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Physicochemical Evaluation and Phytochemical Characterization of Baicalin in Neeradimuthuvallathy Mezhugu: A Herbomineral Siddha Formulation for Diabetes Management

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ABSTRACT:

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, insulin resistance, and oxidative stress, affecting millions worldwide. Traditional medicine, particularly the Siddha system, offers holistic and natural therapeutic approaches for diabetes management. Neeradimuthuvallathy Mezhugu (NM), an herbomineral Siddha formulation, has been traditionally used to manage chronic ailments, including diabetes. This study aims to scientifically evaluate the physicochemical, phytochemical, and safety parameters of Neeradimuthuvallathy Mezhugu (NM), with a special focus on baicalin, a bioactive flavonoid known for its anti-diabetic properties. Physicochemical analysis of Neeradimuthuvallathy Mezhugu revealed optimal moisture content (14.81%), ash values- total ash (5.21 %) & acid-

insoluble ash (1.28 %), and pH (5.63), Alcohol soluble Extractive (39.96%), ensuring the formulation's stability and quality. Phytochemical screening confirmed the presence of flavonoids, phenolic compounds, tannins, and alkaloids, all of which contribute to the drug's pharmacological effects. Chromatographic analysis using TLC and HPTLC confirmed baicalin's presence, reinforcing the drug's bioactive potential. Safety analysis established that Neeradimuthuvallathy Mezhugu was free from toxic heavy metals, pesticide residues, microbial contaminants, and aflatoxins. It also exhibited significant antimicrobial activity against Gram-positive and Gram-negative bacterial pathogens, suggesting a protective role against infections commonly associated with diabetes. The study provides strong scientific validation for NM as a safe and effective Siddha formulation.

Keywords: Siddha medicine, Neeradimuthuvallathy Mezhugu, diabetes mellitus, baicalin, phytochemical analysis, antimicrobial activity, traditional medicine.

1. INTRODUCTION:

Diabetes mellitus, a common metabolic disorder marked by chronic hyperglycemia, affected around 415 million adults worldwide in 2017, with projections estimating this figure will rise to 693 million by 2045. This condition brings about considerable health burdens and heightens the risk of systemic complications, such as cardiovascular diseases [1], [2]. Diabetes mellitus has developed into a global health crisis, with recent research showing a significant increase in its prevalence. In 2022, the number of adults globally living with diabetes exceeded 800 million, a figure that has increased more than fourfold since 1990. This increase is especially evident in countries with low and middle incomes, as treatment options are scarce in these places. Consequently, a great number of people are at risk for serious complications. [3]

In India, the prevalence of diabetes has reached alarming levels, positioning the country among those with the highest number of cases globally. This escalating health burden has prompted a renewed interest in traditional medical systems, such as Siddha medicine, for potential solutions. Traditional medicinal systems, such as Siddha medicine, have long utilized herbomineral formulations to manage various ailments, including diabetes. One such formulation is Neeradimuthuvallathy Mezhugu, which incorporates both herbal and mineral components believed to synergistically enhance therapeutic efficacy. Among its constituents, baicalin, a flavonoid, has garnered attention for its potential anti-diabetic properties. [4], [5]

Recent studies have elucidated the mechanisms by which baicalin exerts its antidiabetic effects. It has been reported that baicalin has been shown to interact with the pleckstrin homology domain of AKT, leading to the activation of the AKT/GSK3β signaling pathway, which plays a crucial role in glucose metabolism. Additionally, baicalin has demonstrated inhibitory activity against α-glucosidase, an enzyme involved in carbohydrate digestion, thereby contributing to improved postprandial blood glucose levels. [6]

Traditional medicine, especially the Siddha system of medicine, traces its origins to South India and is one of the major global healthcare providers. The efficacy of Indian traditional medical systems like Siddha has a long history comprising Polyherbal and Herbomineral Formulations. The Siddha system of medicine encompasses many preparations for treating diabetes, and one such Herbomineral formulation is Neeradimuthuvallathy Mezhugu (NM). The reference for the drug NM is taken from the Siddha text Anuboga Vaidya Navaneetham, authored by Abdulla Saibu, with the indication of diabetes [7]. A comprehensive physicochemical and phytochemical characterization of this formulation, with a focus on its baicalin content, could provide valuable

insights into its therapeutic potential and enlighten its integration into contemporary diabetes management procedures.

1.1 INGREDIENTS OF NEERADIMUTHUVALLATHY MEZHUGU (NM):

The ingredients of the drug Neeradimuthuvallathy Mezhugu (NM) are given in Table 1.

S.NO.	VERNACULAR NAME (TAMIL NAME)	BOTANICAL NAME
01	Serankottai	Semicarpus anacardium
02	Purified Neeradimuthu	Hydnocarpus kurzii
03	Parangipattai	Smilax china
04	Pirappan kizhangu	Calamus rotang
05	Karunseeragam	Nigella sativa
06	Seeragam	Cuminum cyminum
07	Vasambu	Acorus calamus
08	Sivanarvembu	Indigofera aspalathoides
09	Sanganver	Azima tetracantha
10	Karudan Kizhangu	Corallocarpus epigaeus
11	Amukkura Kizhangu	Withania somnifera
12	Vellarugu	Enicostemma littorale
13	Erukkanver	Calotropis gigantean
14	Athipattai	Ficus racemosa
15	Saranaiver	Boerhaavia diffusa
16	Milakaranai Ela	Toddalia asiatica
17	Veppam paruppu	Azadiracta indica
18	Vetpalai arishi	Wrightia tinctoria
19	Ellu	Sesamum indicum
20	Neeli samoolam	Indigofera tinctoria
21	Purified rasam	Hydragyrum
22	Purified Ganthagam	Elemental Sulfur
23	Purified thurusu	Copper Sulfate
24	Purified pal thuththam	Zinc Sulfate
25	Nei	Bos indicus

Table 1. Ingredients of Neeradimuthuvallathy Mezhugu (NM)

2. MATERIALS & METHODS:

2.1 Procurement of the Medicine:

The drug Neeradimuthuvallathy Mezhugu (NM) was purchased from a GMP-certified Siddha Drug Manufacturing Company in Chennai, Tamil Nadu, India.

2.2 Physicochemical Analysis of NM:

Physicochemical investigations were carried out in accordance with WHO and PLIM guidelines, including loss on drying, total Ash value, acid-insoluble ash, water-insoluble ash, alcohol soluble extractive, pH, reducing sugar, and total sugar [8], [9]. The Physicochemical analysis was carried out at Siddha Central Research Institute, Arumbakkam, Chennai.

2.3 Phytochemical analysis of NM:

The preliminary Phytochemical analysis, TLC & HPTLC, Residual analysis such as Pesticide residues, Heavy metals and Aflatoxins, Microbial load, and the antimicrobial activity of NM were carried out at the Regional Research Centre for Unani Medicine, Chennai.

2.3.1 Preliminary Qualitative Analysis of Phytochemicals:

NM Ethanol extract was subjected to preliminary phytochemical analyses such as flavonoids, terpenes, saponins, phenolic compounds, tannins, steroids, proteins, monosaccharides, reducing sugars, carbohydrates, and alkaloids by standard conventional protocols. [10], [11], [12].

2.3.2 TLC & HPTLC fingerprint of NM:

A soxhlet apparatus was used to accurately dry 5 g of NM with 50 mL of ethanol solvent for 30 minutes at 40 °C. This process was done three times to ensure full extraction. Following completion, the extract was stored in closed glass vials for examination after being filtered via Whatman's filter paper.

As mobile phase, the TLC plate was developed using Toluene: Ethyl acetate: Formic acid (6.5: 3.5: 0.01). TLC plates coated with silica gel 60 F254 for HPTLC (0.2 mm thickness, 5–6 µm particle size) were purchased from Merck, Darmstadt, Germany. All the solvents and chemicals were purchased from manufacturers- Ethanol, Sigma Aldrich, Darmstadt, Germany; Methanol, Sigma Aldrich, Darmstadt, Germany; Lead, Merck, Life Sciences Pvt Ltd, Mumbai, India; Cadmium, Arsenic and Mercury, Sigma Aldrich, St. Louis, USA; L-Ascorbic acid, Sisco Research Laboratories, Pvt Ltd, Maharashtra India; DPPH (2,2- diphenyl-1-picryl hydrazyl) Sisco Research Laboratories Pvt Ltd, Maharashtra, India; Nitric acid, Merck Life Sciences Pvt Ltd., Mumbai India. Standard Baicalin was purchased from Sigma Aldrich, Bangalore, India.

The ethanolic extract of NM was subjected to HPTLC fingerprinting to obtain the nature of different metabolites present along with the standard Baicalin. The number of peaks, areas of the peak, and Rf values were recorded [12], [13]. The best resolution was developed in the Toluene: ethyl acetate: formic acid (7:3:0.01, v/v/v) and was the finest qualitative analysis combination. HPTLC fingerprinting was carried out by using the CAMAG HPTLC system (Switzerland) with CAMAG Automatic TLC sample applicator (ATS 4). The entire study was performed in an air-conditioned room maintained at 25ºC and 55 % humidity. A spectro-densitometer (Scanner 3, CAMAG) equipped with Win CATS- planar chromatography manager (version 1.4.9.2001) software was used for the densitometry measurements, spectra recording, and data processing.

2.4 Residual Analysis of NM:

2.4.1 Pesticide Residues:

Pesticide residues, which build up from agricultural activities like spraying, treating soils during cultivation, and using fumigants during storage, may be present in herbal medicines. Hence, a test for pesticide residues was performed using the LCMS method.

2.4.2 Heavy Metal Analysis:

Heavy metal analysis was carried out to detect toxic metals that might be present in the formulation. Metals such as Lead (Pb), Cadmium (Cd), Arsenic (As), and Mercury (Hg) were analyzed using atomic absorption Spectropotometry (AAS) (Thermo Scientific, iCE 3000 model) using SOLAAR, AA Software, Version 11.10. The analysis was carried out at RRIUM, Chennai.

2.4.3 Determination of Aflatoxin:

Aflatoxins are a group of naturally occurring toxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, two common mold species. AflaTest is a quantitative method for the detection of aflatoxin in B1, B2, G1, G2, M1, and M2. The test was carried out using afla-test fluorometer

2.5 Determination of Microbial load:

The microbial quality was tested according to the regulations, including the isolation and identification of pathogenic bacteria [8]. The tests were used to quantify the number of bacteria and fungi isolated that can grow aerobically in 1 g of the sample. All dehydrated media were prepared according to the manufacturer's instructions and seeded and incubated at 37 °C for 24 to 48 hours for bacterial screening and at 25 °C for 48 to 72 hours for fungal screening. At the end of the incubation period, the number of colony-forming units per gram (CFU/g) was calculated by multiplying the average number of colonies by the dilution factor. The obtained CFU/g of the sample was compared with WHO standards.

2.5.1 Test for specific Pathogens:

For bacterial isolation and identification, the samples were diluted in water or Tween according to their solubility and homogenized by vigorously mixing. The 1 mL aliquots were transferred to 9 mL of peptone broth and cultured at the recommended time and temperature. All microbial analyses were carried out in triplicate. For investigating *Escherichia coli*, *Salmonella spp*., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* EMB agar, MacConkey agar, Deoxycholate citrate agar, Cetrimide agar, and Mannitol salt agar culture media were used respectively. At the end of the incubation period, pathogenic bacterial isolates were preliminarily characterized by colony morphology, Gram staining, and biochemical tests (oxidase, gas, and catalase production).

2.6 Antibacterial Study

The solvent and chemicals used in this study were analytical and laboratory grade and procured from Merck chemicals; the media for this study were purchased from Hi-media. To evaluate the microbial studies, a few cultures were procured from the Clinical Laboratory in and around Chennai. The organisms used were *Staphylococcus aureus* (ATCC-29213), *Bacillus cereus* (Clinical Cochin University) (Gram-positive), *Escherichia coli* (ATCC-25922), *Klebsiella pneumonia* (ATCC-700603) (Gram-negative), and *Candida albicans* (Clinical Cochin University). All the organisms were confirmed using specific biochemical tests [14].

2.6.1 Medium Preparation

38 g of Mueller Hinton Agar (M-H Agar) Sigma-70191 was suspended in 1000 ml of distilled water. Boiled to dissolve the medium completely and sterilized by autoclaving at 121°C for 15 minutes. A lag phase uniform suspension of all the organisms listed above was prepared by inoculating a loopful of each culture in 10 ml of nutrient broth, incubated at 37ºC for about 6 to 8 hours. 65 g of Sabouraud dextrose agar (SDA) powder was suspended in 1000 ml of distilled or deionized water. It was mixed well and heated to boil, shaking frequently until completely dissolved and sterilized in an autoclave at 121°C for 15 minutes.

2.6.2 Sample preparation

10 g of Siddha formulation NM were dissolved in 50 ml of hydroalcohol (1:1), and the sample was kept in a shaking incubator for 24 to 48 hours. After the complete solubility of the formulation, the solution was filtered through Whatman no.1 filter paper. The filtered extract was evaporated in a hot air oven at 40°C for one day. The final extract was weighed and dissolved using the same solvent at 25 & 50 mg/ml concentration used for further assay.

2.6.3 Antimicrobial activity of formulation:

The Agar well diffusion method tested the drug NM for its antimicrobial activity. The autoclaved media was mixed thoroughly while still hot and then added to 100 mm Petri plates (25-30 ml/plate). The bacterial strains that had been cultured for 24 hours were transferred to the medium. The wells, which were 10 mm in diameter and made from agar, were filled with 100 µl of diluted formulation at different concentrations (25 & 50 mg/mL). The commercially available drugs Amp: Ampicillin 10mcg; Nx: Norfloxacin 10mcg; Ap: Amphotericin 20mcg were used as controls. The plates were then incubated at 37 °C for bacteria and 30 °C for fungus for 12 to 24 hours. After 24 hours of incubation, the zone of inhibition of the organism were measured [15].

3 RESULTS:

3.1 Physicochemical

The Physicochemical analysis of the drug NM is given in Table 2.

S.No	Name of the Experiment	Mean value
1.	Loss on Drying	14.81%
2.	Total Ash	5.21 %
3.	Acid Insoluble Ash	1.28 %
4.	Water soluble Extractive	39.18%
5.	Alcohol soluble Extractive	39.96 %
6.	pH (10 % Solution)	5.63
7.	Reducing sugar	11.27%
8.	Total sugar	31.87%

Table 2. Physicochemical analysis of NM

3.2 Phytochemical Screening:

The Preliminary Phytochemical screening of the drug NM is given in Table 3.

S.NO.	Preliminary Test	Methanol extract	Hydro-alcohol extract
1	Alkaloids	+	-
2	Carbohydrates	+	+
3	Glycosides	-	-
4	Proteins & amino acids	-	-
5	Flavonoids	-	+
6	Phenolic compounds	-	+
7	Tannins	-	+
8	Phytosterols	-	+
9	Cholesterol	-	-
10	Terpenoids	-	-
11	Quinones	-	-
12	Anthocyanin	-	-
13	Carboxylic acid	-	-
14	Gums & mucilage	-	-
15	Fixed oil & fat	-	+

Table 3. Preliminary Phytochemical Screening of NM

3.3 Thin Layer Chromatography:

The TLC and HPTLC Fingerprint of the drug NM at 254nm is given in Figures 1, 2, 3, and the densitometric chromatogram of NM is given in Figure 4

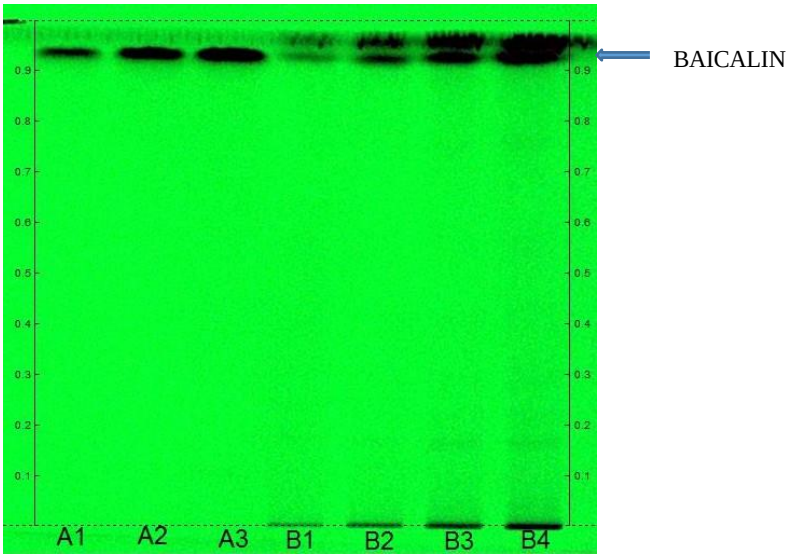
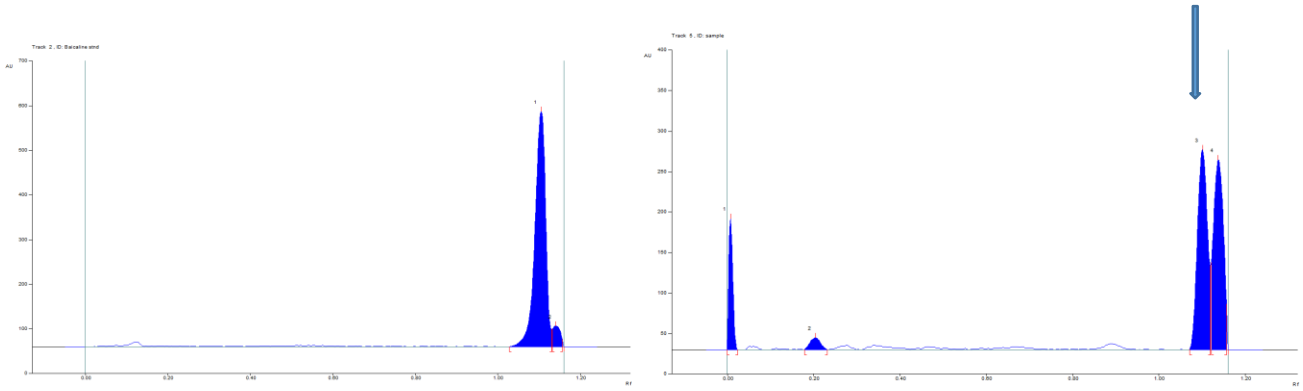


Fig 1. Presence of band in NM (B1- 2.0 µl, B2- 4.0 µl, 6.0 µl, B4- 8.0 µl) corresponding to standard Baicalin (A1-1.0 µl, B2- 2.0 µl, A3- 3.0 µl) at UV- 254 nm (Absorbance mode)

BAICALIN



a b

Fig 2. HPTLC Fingerprint of Standard Baicalin- a & NM - b, UV – 254 nm (Absorbance mode)

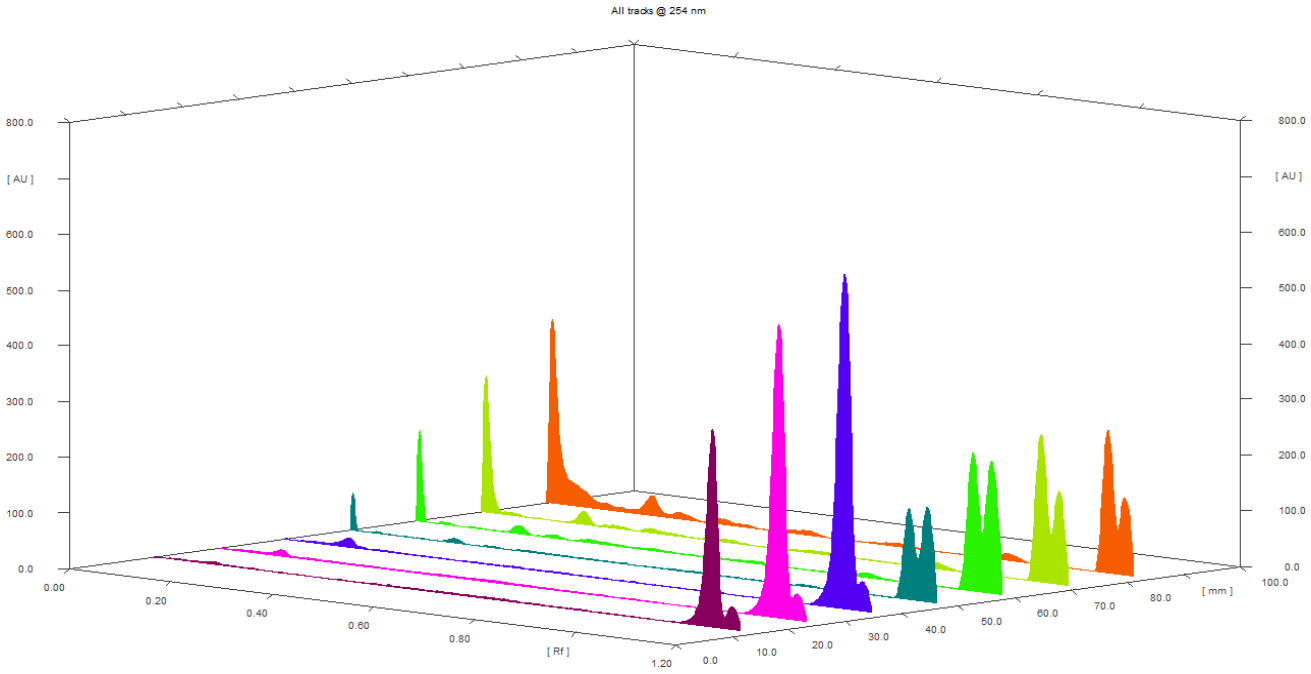


Fig 3. HPTLC fingerprint of NM at 254 nm (Absorbance mode)

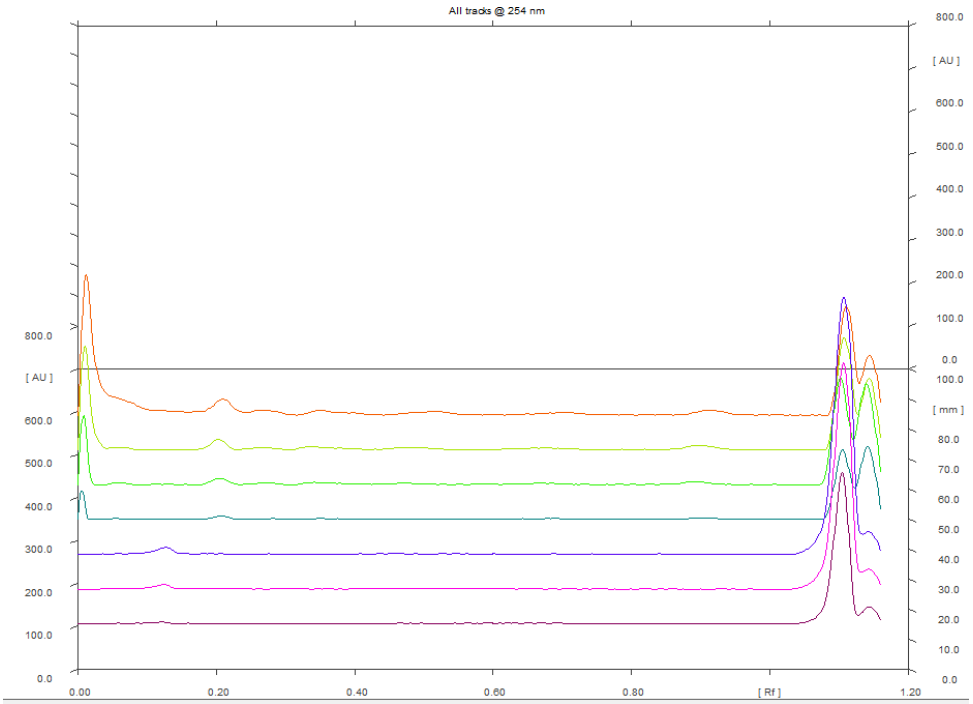
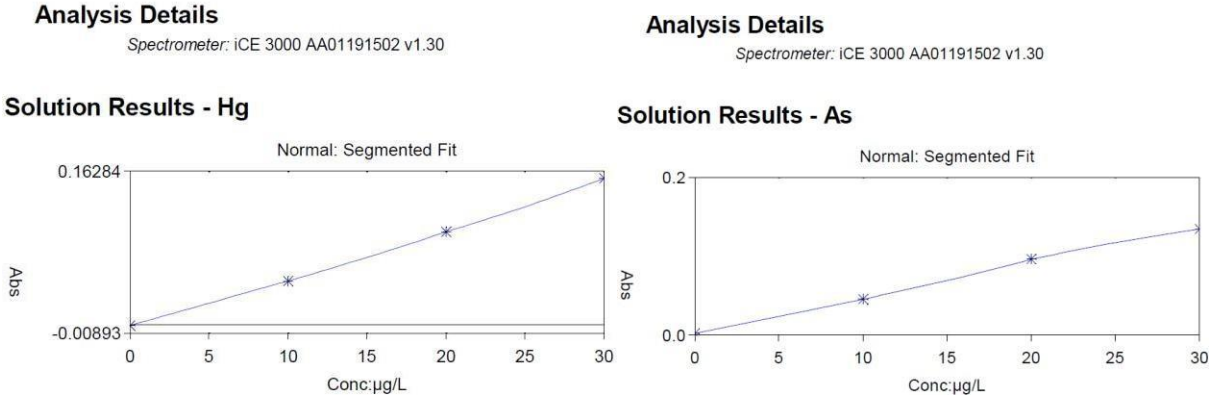


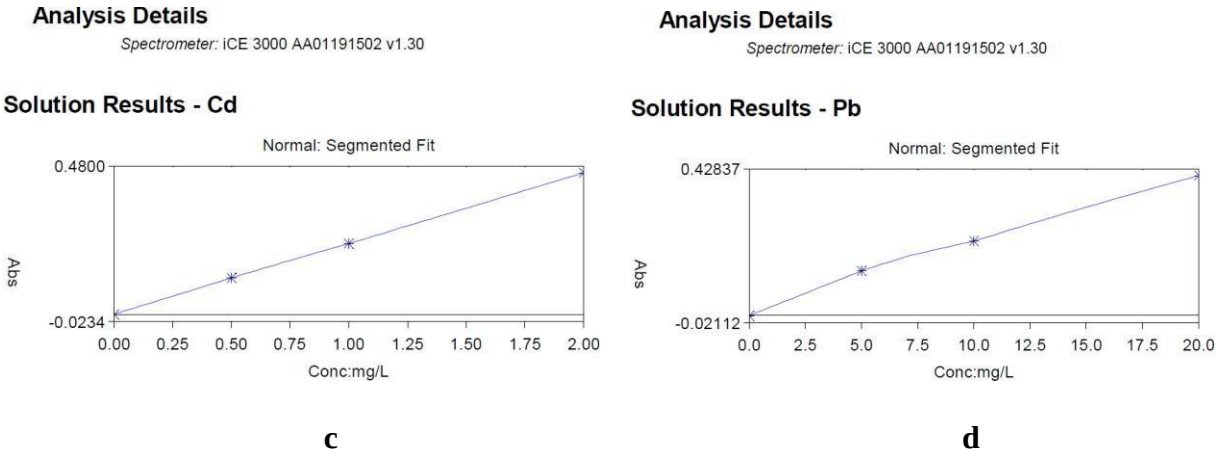
Fig 4. Densitometric chromatogram of NM at 254 nm (Absorbance mode)

3.4 Heavy Metal Analysis:

The linearity graph of the standard are given in Figure 5 and the Heavy metal analysis of the drug NM is given in Table 4



a **b**



c **d**

Figure 5. Linearity Graphs of Standard Solutions - a. Mercury, b. Arsenic, c. Cadmium, d. Lead

Name of the Element	Result mg/L	Permissible limit (ppm)	Inference
Lead	0.0539	10	Within the permissible limit
Cadmium	0.0026	0.3	
Arsenic	0.0365	3	
Mercury	0.7992	1	

Table 4. Heavy metal analysis of NM

3.5 Pesticide Residues:

The Pesticide residue analysis of the drug NM is given in Table 5.

S. No.	Test Parameter	Inference
01.	Organochlorine group	ND
	Aldrin	ND
	Dieldrin	ND
	P, P – DDT	ND
	O, P – DDT	ND
	P, P – DDE	ND
	O, P – DDE	ND
	P, P – DDD	ND
	O, P – DDD	ND
	Alpha Endosulfan	ND
	Beta Endosulfan	ND
	Endo Sulfansulphate	ND
	Endrin	ND
	Alpha HCH	ND
	Beta HCH	ND
	Delta HCH	ND

	Gamma HCH (Lindane)	ND
	Heptachlor	ND
02.	Organophosphorous group	
	4-Bromo- 2-Chloro Phenol	ND
	Acephate	ND
	Cis-Chlorfenvinphos	ND
	Trans-Chlorfenvinphos	ND
	Chlorpyrifos	ND
	Diazinon	ND
	Dichlorvos	ND
	Dimethoate	ND
	Omethoate	ND
	Ethion	ND
	Etrimfos	ND
	Fenitrothion	ND
	Iprobenfos	ND
	Malathion	ND
	Methamidophos	ND
	Monocrotophos	ND
	Oxydemeton-methyl	ND
	Methyl parathion	ND
	Ethyl Parathion	ND
	Phorate	ND
	Phosalone	ND
	Profenofos	ND
	Phosphamidon	ND
	Quinalphos	ND
	Triazophos	ND
03.	Synthetic Pyrethroids	
	Cypermethrin	ND

Table 5. Pesticide Residues of NM

3.6 Microbial Load:

The Microbial load and the test for specific pathogens of the drug NM is given in Table 6

S. No.	Parameters	Results	Inference
1	Total Bacterial Count (TBC)	Less than 10 cfu/g	Within permissible limits
2	Total Fungal Count (TFC)	Less than 1 cfu/g	
3	Enterobacteriaceae	Absent	
4	*Escherichia coli*	Absent	
5	Salmonella Spp	Absent	
6	*Staphylococcus aureus*	Absent	
7	*Pseudomonas aeruginosa*	Absent	

Table 6. Microbial load & Test for Specific Pathogens of NM

3.7 Aflatoxin:

The analysis of Aflatoxins in the drug NM is given in Table 7

S.No.	Parameters	Inference
1.	Total Aflatoxin B1+B2+G1+G2	1ppb

Table 7. Aflatoxin analysis of NM

3.8 Antimicrobial activity:

The antimicrobial activity of the drug NM is given in Table 8, and its zone of inhibition is given in Fig. 6

Test samples	**CONC (mg/ml)**	***S A***	***B C***	***KP***	***EC***	***CA***
		(Zone of inhibition mm in Dm)				
Siddha formulation NM	**25**	16	14	15	22	14
	50	20	17	19	25	18
	SC	-	-	-	-	-
	Std	14	-	24	31	8
Standard (Std)	Amp: Ampicillin 10mcg (Gram positive organisms); Nx: Norfloxacin10mcg (Gram negative organisms) ; Ap: Amphotericin 20mcg (Fungi)					
Organisms Abbreviation	*SA: Staphylococcus aureus* (ATCC-29213)*; BC Bacillus cereus* (Clinical Cochin University)*; KP: Klebsiella pneumonia* (ATCC-700603)*; EC: Escherichia coli* (ATCC 25922) and *CA: Candida albicans* (Clinical Cochin University). SC-Solvent control					

Table 8. Antimicrobial activity of NM

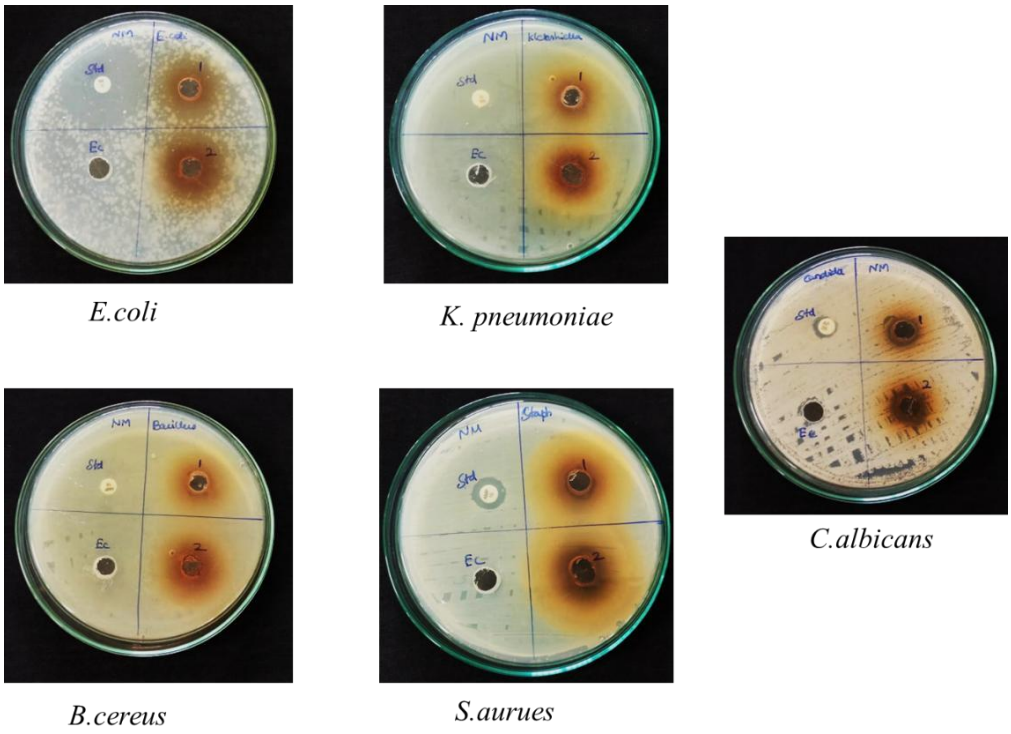


Fig 6. Agar plates showing the Zone of Inhibition of NM against E. coli, K. pneumoniae, B. cereus, S. aureus and C. albicans

4. SUMMARY:

The analysis of the sample NM reveals important physicochemical, phytochemical, and microbial characteristics, along with its safety profile, determination of the phytocomponent Baicalin, and antimicrobial activity. Physicochemical properties show that the sample has a moisture content of 14.81%, a total ash of 5.21%, and a slightly acidic pH of 5.63. It has significant levels of water and alcohol-soluble extractives (39.18% and 39.96%, respectively), and the sugar content includes 11.27% reducing sugar and 31.87% total sugar. In phytochemical analysis, the methanol extract contains alkaloids and carbohydrates in phytochemical analysis; the methanol extract contains alkaloids and carbohydrates, while the hydro-alcohol extract contains carbohydrates, flavonoids, phenolic compounds, phytosterols, and fixed oils. Tannins are present in both extracts, but other compounds like glycosides, proteins, and terpenoids were absent. The heavy metal analysis indicates that the sample is safe, with lead and cadmium, arsenic, or mercury levels found to be within the permissible limits. Pesticide residue tests showed no detectable traces of organochlorine, organophosphorus, or synthetic pyrethroid pesticides, confirming the safety of the drug NM in terms of chemical contamination. The test for Microbial load and specific pathogens demonstrated that the drug is safe with negligible bacterial and fungal counts below the permissible limits of AYUSH and no presence of harmful pathogens such as *E. coli, Salmonella*, or *Staphylococcus aureus*. The aflatoxin levels were found to be within the permissible limits. The antimicrobial activity of the formulation NM was promising, showing significant inhibition against the tested organisms, specifically against the Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* and Candida albicans.

5. DISCUSSION:

Diabetes mellitus, a long-lasting metabolic disorder marked by high blood sugar levels, insulin resistance, and oxidative stress, remains a significant global health issue. The IDF (2022) reports that over 800 million individuals globally are affected by diabetes, with India being among the leading countries contributing to the highest number of cases [16]. Conventional treatments for diabetes, such as insulin and oral hypoglycemic agents, effectively manage blood sugar levels but come with side effects like hypoglycemia, weight gain, and long-term reliance on medication [17].

One of the oldest known traditional medical systems, 'Siddha' medicine emphasizes the maintenance of bodily metabolism. Siddha formulations employ a multi-component therapeutic strategy, using synergistic combinations of herbs, minerals, and metals to restore metabolic homeostasis [18]. Siddha formulations primarily address diabetes by increasing insulin sensitivity, enhancing pancreatic function, decreasing oxidative stress, and regulating glucose metabolism [19].

Neeradimuthuvallathy Mezhugu (NM) is a herbomineral Siddha formulation used traditionally to treat chronic conditions such as diabetes. This research provides scientific validation of NM through physicochemical, phytochemical, and chromatographic analyses. A particular emphasis is placed on baicalin, a flavonoid recognized for its anti-diabetic effects. Baicalin, a flavonoid with bioactive properties, has undergone extensive research regarding its antidiabetic effects. Research has demonstrated that baicalin improves insulin sensitivity, facilitates glucose absorption in skeletal muscles, and decreases the apoptosis of pancreatic beta-cells [20]. Additionally, it has antioxidant effects by scavenging free radicals and lessening oxidative stress-induced insulin resistance, a key factor in diabetic complications [21]. Baicalin's presence in NM was verified using thin-layer chromatography (TLC) and High-Performance thin-layer chromatography (HPTLC).

The research also showed that NM has high extractive values, suggesting a rich composition of flavonoids, phenolic compounds, tannins, and alkaloids, all associated with hypoglycemic and free radical-scavenging activities [22]. The findings provide robust evidence for NM's role as a natural antidiabetic formulation.

Standardization is essential to guarantee that herbal formulations are effective, safe, and high-quality. As outlined by Sharma et al. (2020) [23], this research identifies important physicochemical parameters—such as moisture content, total ash, acid-insoluble ash, and extractive values—crucial for ensuring consistency in NM preparation. NM also exhibited considerable antimicrobial effects against Gram-positive and Gram-negative bacterial pathogens, such as Staphylococcus aureus and Escherichia coli, which are frequently linked to infections in diabetics [24].

The present study's findings add that the drug Neeradimuthuvallathy Mezhugu (NM) is a Siddha formulation that has been scientifically validated and meets modern phytopharmaceutical standards. With additional research revealing its antidiabetic potential and establishing clinical standards, NM can be incorporated into conventional diabetes management with further pharmacological investigations.

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8. DATA AVAILABILITY STATEMENT:

The authors confirm that the data supporting the findings of the study are available within the article