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Qualitative and Quantitative analysis of classical siddha medicine 'Veera Mezhugu'

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Abstract
Veera Mezhugu (VM) is a poly-herbo-mineral formulation used in the Siddha system of medicine, traditionally indicated for tumour and cancer-related conditions. Since such formulations combine purified metallic salts (mercury- and arsenic-based paasaanams) with herbal and resinous excipients, batch-to-batch consistency and safety cannot be assumed and must be established through systematic standardization. This study evaluated VM using organoleptic assessment, physicochemical parameters (ash values, extractive value, loss on drying, pH), preliminary biochemical and phytochemical screening, microbial limit testing, pesticide-residue and aflatoxin analysis, and instrumental characterization by SEM, FTIR, XRD, and TGA. The drug was found to be a dark brown, bitter, aromatic, fine wax-like preparation with ash and extractive values within acceptable limits, a near-neutral pH, and low moisture content. Biochemical screening revealed the presence of albumin, ferrous iron, calcium, phosphate, chloride, and sulphate, while phytochemical screening confirmed alkaloids, glycosides, and proteins. No microbial growth, pathogenic contamination, pesticide residues, or aflatoxins were detected, and all values fell within AYUSH-specified limits. SEM and XRD indicated a nanoparticulate, partially crystalline structure, FTIR confirmed a complex mixture of alcohols, esters, and fatty compounds, and TGA showed thermal stability up to approximately 100°C. These findings establish reference quality-control parameters for VM and support its safety profile ahead of pre-clinical evaluation.

Keywords: Siddha Medicine, Veera Mezhugu, Standardization, SEM, FTIR, TGA, XRD, Physicochemical Analysis.

Introduction

Standardization is the process of establishing documented evidence of identity, purity, quality, and consistency for a drug, and it is a mandatory prerequisite before any pharmaceutical formulation — herbal, mineral, or herbo-mineral — proceeds to pre-clinical trial. Traditional Siddha formulations pose a particular standardization challenge because they are prepared from multiple sources (plant, mineral, and sometimes animal-derived materials) through processes such as purification (suddhi), calcination, and trituration, all of which can introduce batch-to-batch variability.

The World Health Organization has set up guidelines for standardization of such drugs, which are used as a standard by the majority of countries, and these guidelines cover external examination alongside internal parameters such as ash values, extractive values, and other tests used to identify, authenticate, and

characterize the chemical composition of the drug. According to this framework, standardization and quality control of herbal and herbo-mineral drugs involves organoleptic evaluation, quantitative evaluation such as ash and extractive values, phytochemical evaluation, screening for xenobiotics, microbial load testing, and toxicity testing.

Materials and methods

ACQUISITION OF THE INGREDIENTS:

All the ingredients were acquired from a certified "raw drug store" at Thukkalay, Kanyakumari, Tamilnadu.

IDENTIFICATION AND AUTHENTICATION:

The faculties of Gunapadam department of our college GSMC, Palayamkottai had identified and authenticated all raw ingredients.

INGREDIENTS OF MEDICINE –*VEERA MEZHUGU*

1. Veeram (Hydragyrum perchloride) - 1 varaagan (3 ½ grams)
2. Navachaaram (Ammonium chloride) - 1 varaagan (3 ½ grams)
3. Rasa karpooram (Hydragyrum subchloride) - 1 varaagan (3 ½ grams)
4. Chukku (Zingiber officinalis) - 1 varaagan (3 ½ grams)
5. Milagu (Piper nigrum) - 1 varaagan (3 ½ grams)
6. Arisi thippili (Piper longum) - 1 varaagan (3 ½ grams)
7. Anda thailam(egg yolk oil) - Needed quantity

Purification of Ingredients:

Veeram:

Veeram have been tied to the stick using a cotton cloth and had suspended over a sand pot filled with tender coconut water without submerging the Veeram in contact with coconut water. Then the sand pot is heated and the coconut water is boiled for 30 minutes and the Veeram is isolated and then dried. Thus, the purification of Veeram had done.

Rasa Karpooram:

The betel leaf and pepper have taken in same amount of 17.5 g and made into a poultice and then it had dissolved in 2.6 liter of water in a sand pot and pooram of 70 g had taken and tied with a stick using a cotton cloth and had suspended over the sand pot where the pooram had submerged in the solution of betel and pepper and the water is heated till it reduced to $3/4^{th}$ of its volume and then the pooram had taken out and dried.

Navachaaram:

Navachaaram is dissolved in hot water and then filtered. After cooling the water is pored into a vessel with broad opening. Then it had to remain undisturbed until the salt had formed. Hence the salt formed is purified which is isolated and stored for the preparation of medicine.

Chukku:
Chukku had been soaked in calcium carbonate filtrate for 3 hours and dried, Then the outer skin is removed.

Milagu:
Milagu had been soaked in Buttermilk for 1 ½ hours and dried and then had been roasted.

Thippili:
Thippili had been soaked in lemon juice and taken and dried.

Composition of Formulation
The complete list of ingredients, local names, botanical name/ Chemical name, used parts and precise composition weights are tabulated below

Preparation of Veera Mezhugu
Three herbal drugs of Chukku, Milagu and Thippili are powdered separately and the other three mineral drugs of Veeram, Rasa Karpooram and Navachaaram are powdered and mixed with the above mixture of herbal drugs and then grinded well for several hours. Then Anda thailam is added in a little amount and grinded for six hours till the medicine attains a waxy consistency and fine texture which didn't stick in hand while rolled into tablets.

Study methodology
The physicochemical analysis, test for microbial contamination, heavy metals, pesticide residues were performed at a research facility in Sophisticated Analytical Instrumentation Centre (SAIF) at IIT, Madras.

Results and Discussions
Physico-chemical standardization:

Table 1 : Physicochemical analysis of VM:

S.No.	PARAMETERS		RESULTS
1.	**Organoleptic characters**		
	a. Color		Dark Brown
	b. Odour		Aromatic odour
	c. taste		Bitter
	d. Sense of touch		Smooth
	e. Appearance		Fine wax
2.	**Physico-chemical standardization**		
	b. Ash	Total ash	10.08 ± 0.110
		Acid insoluble ash	7.70 ± 0.010
		Water soluble ash	9.01 ± 0.030
	c. Water soluble extractive value		9.70 ± 0.520
	a. Loss on drying at 110□c		0.55 ± 0.510

	d. pH value (1% solution)	6.90
	e. Moisture	0.90

(SEM - singularity expansion method) [Values are mean of three determinations ± SEM]

INTERPRETATION:

The organoleptic characteristics such as color, taste, odour, touch and appearance of *Veera Mezhugu* were dark brown color, bitter taste, Aromatic odour and smooth and fine wax respectively.

Determination of Loss on Drying:

The loss on drying of Veera Mezhugu at 110°C was found to be 0.55%. A low moisture content is crucial for maintaining the drug's quality**.**

Determination of Ash Value:

- **Total Ash:** The total ash value for Veera Mezhugu is 10.08%. This indicates that the inorganic content is within acceptable limits, which can be used to assess the purity and genuineness of the formulation.
- **Water Soluble Ash**: The water-soluble ash value is 9.01%. This value is higher than the acid-soluble ash, suggesting high quality of the drug.
- **Acid Insoluble Ash:** The acid insoluble ash value is 7.70%, representing the amount of ash that is insoluble in dilute hydrochloric acid.

Determination of Water-Soluble Extractive Value:

The water-soluble extractive value is 9.70 suggests that the drug contains a moderate number of water-soluble compounds such as sugars, glycosides, tannins, and other phytoconstituents

Determination of pH:

The pH value of Veera Mezhugu is 6.90 which is close to neutral, but slightly on the acidic side. This could indicate the presence of weak acids, such as organic acids or other acidic components, which are common in plant-based drugs.

BIO CHEMICAL ANALYSIS OF *VEERA MEZHUGU:*

Table 2 : Preliminary investigation of basic and acidic radicals in the drug:

S.No	Test	INFERENCE
1.	Carbonate test	Absence (-)
2.	Albumin test	**Presence (+)**
3.	Ferrous iron test	**Presence (+)**
4.	Calcium test	**Presence (+)**
5.	Starch test	Absence (-)
6.	Phosphate test	**Presence (+)**
7.	Chloride test	**Presence (+)**
8.	Ferric iron test	Absence (-)
9.	Sulphate test	**Presence (+)**
10.	Zinc test	Absence (-)
11.	Test for reducing sugar	Absence (-)
12.	Test for the unsaturation	**Presence (+)**
13.	Amino acid test	Absence (-)

14.	Tannic acid test	Absence (-)

Interpretation:

The trial drug of *Veera Mezhugu* contains albumin (protein), ferrous iron, calcium, phosphate, chloride, and sulphate ions, and has unsaturated compounds.

1. **Albumin (Protein)**

- **Function**: Albumin is a major protein commonly found in blood plasma. It helps maintain osmotic pressure and plays a role in transporting hormones, vitamins, and other substances.
- **Significance in Drugs**: The presence of albumin suggests the drug may have a proteinaceous component, potentially aiding in tissue repair, healing, or maintaining bodily functions.

2. **Ferrous Iron (Fe^{2+})**

- **Function**: Ferrous iron is a bioavailable form of iron essential for hemoglobin formation, oxygen transport in the blood, and enzyme functions.
- **Significance in Drugs**: The presence of ferrous iron in the drug may contribute to the treatment of iron deficiency or anemia, improving overall energy and metabolic functions.

3. **Calcium**

- **Function**: Calcium is vital for bone health, muscle contraction, nerve transmission, and blood clotting.
- **Significance in Drugs**: Calcium in the drug can support bone health and prevent conditions like osteoporosis. It is also essential in various cellular processes.

4. **Phosphate (PO_4^{3-})**

- **Function**: Phosphate is a key component of DNA, RNA, and ATP, and it is essential for energy storage and transfer within cells.
- **Significance in Drugs**: Phosphate presence indicates that the drug may aid in energy metabolism, cellular repair, and bone health, as phosphates contribute to bone mineralization.

5. **Chloride (Cl^-)**

- **Function**: Chloride is essential for maintaining fluid balance, blood pressure, and proper nerve and muscle function.
- **Significance in Drugs**: The presence of chloride suggests the drug may help in maintaining electrolyte balance, which is crucial for hydration and acid-base homeostasis.

6. **Sulphate (SO_4^{2-})**

- **Function**: Sulphate is involved in detoxification, protein synthesis, and maintaining healthy joints and cartilage through its role in glycosaminoglycans.
- **Significance in Drugs**: Sulphates in the drug might help detoxify the body, improve joint health, and support metabolic processes.

7. **Unsaturated Compounds**

- **Function**: Unsaturated compounds, such as unsaturated fatty acids, are important for heart health, brain function, and reducing inflammation.
- **Significance in Drugs**: The presence of unsaturated compounds suggests potential benefits for cardiovascular health and anti-inflammatory properties, which could enhance the drug's therapeutic action.

ANALYSIS OF *VEERA MEZHUGU* FOR PHYTOCHEMICALS

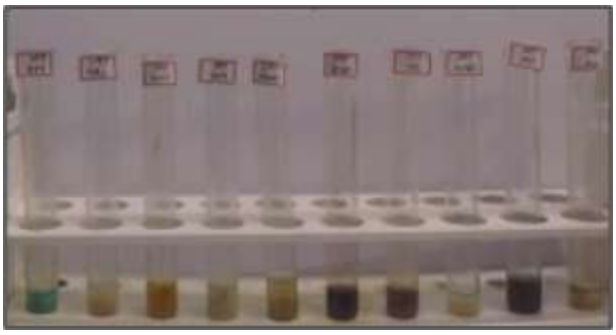


Table 3 : Experiments to check for the presence of phytoconstituents

S.No	Tests Performed	Results Obtained
1.	Coumarins	Negative (-)
2.	Tannins	Negative (-)
3.	Alkaloids (Mayer's Test)	**Positive (+)**
4.	Saponins	Negative (-)
5.	Glycosides (Molisch Test)	**Positive (+)**
6.	Flavonoids (Alkaline reagent test)	Negative (-)
7.	Steroids	Negative (-)
8.	Phenols (Lead acetate test)	Negative (-)
9.	Triterpenoids (Liebermann–Burchard test)	Negative (-)
10.	Proteins (Biuret Test)	**Positive (+)**
11.	Betacyanin	Negative (-)
12.	Anthocyanin	Negative (-)

INTERPRETATION:

The drug contains **alkaloids**, **glycosides**, and **proteins**, indicating potential therapeutic properties. Presence of alkaloids suggests potential pharmacological activity, as many alkaloids have medicinal properties, including analgesic, antimalarial, or sedative effects. Presence of glycosides indicates that the drug contains sugar moieties attached to other functional groups, often associated with various therapeutic benefits, including cardiotonic effects. Presence of proteins indicates that the drug may have structural components and potential nutritional benefits or therapeutic properties.

Investigation of Microbial Limits in *Veera Mezhugu:*

Microbial Limit Testing for *Veera Mezhugu*

Fig 8: VM's Microbial limit test

Result:

1. No growth or colonies were observed on any of the plates inoculated with *Veera Mezhugu* after the incubation period.
2. After the incubation period, all inoculated plates with *Veera Mezhugu* showed no signs of microbial growth.

Table 5: Microbial limit testing of *VM*

S.No	Test	Specification	Result
1.	Total Fungal Count	NMT 10^3CFU/g	Nil
2.	Total Bacterial Count	NMT 10^5CFU/g	Nil

Table-6: Test for specified Pathogens:

S.NO	Test for specific pathogens	Colony counts (CFU/g)	Limits Value (CFU/g)
1	Salmonella sp.	No growth	-
2	Staphylococcus aureus	No Growth	-
3	E-coli	No Growth	-
4	Pseudomonas aeruginosa	No Growth	-

INTERPRETATION:

Based on the findings, *Veera Mezhugu* appears to be free from microbial contamination, as no bacterial or fungal counts were recorded.

Results confirm that the sample does not contain any of the tested pathogens, aligning with safety standards for microbial contamination.

Pesticide Residue Analysis:

Table - 7: Residues of Pesticides in *VM*

S.No	Pesticide Residue	*VM*	AYUSH Limit (mg/kg)
1	Organo Phosphorus Pesticides		
	i) Chlorpyrifos	[BQL]	0.2 mg/kg

S.No	Pesticide Residue	*VM*	AYUSH Limit (mg/kg)
	ii) Malathion	[BQL]	1 mg/kg
	iii) Dichlorvos	[BQL]	1 mg/kg
2	Organo Chlorine Pesticides		
	i) Endosulfan	[BQL]	3 mg/kg
	ii) Delta BHC	[BQL]	0.1 mg/kg
	iii) Beta BHC	[BQL]	0.1 mg/kg
	iv) DDT	[BQL]	1 mg/kg
	v) Gamma BHC	[BQL]	0.1 mg/kg
	vi) Alpha BHC	[BQL]	0.1 mg/kg
3	Pyrethroid		
	i) Cypermethrin	[BQL]	1 mg/kg
4	Organo Carbamates		
	i) Carbofuran	[BQL]	0.1 mg/kg

BQL - Below Quantification Limit

Interpretation:

- The analysis indicates that all tested pesticide residues, including various organophosphorus and organochlorine pesticides, as well as pyrethroids and Organo carbamates, were found to be below the quantification limit (BQL).
- These results suggest that the pesticide residues in TPC are within safe limits as per the AYUSH standards.

Aflatoxin Assay:

S.No	Aflatoxins	VM	AYUSH Specification Limit
1	G1	[Nil]	0.5 ppm (0.5 mg/kg)
2	G2	[Nil]	0.1 ppm (0.1 mg/kg)
3	B1	[Nil]	0.5 ppm (0.5 mg/kg)
4	B2	[Nil]	0.1 ppm (0.1 mg/kg)

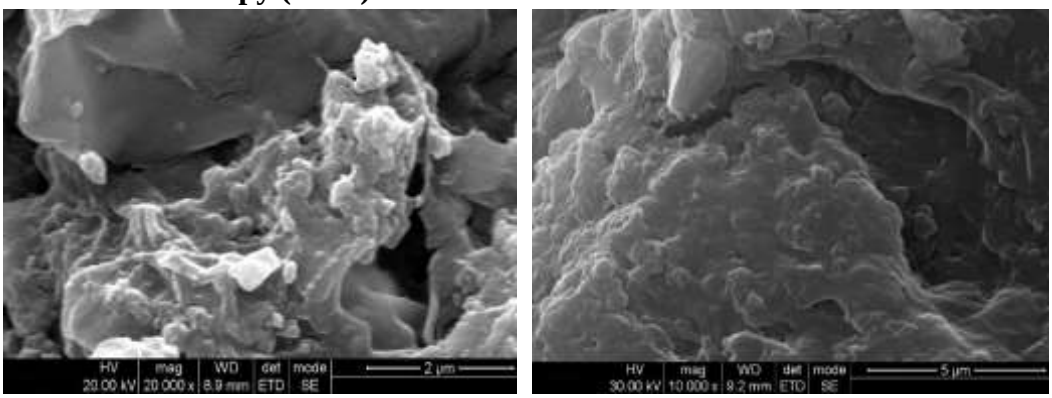
Table 9: Aflatoxin Analysis for *VM*

Interpretation:

The results indicate that the trial drug of *VM* placed on TLC plates showed no spots when compared to the standard. This demonstrates that the medication is free from the aflatoxins B1, B2, G1, and G2.

Instrumental Analysis:

Scanning Electron Microscopy (SEM) of *VM:*



SEM Morphology Analysis of VM

The morphology and elemental composition of the VM sample can be analyzed using Scanning Electron Microscopy (FEI Quanta). To prepare the sample for SEM analysis, a representative portion should be sprinkled onto a double-sided carbon tape and mounted on aluminum stubs, ensuring a high-quality secondary electron image for better examination.

Discussion of SEM Results:

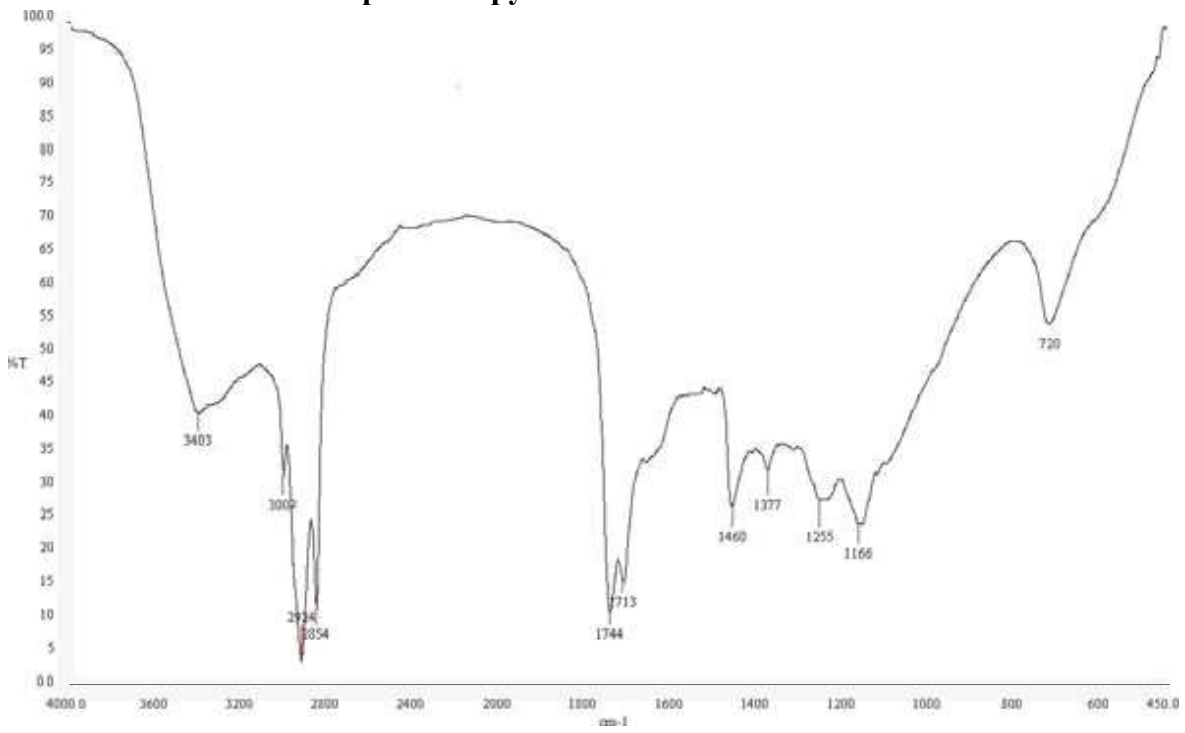
Although particle sizes appear similar across different batches, the particles seem to be aggregates of much smaller individual particles. When dispersed in an aqueous medium, these preparations create a negatively charged hydrophobic suspension. This hydrophobic nature causes the particles to aggregate and form larger clusters. The relatively larger particle size observed could result from repeated cycles of calcination during preparation, leading to agglomeration.

Particles with a high positive surface charge, like chitosan, tend to be attracted to the intestinal mucosa, aiding in the enhanced intestinal absorption of the encapsulated drug. However, the strong electrostatic interaction between these positively charged particles and the negatively charged glycocalyx can slow their movement and reduce penetration toward the epithelial cell surface, thus decreasing their uptake. Additionally, it has been observed that non-ionized particles have a higher affinity for M cells compared to ionized or positively charged particles.

Conclusion:

- Nanoparticles are present in VM formulation.
- Nano-emulsions can be synthesized due to the abundant use of oils.
- Successful development of these particles could lead to higher absorption, reduced toxicity, and increased cost-effectiveness.

Fourier Transformed Infrared Spectroscopy:



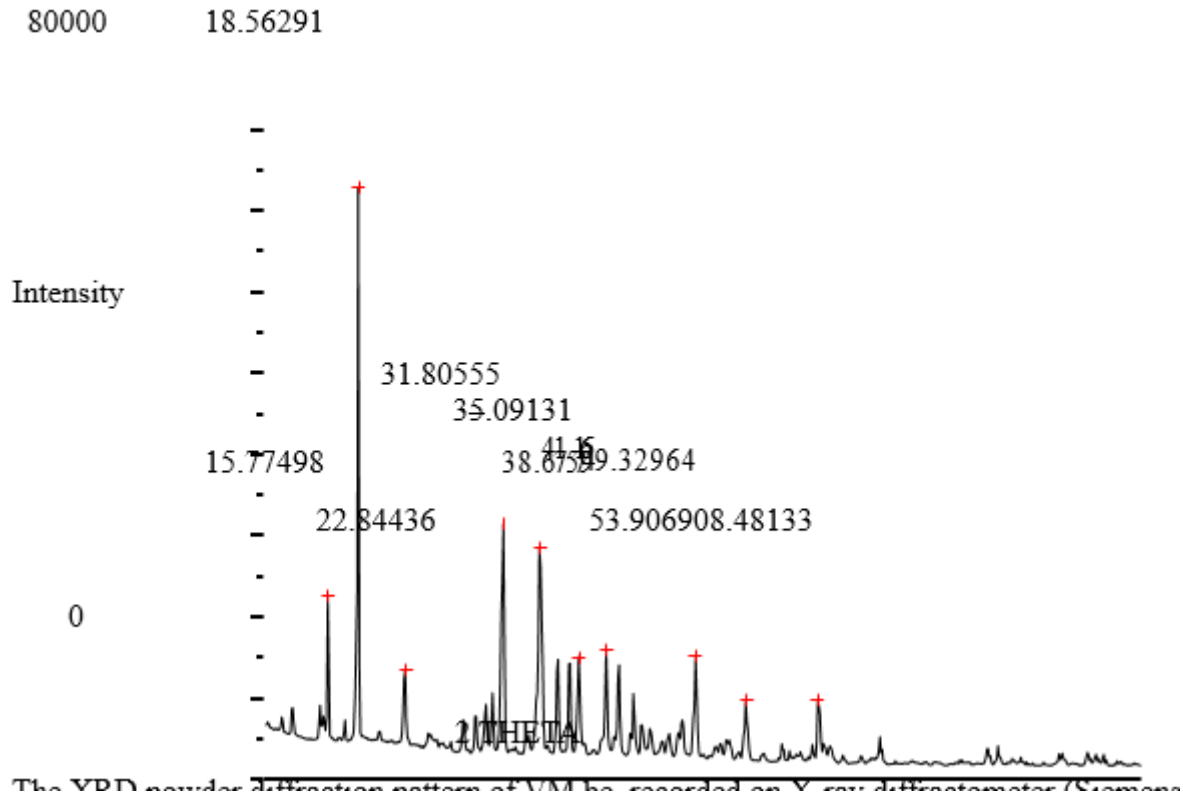
S.No	Frequency	Type and nature of Bonds	Functional group
1	3403	O-H Stretching with weaker hydrogen bonding	Alcohol
2	3009	Olefinic C-H Stretching	Alkene
3	2924	C-H Stretching	Alkyl
4	2854	C-H Stretching with strong intensity	Symmetric Alkyl
5	1744	C=O Stretching with strong intensity	Carbonyl
6	1713	C=O Stretching with strong intensity	Carbonyl
7	1460	C-H bending vibration	Methylene
8	1377	C-H Bending vibration	Methyl
9	1255	C-O Stretching	Carbonyl Ester
10	1166	C-O Stretching	Ether
11	720	C-Cl stretching	Alkyl Halides

Interpretation:

- The presence of O-H (alcohol) and C=O (carbonyl) groups suggests that the sample contains alcohols, esters, or possibly carboxylic acids.
- The alkyl and olefinic C-H stretching peaks indicate both saturated (alkyl) and unsaturated (alkene) hydrocarbons, implying that the sample might contain fatty acids or oils with both saturated and unsaturated components.
- The C-O stretching peaks suggest the presence of esters and ethers, which are commonly found in oils and fatty compounds.
- The C-Cl stretching suggests that halogenated hydrocarbons or alkyl halides may be present, possibly due to synthetic modifications or additives.

Overall, the FTIR data indicates a complex mixture of organic compounds, likely containing alcohols, esters, fatty acids, and possibly halogenated hydrocarbons, which could be consistent with a formulation rich in oils, emulsifiers, or nutraceutical components.

X-RAY Diffraction (XRD) Analysis:



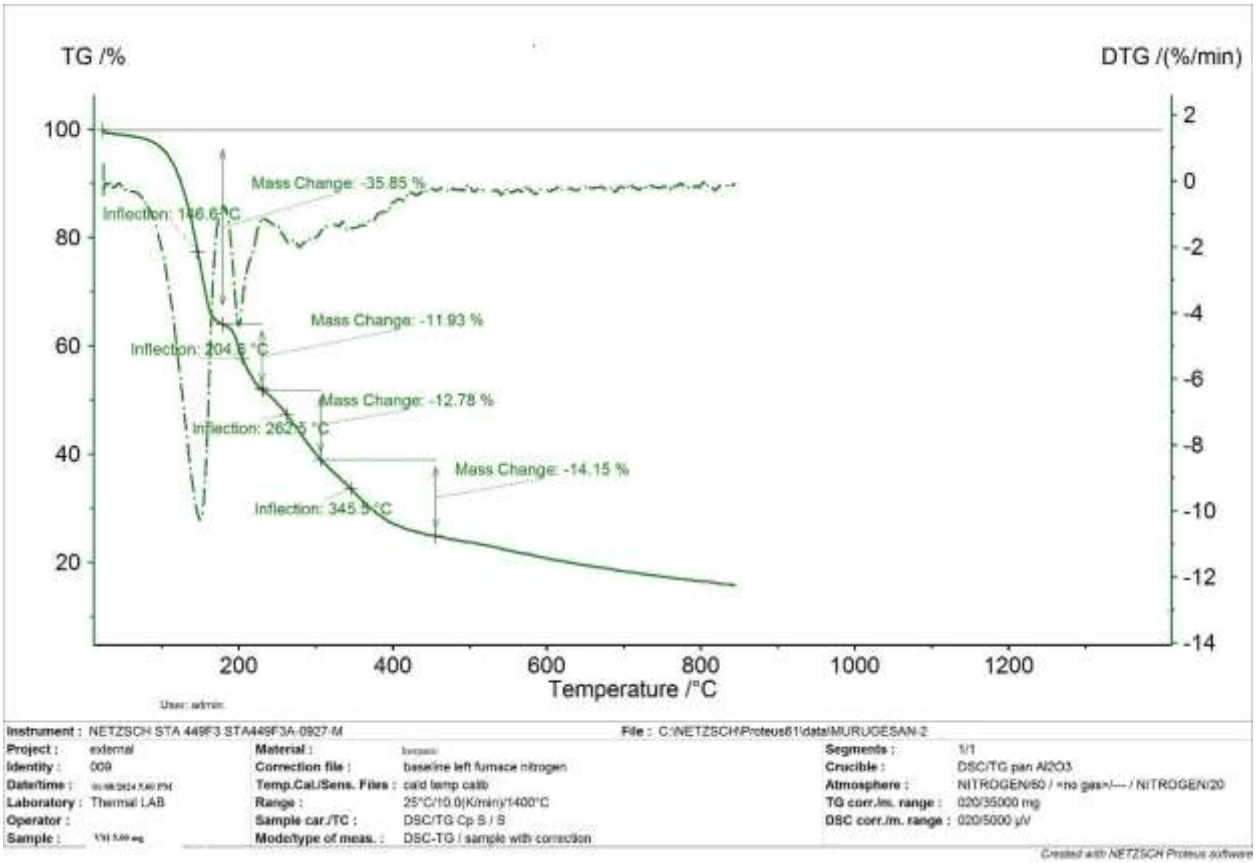
The XRD powder diffraction pattern of VM be recorded on X-ray diffractometer (Siemens D5005 Diffractometer) using Cu Ka radiation, Lambda = 1.5406 Angstrom] over the range 10.0-80.0[degrees].

Interpretation:

The XRD data for the VM drug indicates a mixture of low, mid, and high-angle peaks, suggesting that the sample contains both crystalline and possibly amorphous components. The presence of distinct sharp peaks at high 2θ values points to a high degree of crystallinity. The lower angle peaks likely correspond to the active pharmaceutical ingredient (API) or other organic compounds within the drug.

Additionally, the crystallinity of the drug formulation may play an important role in its bioavailability and pharmacokinetics.

Thermogravimetric Analysis (TGA):



Interpretation:

Overall Mass Loss Profile:

Total Mass Loss: Adding up all the stages results in a cumulative mass loss of around 74.71% by the time the sample reaches around 350°C.

This indicates that a large portion of the VM sample consists of volatile components, organic matter, or decomposable materials, while the remaining 25-26% could be stable residuals, possibly inorganic fillers, or non-decomposable materials.

Final Thoughts on Thermal Stability:

- Thermal Stability: The sample shows a significant thermal decomposition starting around 100°C and continuing through to 350°C. This suggests that the material is not thermally stable beyond 100°C, which could impact its processing or formulation if exposed to higher temperatures.
- Residual Mass: Beyond 350°C, the mass loss curve flattens, indicating that no further significant decomposition occurs at higher temperatures, suggesting that the remaining material may be inorganic or thermally resistant.

The TGA results provide insight into the thermal degradation behavior of the VM sample, which can be important for understanding its stability, formulation, and potential use in applications where temperature sensitivity is critical.

SUMMARY:

The physico-chemical profile of VM showed a dark brown, aromatic, bitter, smooth, fine-wax preparation with the following key parameters:

- Total ash: 10.08 ± 0.110% | Water-soluble ash: 9.01 ± 0.030% | Acid-insoluble ash: 7.70 ± 0.010%
- Water-soluble extractive value: 9.70 ± 0.520%
- Loss on drying (110°C): 0.55 ± 0.510%
- pH (1% solution): 6.90 (near-neutral, slightly acidic)

Preliminary biochemical testing detected albumin, ferrous iron, calcium, phosphate, chloride, and sulphate, while phytochemical screening confirmed the presence of alkaloids, glycosides, and proteins, with coumarins, tannins, saponins, flavonoids, steroids, phenols, and triterpenoids absent.

Microbial limit testing showed no fungal or bacterial growth and no detectable Salmonella sp., Staphylococcus aureus, E. coli, or Pseudomonas aeruginosa. Pesticide-residue analysis found all tested organophosphorus, organochlorine, pyrethroid, and carbamate residues below the quantification limit (BQL), and the aflatoxin assay (B1, B2, G1, G2) was negative — both within AYUSH specification limits. Instrumental analysis added a further layer of characterization: SEM suggested the presence of nanoscale aggregated particles; FTIR revealed O–H, C=O, C–H, C–O, and C–Cl bonds consistent with alcohols, esters, and fatty/oily constituents; XRD showed a mixed crystalline–amorphous structure; and TGA demonstrated a cumulative mass loss of approximately 74.71% by 350°C, with decomposition onset near 100°C, indicating limited thermal stability above that temperature.

Conclusion

Taken together, the organoleptic, physicochemical, biochemical, phytochemical, microbiological, pesticide-residue, aflatoxin, and instrumental data indicate that VM, as prepared, meets acceptable pharmacopoeial and AYUSH-specified quality benchmarks and is free from microbial, pesticide, and mycotoxin contamination. The presence of nanoscale particles on SEM, together with the oil/ester-rich FTIR profile, suggests that VM may exist partly as a nano-emulsion system, which could favour enhanced absorption if confirmed and optimized in further studies. The TGA-derived thermal-stability limit (~100°C) is a practically important finding for storage and processing conditions. Overall, this standardization dataset provides a reproducible quality-control reference for VM and establishes the analytical foundation required before the formulation can be advanced to pre-clinical safety and efficacy evaluation.

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