058	138831	1.17	2.05	87	Benzenepropanoic acid, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy-, methyl ester
25	27.206	225865	1.90	2.86	94	Benzothiazole, 2-(2-hydroxyethylthio)-
26	27.555	169437	1.43	2.43	91	Octadecanoic acid
27	28.243	59683	0.50	2.80	66	2,2'- Methylenediphenol, trimethylacetate
28	28.564	119250	1.00	2.06	93	Eicosanal-
29	29.276	32587	0.27	2.02	72	Phenol, 4,4'-methylenebis-
30	29.670	775508	6.54	2.33	95	9,12-Octadecadienoic acid, methyl ester
31	29.788	2672859	22.52	2.35	95	9-Octadecenoic acid (z)-, methyl ester
32	29.883	63039	0.53	2.57	77	8- Octadecenoic acid, methyl ester
33	30.243	1663668	14.02	2.52	97	Methyl stearate
34	30.336	139473	1.18	3.19	75	Undec-10-ynoic acid, octadecyl ester
35	30.423	434397	3.66	4.82	87	cis-Vaccenic acid
36	38.697	93093	0.78	3.01	82	Methyl 11-docosenoate
37	39.170	53706	0.45	2.81	81	Bis(2-ethylhexyl) phthalate
38	39.900	90764	0.76	2.81	78	(E)-9-Octadecenoic acid ethyl ester
39	41.162	69411	0.58	2.61	70	4,4'- ((p-phenylene) di isopropylidene) diphenol
40	43.647	55552	0.47	2.12	52	5. alpha.- Cholestan-6.beta.-amine, N, N-dimethyl-
41	44.836	274989	2.32	3.61	88	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z,Z)-
42	47.181	64376	0.54	3.56	41	Nonadecanoic acid
		11866456	100.00			

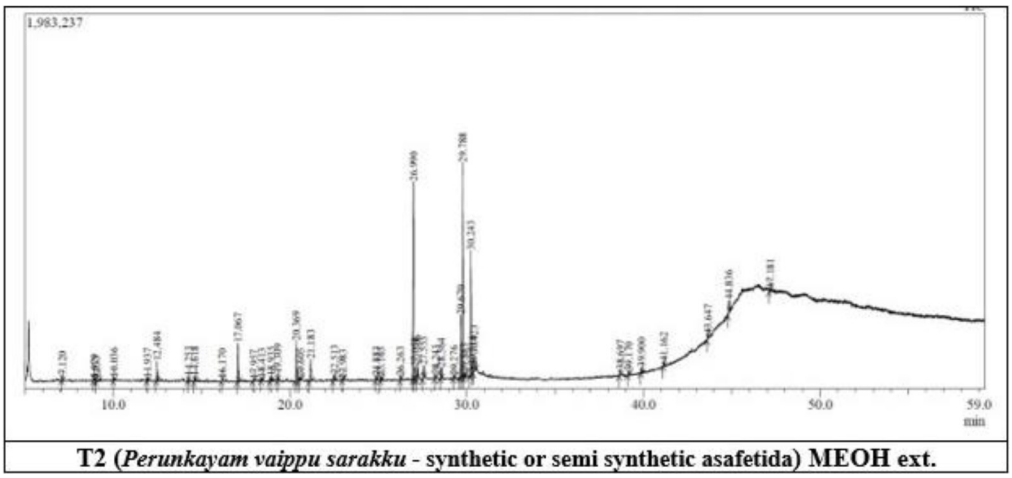


Figure 3. Chromatogram of *Perunkayam Vaippu Sarakku* in GC-MS.

Table 6. Antioxidant activity of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) in DPPH assay

Group	% antioxidant activity	Group	% antioxidant activity
MES (50 µg/mL)	20.541±1.749	BHT (2 µg/mL)	32.201±2.232
MES (100 µg/mL)	27.674±1.145	BHT (4 µg/mL)	36.831±1.414
MES (150 µg/mL)	35.733±1.080	BHT (6 µg/mL)	44.753±1.597
MES (200 µg/mL)	46.056±1.404	BHT (8 µg/mL)	52.023±0.831
MES (250 µg/mL)	54.252±1.547	BHT (10 µg/mL)	60.356±1.030
EC_{50}	235.9µg/mL	EC_{50}	6.597µg/mL

The values were expressed as mean±SD (n=3)

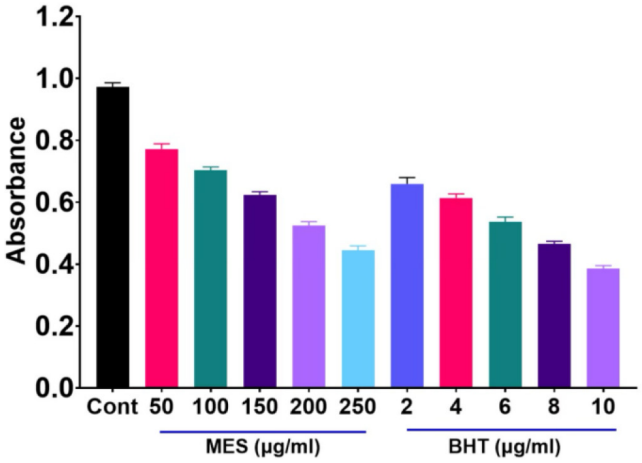


Figure 4. Antioxidant potential of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) in DPPH assay.

3.7 Vasodilatory Activity of *Perunkayam Vaippu Sarakku*

At a concentration of 1 µg/mL, *Perunkayam Vaippu Sarakku* induced a slight relaxation of 3.522 ± 0.547% in en+ conditions and 1.051 ± 0.344% in en- conditions. As the concentration increased to 5 µg/mL, the relaxation percentages were 6.108 ± 1.563% (en+) and 2.329 ± 0.709% (en-), with a significant difference observed in en+ ($p<0.05$). At the higher concentrations of 250 µg/mL and 500 µg/mL, the relaxation percentages were 69.376 ± 2.151% (en+, $p<0.001$) and 83.468 ± 1.608% (en+, $p<0.001$) respectively, compared to 41.279 ± 2.105% (en-) and 58.041 ± 1.433% (en-). These results indicate that formulation exhibits significant vasodilatory activity, with a greater effect observed in the presence of endothelium as shown in Figure 6 and Table 8.

3.8 *In Vitro* Cytotoxicity Activity of *Perunkayam Vaippu Sarakku* on Cell Lines

The cytotoxic effects of Methanol Extract of *Perunkayam Vaippu Sarakku* (MES) were evaluated on HCT-116 colon cancer, HepG2 Human Liver cancer, and Vero cell lines by measuring cell viability (Figure 7). At the lowest concentration of 1 µg/mL, MES exhibited minimal cytotoxicity with a cell viability of 97.449 ± 2.869% of HCT cell lines. As the concentration increased to 5 µg/mL and 10 µg/mL, the cell viability decreased to 91.137 ± 2.562% and 85.214 ± 2.474%,

Table 7. Antispasmodic activity of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) on chicken ileum

Dose (ml)	Height of the response in CDC (mm)	
	Acetylcholine	Ach+MES
0.1	6.416±1.010	3.416±1.127
0.2	10.416±0.877	7.754±0.661
0.4	14.333±0.763	12.166±1.127
0.8	20.254±0.901	16.836±0.800
1.6	25.083±0.629	20.833±0.803
EC_{50}	**3.678**	**4.782**
Dose ratio	**1.300**	

The values were expressed as mean±SD (n=3)

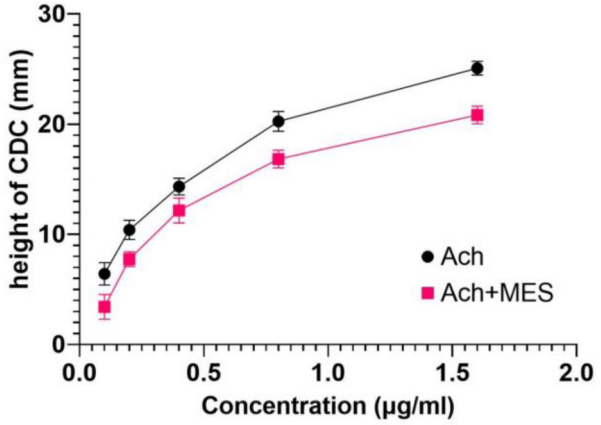


Figure 5. Antispasmodic activity of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) on chicken ileum.

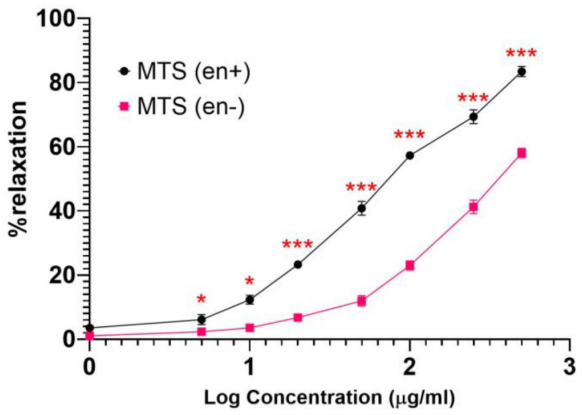


Figure 6. Concentration-response curves of MTS in vasodilatory assay.

en+=with endothelium; en-=without endothelium; *p<0.05, ***p<0.001 is significant compared to the en-

Table 9. Cytotoxic effect of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) on HCT-116 colon cancer cell lines

Concentration	Absorbance	% cell viability
Control	0.771±0.009	100±1.1887
MES 1µg/ml	0.751±0.022	97.449±2.869
MES 5µg/ml	0.702±0.019	91.137±2.562
MES 10µg/ml	0.657±0.019	85.214±2.474
MES 50µg/ml	0.526±0.011	68.309±1.492
MES 100µg/ml	0.409±0.012	53.091±1.611
MES 500µg/ml	0.185±0.011	24.038±1.457
MES 1000µg/ml	0.112±0.008	14.526±1.108
IC_{50}	**116µg/ml**	

The values were expressed as mean±SD (n=3)

Table 8. The vasodilatory activity of Methanol extract of *Perunkayam Vaippu Sarakku* in Tyrode's Solution (MTS)

Concentration (µg/ml)	Tension of the tissue (mN/mm)		%relaxation	
	MTS (en+)	MTS (en-)	MTS (en+)	MTS (en-)
1	11.316±0.064	11.606±0.04	3.522±0.547	1.051±0.344
5	11.013±0.183	11.456±0.083	6.108±1.563*	2.329±0.709
10	10.286±0.162	11.313±0.066	12.301±1.385*	3.551±0.567
20	9.002±0.141	10.936±0.061	23.267±1.208***	6.761±0.52
50	6.940±0.252	10.330±0.19	40.825±2.151***	11.932±1.626
100	5.010±0.132	9.033±0.168	57.274±1.127***	22.983±1.437
250	3.590±0.252	6.886±0.247	69.376±2.151***	41.279±2.105
500	1.936±0.188	4.920±0.168	83.468±1.608***	58.041±1.433
EC_{50}			79.31µg/ml	352.4µg/ml

The values were expressed as mean±SD (n=3); en+=with endothelium; en-=without entdothelium; *p<0.05, ***p<0.001 is significant compared to the en-

respectively. More substantial cytotoxic effects were observed at higher concentrations. At 50 µg/mL, the cell viability was reduced to 68.309 ± 1.492%, and at 100 µg/mL, it further decreased to 53.091 ± 1.611%. The most pronounced effects were seen at 500 µg/mL and 1000 µg/mL, where the cell viability was 24.038 ± 1.457% and 14.526 ± 1.108%, respectively as shown in Table 9.

For HepG2 cell lines, MES demonstrated a dose-dependent decrease in cell viability. At the lowest concentration of 3.125 µg/mL, cell viability was 98.207 ± 1.127%. This slightly reduced to 96.908 ± 1.231% at 6.25 µg/mL and further to 94.847 ± 0.787% at 12.5 µg/mL. At 25 µg/mL and 50 µg/mL, the cell viability decreased to 92.652 ± 2.486% and 89.919 ± 1.635%, respectively. A more substantial decrease was observed at 100 µg/mL (82.392 ± 1.148%, p<0.001) and 200 µg/mL (75.940 ± 2.647%, p<0.001) as shown in table 10. The IC50 value for HepG2 cells was calculated to be 538.5 µg/mL, indicating moderate cytotoxicity at higher concentrations.

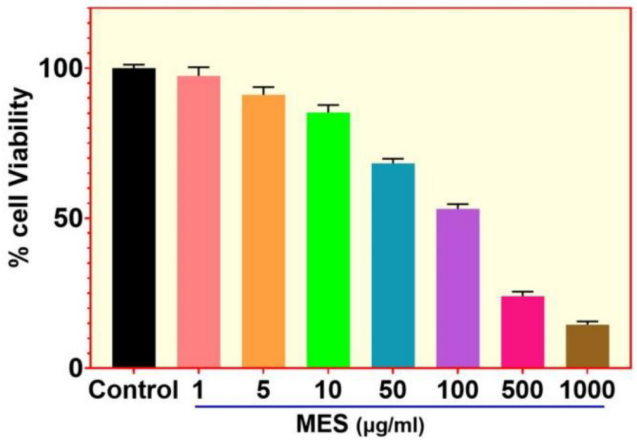


Figure 7. Cytotoxicity of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) on HCT-116 colon cancer cell lines.

Table 10. Effect of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) on the % cell viability of various cell lines

Concentration	% cell viability	
	HepG2 cell lines	Vero cell lines
Control	100±0	100±0
MES 3.125µg/ml	98.207±1.127	98.507±1.377
MES 6.25µg/ml	96.908±1.231	96.517±0.564
MES 12.5µg/ml	94.847±0.787	95.477±1.137
MES 25µg/ml	92.652±2.486	95.296±1.572
MES 50µg/ml	89.919±1.635	94.708±0.489
MES 100µg/ml	82.392±1.148***	92.672±0.755
MES 200µg/ml	75.940±2.647***	89.959±1.336
IC_{50}	**538.5µg/ml**	**1458µg/ml**

The values were expressed as mean±SD (n=3); ***p<0.001 is significant compared to the control group

For Vero cell lines, MES exhibited less cytotoxicity. At 3.125 µg/mL, cell viability was 98.507 ± 1.377%, which slightly decreased to 96.517 ± 0.564% at 6.25 µg/mL and 95.477 ± 1.137% at 12.5 µg/mL. The cell viability remained relatively stable at 25 µg/mL (95.296 ± 1.572%) and 50 µg/mL (94.708 ± 0.489%). Even at higher concentrations, the decrease in cell viability was gradual, with 92.672 ± 0.755% at 100 µg/mL and 89.959 ± 1.336% at 200 µg/mL. The IC50 value for Vero cells was determined to be 1458 µg/mL, suggesting a higher tolerance than HepG2 cells.

3.9 Hepatoprotective Activity of *Perunkayam Vaippu Sarakku* on Ethanol-induced Liver Damage

The hepatoprotective activity of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) was evaluated against ethanol-induced damage in HepG2 cell lines. Ethanol significantly reduced cell viability to 59.050 ± 2.833% compared to the control group (p<0.001), indicating substantial hepatocellular damage (Figure 8). MES treatment at various concentrations showed a dose-dependent protective effect against ethanol-induced cytotoxicity. At 5 µg/mL, cell viability increased

Table 11. Effect of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) on ethanol-induced damage in HepG2 cell lines

Concentration	%cell viability
Ethanol	59.050±2.833###
MES 1µg/ml	60.304±1.044ns
MES 2µg/ml	63.620±1.834*
MES 5µg/ml	65.501±1.486**
MES 10µg/ml	73.924±2.816***
MES 25µg/ml	81.989±1.323***
MES 50µg/ml	88.933±1.552***

The values were expressed as Mean±SD (n=3); ***p<0.001; **p<0.01; *p<0.05 nonon-significant compared to Ethanol group; ###p<0.001 compared to control group

to 65.501 ± 1.486% ($p<0.01$), and at 10 µg/mL, it significantly improved to 73.924 ± 2.816% ($p<0.001$). Further increases in MES concentration to 25 µg/mL and 50 µg/mL resulted in cell viabilities of 81.989 ± 1.323% and 88.933 ± 1.552%, respectively, both highly significant ($p<0.001$) compared to the ethanol group as shown in Table 11.

3.10 Antiviral Activity of *Perunkayam Vaippu Sarakku*

The antiviral activity of the Methanol extract of *Perunkayam Vaippu Sarakku* (MES) was assessed by measuring the viral viability in Vero cell lines at various concentrations (Figure 9). In the control group, viral viability was 100.904 ± 1.494%. Treatment with MES showed a concentration-dependent reduction in viral viability. At 3.125 µg/mL, MES reduced viral viability to 94.708 ± 2.385%, and further to 88.647 ± 3.33% at 6.25 µg/mL. As the concentration increased to 12.5 µg/mL, viral viability decreased to 82.767 ± 1.336%. Higher concentrations of MES resulted in more pronounced antiviral effects. At 25 µg/mL, the viral viability was reduced to 74.626 ± 2.842%, and at 50 µg/mL, it further decreased to 66.123 ± 1.5%. Significant reductions were observed at 100 µg/mL and 200 µg/mL, with viral viability of 58.38 ± 2.07% and 51.876 ± 2.176%, respectively. The IC_{50} value, indicating the concentration required to inhibit 50% of viral viability was 129.4 µg/mL (Table 12).

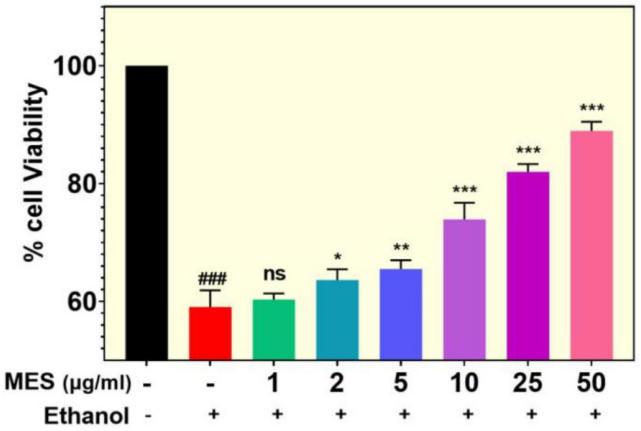


Figure 8. Hepatoprotective effect of MES on ethanol-induced damage in HepG2 cell lines.

Table 12. Effect of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) on the viral viability

Concentration	% viral viability in vero cell lines
Control	100.904±1.494
MES 3.125µg/ml	94.708±2.385
MES 6.25µg/ml	88.647±3.33
MES 12.5µg/ml	82.767±1.336
MES 25µg/ml	74.626±2.842
MES 50µg/ml	66.123±1.5
MES 100µg/ml	58.38±2.07
MES 200µg/ml	51.876±2.176
IC_{50}	129.4µg/ml
Selectivity index (SI)	11.26

The values were expressed as Mean±SD (n=3);

4. Discussion

Research in *Gunapaadam Mooligai Vaguppu* on original *Perunkayam* and ingredients of synthetic *Perunkayam* revealed a significant similarity in the pharmacological activities like the stimulant activity with *chukka, kagu* and *omam*, carminative activity with *milagu* and *omam*, antispasmodic activity with *omam*, aphrodisiac activity with *ulundu,* and emmenagogue activity with *murungaipattai*.

Natural asafoetida comes from plants of the *Ferula* genus of the *Umbelliferae*. It is made up of resins of ferulic acid, coumarins, sesquiterpene coumarins, and other terpenoids, sulfur-containing compounds, *monoterpenes,* and other volatile terpenoids in addition to umbelliferone, farnesiferols A, B, and C, vanillin, and traces of valeric acid being the most common. There

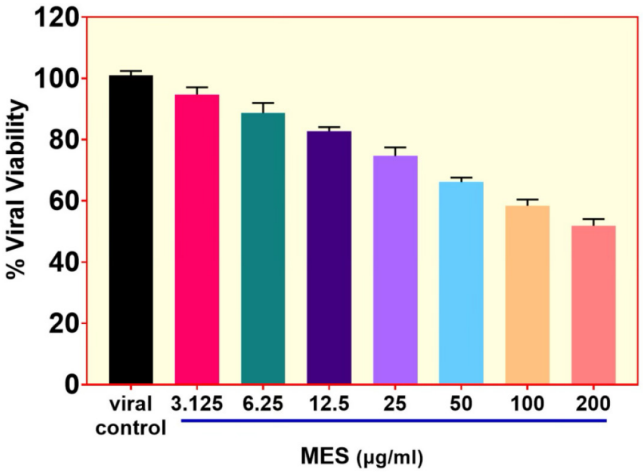


Figure 9. Antiviral activity of Methanol extract of *Perunkayam Vaippu Sarakku* (MES) in Vero cell lines.

were also similarities in the phytochemistry of natural asafoetida and prepared *Perunkayam Vaippu Sarakku* (PVS) with the presence of carbohydrates, phytosterols, phenols, protein and amino acids, gum, mucilage, fixed oil, and terpenoids. Natural *Perunkayam* contains saponins and essential oils which were absent in PVS and alkaloids, tannins and flavonoids are present in PVS but not in original *Perunkayam*.

As a part of standardization, the physiochemical properties of the prepared PVS were established and were found under limits as per the *Siddha* Pharmacopoeia and Indian Pharmacopoeia. The HPTLC fingerprinting profile of *Perunkayam Vaippu Sarakku* (T2) shows eight spots at R_f values of 0.09, 0.24, 0.32, 0.42, 0.67, 0.80, 0.86, 0.93. GC-MS of *Perunkayam Vaippu Sarakku* (T2) Methanol extract showed 42 compounds. The GC-MS study shows seven similar compounds in both original asafetida and *Perunkayam Vaippu Sarakku* samples, which are pentadecane, 2,4-di-tert-butylphenol, hexadecane, hexadecanoic acid methyl ester, 8-octadecenoic acid methyl ester eicosanal-, and methyl stearate significantly[34]. Further, some of the new compounds found only in PVS are Omega-7 fatty acid (cis-vaccenic acid), a type of trans-fatty acid that is present in human milk and is known for antimicrobial and lipid-lowering effects in rats[35]. Heptadecane showed its ability against inflammation in mature kidney tissues[36].

As a part of biological standardization, our study proved the anti-cancer effects of PVS on colon cancer cell lines and liver carcinoma with significant IC_{50} values which might be due to the presence of Nonadecanoic acid, an intermediate in the biodegradation of n-icosane, which was proven to inhibit cancer growth. Also, PVS significantly inhibited ethanol-induced liver damage and exhibited a relaxation effect on vascular tissue which might be due to the presence of Benzaldehyde 3-hydroxy, which possesses vasculoprotective effects by lowering inflammation in endothelial cells and proliferation of vascular smooth muscle cells[37]. The prepared formulation produced significant anti-viral activity in vero cell lines due to Oleo gum and terpenoids[38].

The prepared PVS showed antispasmodic activity which was investigated based on the previous work that proved the activity of natural asafoetida[39]. Antioxidant activity was also significant due to the presence of some compounds similar to the essential oils that are present in the original asafoetida[40].

Based on the results of the present study it can be advocated that the prepared *Perunkayam Vaippu Sarakku* had so many similarities in terms of phytochemistry and pharmacological profile to the natural *Perunkayam*. Thus, it can be claimed that the prepared formulation can be used as an alternative to the natural drug which shows similar therapeutic activity. Also, as the standardization tests resulted in the values under acceptable limits the high quality of *Perunkayam Vaippu Sarakku* is established.

5. Conclusion

In conclusion, the current scientific investigation into the *Siddha* concept of *Vaippu Sarakku,* for preparing synthetic medications, has served as an invaluable and potential alternative for costly and scarcely available original herbal drugs. Our research has underscored the remarkable similarity between the natural and synthetic forms of *Perunkayam* (asafoetida), while also uncovering novel compounds in synthetic alternatives. Further, this study lays the background for future clinical trials, ensuring a promising era in synthetic drug development in *Siddha* medicine aimed to address the scarcity and ensure safe and standard medications.

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7. Author Contribution

JV: Conception and design, Writing; SS: Software creation; RK: Oversight and leadership; RR: Research data management, visualization; PK: Study materials; AK: Data acquisition, analysis, and interpretation. All authors have read and agreed to the published version of the manuscript.

8. References

1. Barzegar A, Salim MA, Badr P, Khosravi A, Hemmati S, Seradj H, Iranshahi M. Persian asafoetida vs. sagapenum: challenges and opportunities. Res J Pharmacogn. 2020; 7(2):71-80.

2. Amalraj A, Gopi S. Biological activities and medicinal properties of Asafoetida: a review. J Tradit Complement Med. 2017; 7(3):347-359. https://doi.org/10.1016/j.jtcme.2016.11.004 PMid:28725631 PMCid:PMC5506628
3. Sudhaadevi PV, Chitra SM, Anbu N. Standardization of *Siddha* herbo mineral formulation Chanthirothaya Tablet (CHTM) for Rheumatoid Arthritis (RA). Int J Health Sci. 2022; 6(S2):2579-2588. https://doi.org/10.53730/ijhs.v6nS2.5764
4. Kandasamy Pillai K. History of *Siddha* Medicine. 12th ed. Thamarai Noolagam; 1979. p. 68.
5. Subbarayappa BV. *Siddha* medicine: an overview. Lancet. 1997; 350(9094):1841-1844. https://doi.org/10.1016/S0140-6736(97)04223-2 PMid:9428267
6. Sigicha Rathna Deepam. Rathnanayakkar and Sons; 2007. p. 223.
7. Thiyagarajan R, Ramanan G, Kalaimohan P, Iyswarya S, Tamilselvi S, Mathukumar S. *In-vitro* carminative activity and screening of heavy metals in *Vaippu Perungayam* - A *Siddha* polyherbal formulation. Eur Chem Bull. 2020; 12(7):1169-1175.
8. Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine (IUPAC Technical Report). Pure Appl Chem. 2008; 80(10):2195-2230. https://doi.org/10.1351/pac200880102195
9. Pandey A, Tripathi S. Concept of standardization, extraction and pre-phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem. 2014; 2(5):115-119.
10. Chitra SM, Anbu N. Heavy metal, aflatoxin, pesticide residue, microbial analysis of *Siddha* polyherbal formulation *Veppampoo Mathirai*. J Pharm Res Int. 2021; 33(54B):180-186. https://doi.org/10.9734/jpri/2021/v33i54B33778
11. *Siddha* Pharmacopoeia of India (SPI). 2008; 1(1):137.
12. India Pharmacopoeia (IP). Government of India, Ministry of Health and Family Welfare, Indian Pharmacopeia Commission. 2007; 1(1).
13. Lohar DR, Singh R. Quality control manual for Ayurvedic, *Siddha*, and Unani medicine. Ghaziabad: Department of AYUSH, Ministry of Health and Family Welfare, Pharmacopoeial Laboratory for Indian Medicine; 2008. p. 28-45.
14. Brain KR, Turner TD. The practical evaluation of phytopharmaceuticals. Bristol: Wright Scientechnica; 1975. p. 36-45.
15. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall; 1998.
16. Wagner H, Bladt S. Plant drug analysis: a thin-layer chromatography atlas. Springer Science and Business Media; 1996. https://doi.org/10.1007/978-3-642-00574-9
17. Konappa N, Udayashankar AC, Krishnamurthy S, Pradeep CK, Chowdappa S, Jogaiah S. GC-MS analysis of phytoconstituents from *Amomum nilgiricum* and molecular docking interactions of bioactive serverogenin acetate with target proteins. Sci Rep. 2020; 10(1):16438. https://doi.org/10.1038/s41598-020-73442-0 PMid:33009462 PMCid:PMC7532471
18. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. J Food Sci Technol. 2015; 52:1212-1217. https://doi.org/10.1007/s13197-013-1105-9 PMid:25694742 PMCid:PMC4325072
19. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules. 2022; 27(4):1326. https://doi.org/10.3390/molecules27041326 PMid:35209118 PMCid:PMC8878429
20. Sharma US, Kumar A. *In vitro* antioxidant activity of *Rubus ellipticus* fruits. J Adv Pharm Technol Res. 2011; 2(1):47-50. https://doi.org/10.4103/2231-4040.79805 PMid:22171292 PMCid:PMC3217685
21. Panaskar AN, Panaskar BA, Jadhav AR, Kale RP, Kale SD. *In-vitro* antispasmodic activity of aqueous leaves extract of *Coriandrum sativum* (*C. sativum*) Linn. Int J Adv Sci Res. 2023; 8(5):3698-3702.
22. Jain G, Bodakse SH, Namdev K, Rajput MS, Mishra S. Development of an *ex vivo* model for pharmacological experimentation on isolated tissue preparation. J Adv Pharm Technol Res. 2012; 3(3):176-181. https://doi.org/10.4103/2231-4040.101013 PMid:23057004 PMCid:PMC3459447
23. Raimundo JM, de Almeida RR, Velozo LS, Kaplan MA, Gattass CR, Zapata-Sudo G. *In-vitro* vasodilatory activity of the hexane extract of leaves and stems from *Piper truncatum* Vell. in rats. J Pharm Pharmacol. 2004; 56(11):1457-1462. https://doi.org/10.1211/0022357044779 PMid:15525454
24. Asaad AM, Kakey IS, Awez DJ, Mhamadsalih MM. *In vitro* study of the relaxant effect of doxazosin in the activity of smooth muscles in isolated goat renal artery. Iraqi J Vet Sci. 2023; 37(4):781-788. https://doi.org/10.33899/ijvs.2023.136609.2597
25. Geleta B, Makonnen E, Debella A, Abebe A, Fekadu N. *In vitro* vasodilatory activity and possible mechanisms of the crude extracts and fractions of *Moringa stenopetala* (Baker f.) Cufod. leaves in the isolated thoracic aorta of guinea pigs. J Exp Pharmacol. 2016; 8:35-42. https://doi.org/10.2147/JEP.S117545 PMid:27785112 PMCid:PMC5067057
26. Fu Q, Lyu D, Zhang L, Qin Z, Tang Q, Yin H, *et al.* Airborne particulate matter (PM2.5) triggers autophagy in human corneal epithelial cell line. Environ Pollut. 2017; 227:314-22. https://doi.org/10.1016/j.envpol.2017.04.078 PMid:28477555
27. Bhowmick D, Bhar K, Mallick SK, Das S, Chatterjee N, Sarkar TS, *et al.* Para-phenylenediamine induces apoptotic death of melanoma cells and reduces melanoma tumour growth in mice. Biochem Res Int. 2016; 2016:3137010.

https://doi.org/10.1155/2016/3137010 PMid:27293892 PMCid:PMC4886052

28. Shen H, Wang H, Wang L, Zhu M, Zhao S, Fan J, *et al*. Ethanol extract from the root of *Prunus persica* inhibited the growth of liver cancer cell HepG2 by inducing cell cycle arrest and migration suppression. Evid Based Complement Alternat Med. 2017; 2017:8231936. https://doi.org/10.1155/2017/8231936 PMid:29234432 PMCid:PMC5660787
29. Han SC, Huang RP, Zhang QY, Yan CY, Li XY, Li YF, *et al.* Antialcohol and hepatoprotective effects of tamarind shell extract on ethanol-induced damage to HepG2 cells and animal models. Foods. 2023; 12(5):1078. https://doi.org/10.3390/foods12051078 PMid:36900595 PMCid:PMC10000874
30. Majrashi TA, El Hassab MA, Mahmoud SH, Mostafa A, Wahsh EA, Elkaeed EB, *et al. In vitro,* biological evaluation and *in silico* insights into the antiviral activity of standardized olive leaves extract against SARS-CoV-2. PLoS One. 2024; 19(4):e0301086. https://doi.org/10.1371/journal.pone.0301086 PMid:38662719 PMCid:PMC11045091
31. Alagarasu K, Patil P, Kaushik M, Chowdhury D, Joshi RK, Hegde HV, *et al*. *In vitro* antiviral activity of potential medicinal plant extracts against dengue and chikungunya viruses. Front Cell Infect Microbiol. 2022; 12:866452. https://doi.org/10.3389/fcimb.2022.866452 PMid:35463636 PMCid:PMC9021897
32. Park JS, Bae J, Jung J, Kim JS, Park SJ. *In vitro* antiviral activity of abietane diterpenoids isolated from *Torreya nucifera* against rotavirus infection. Acta Virol. 2023; 67:11630. https://doi.org/10.3389/av.2023.11630
33. Pizzorno A, Padey B, Dubois J, Julien T, Traversier A, Dulière V, *et al. In vitro* evaluation of antiviral activity of single and combined repurposable drugs against SARS-CoV-2. Antiviral Res. 2020; 181:104878. https://doi.org/10.1016/j.antiviral.2020.104878 PMid:32679055 PMCid:PMC7361110
34. Prasathkumar M, Anisha S, Dhrisya C, Becky R, Sadhasivam S. Therapeutic and pharmacological efficacy of selective Indian medicinal plants - a review. Phytomedicine Plus. 2021; 1(2):100029. https://doi.org/10.1016/j.phyplu.2021.100029
35. Qadir A, Aqil M, Ali A, Ahmad FJ, Ahmad S, Arif M, *et al*. GC-MS analysis of the methanolic extracts of *Smilax china* and *Salix alba* and their antioxidant activity. Turk J Chem. 2020; 44(2):352-363. https://doi.org/10.3906/kim-1907-5 PMid:33488162 PMCid:PMC7671229
36. Kim DH, Park MH, Choi YJ, Chung KW, Park CH, Jang EJ, *et al*. Molecular study of dietary heptadecane for the anti-inflammatory modulation of NF-kB in the aged kidney. PLoS One. 2013; 8(3):e59316. https://doi.org/10.1371/journal.pone.0059316 PMid:23555655 PMCid:PMC3608635
37. Kong BS, Im SJ, Lee YJ, Cho YH, Do YR, Byun JW, *et al*. Vasculoprotective effects of 3-hydroxybenzaldehyde against VSMCs proliferation and ECs inflammation. PLoS One. 2016; 11(3):e0149394. https://doi.org/10.1371/journal.pone.0149394 PMid:27002821 PMCid:PMC4803227
38. Iranshahy M, Iranshahi M. Traditional uses phytochemistry, and pharmacology of asafoetida (*Ferula assa-foetida oleo-gum*-resin): a review. J Ethnopharmacol. 2011; 134(1):1. https://doi.org/10.1016/j.jep.2010.11.067 PMid:21130854
39. Fatehi M, Farifteh F, Fatehi-Hassanabad Z. Antispasmodic and hypotensive effects of *Ferula asafoetida* gum extract. J Ethnopharmacol. 2004; 91(2-3):321-324. https://doi.org/10.1016/j.jep.2004.01.002 PMid:15120456
40. Dehpour AA, Ebrahimzadeh MA, Fazel NS, Mohammad NS. Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. Grasas Aceites. 2009; 60(4):405-412. https://doi.org/10.3989/gya.010109