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RESEARCH ARTICLE

Physicochemical characterization of commercially available Dhanwantharam Mezhupakam - An Ayurvedic oil formulation

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Abstract

As a neuroprotective and nervine tonic, Dhanwantharam Mezhupakam (DM) is a medicated polyherbal oil formulation made using an Ayurvedic method. It is made by steeping herbs in significant water while simmering Taliam oil for a long time with a paste-like herb mixture. The purpose of the current study was to establish its standard criteria in accordance with the monograph to preserve the product's safety, consistency and quality manufacturing while also determining its heavy metal concentration. Organoleptic assessment, physicochemical evaluation, phytochemical evaluation, chromatographic analysis for active ingredients and heavy metal analysis are some of the formulation-related criteria that are assessed. According to pharmacopeia standards, every metric was examined. According to the study's findings, the formulation was well-standardized in terms of its physical consistency, chemical profile, phytoconstituents and antibacterial properties. Additionally, it was discovered that the sample's heavy metal contents were within WHO and API-acceptable standards.

Keywords

Chromatogram; densitometry; *Dhanwantharam Mezhupakam*; heavy metal; HPTLC; phytoconstituents

Introduction

Ayurveda is one of India's oldest medicinal practices (1). From the 4th century B.C. through the 12th century A.D., Ayurveda had been studied and it rose to fame during the Gupta Golden Age (2). The current pharmaceutical industry recognized the importance of Ayurveda, using a range of herbal medicine-derived substances to create potent drugs for a variety of illnesses. Numerous important Ayurvedic drugs and products still haven't had their pharmacology, pharmacokinetics or pharmacovigilance fully investigated (3).

Standardization is necessary to ensure a specific degree of quantity, effectiveness and therapeutic efficacy for each dose of components (4). There is a lot of space for differences among different batches of plant medications, so everyone that is put on the market needs to undergo a standardization procedure. The phytochemical makeup of plant material may differ and this variation is increased by the fact that several plants can be included in a single herbal medicine formulation. This suggests that several quality assurance tests should be carried out to ensure the end

product's quality throughout the manufacturing process (5).

Additionally, the World Health Organization (WHO) needs to emphasize the value of high-quality research development, application and scientific validity of herbal treatments (6). The underlying research behind Ayurveda is among the most significant contributors to its popularity on a global scale. For the accreditation of herbal/ polyherbal Ayurvedic medications, standardization criteria that incorporate unusual analytical techniques are thus required (7). Due to the paucity of thorough phytochemical studies and the mechanisms of action of some components, studies frequently separate the Ayurvedic system from modern ideas. Cutting-edge techniques and research are also available for validation. Thus, it is absolutely necessary that we employ cutting-edge research to support the core concepts and treatments employed in the Ayurvedic system of medicine. Identifying the key phytochemicals and HPTLC fingerprints of the herbal-mineral Ayurveda composition DM oil has therefore been attempted.

The polyherbal formulation is used for the treatment of paralysis, hemiplegia, quadriplegia, physical weakness etc. Additionally, it has nervine and neuroprotective actions, making it essential for neuroprotection and preserving the proper functioning of the nervous system. It supports the musculoskeletal system and is beneficial for painful illnesses, including arthritis, osteoarthritis, knee pain, backaches and spondylosis. More than 43 therapeutic plants are used to form the polyherbal oil, with Taliam (*Seamum indicum*) and Bala (*Sida cordifolia*) being the 2 main ingredients (Table 1).

Ayurvedic medications are well-liked for their all-encompassing approach to treating illness organically. Unfortunately, because Ayurvedic medicines contain a variety of substances and have a complex chemical makeup, standardizing them has proven to be difficult. Therefore, the fact that Ayurvedic drugs and their preparations include active ingredients, but their therapeutic effects have not been scientifically verified is irrelevant. This was mentioned in the draft of the WHO's regional traditional medicine strategy plan (8).

The WHO has explicitly stated that all botanical preparations must have a fingerprint profile developed as part of quality control measures for medicinal plant materials (9,10). Every medicine should contain comprehensive information about its phytoconstituents, physicochemical qualities and organoleptic features. DM's phytochemical, physicochemical and antibacterial activities were not found in the literature. Standardization and characterization research was thus planned for this formulation to describe its whole quality profile, analyze the phytoconstituents in oil extracts, analyze any heavy metals and determine its effectiveness against microorganisms.

Table 1. Llist of ingredients of the polyherbal formulation Dhanwantharam Mezupakam

Official name	Botanical name	Quantity	Official name	Botanical name	Quantity
Taliam	*Sesamum indicum*	10.000 ml	Saileya	*Parmelia perlata*	0.026 g
Bala	*Sida cordifolia*	15.000 g	Vacha	*Acorus calamus*	0.026 g
Yava	*Hordeum vulgare*	0.200 g	Punarnava	*Boerhaavia diffusa*	0.026 g
Kola	*Ziziphus abyssinica*	0.200 g	Aswagandha	*Withania somnifera*	0.026 g
Kulatha	*Macrotyloma uniflorum*	0.200 g	Vari	*Asparagus racemosus*	0.026 g
Vilwa	*Aegle marmelos*	0.200 g	Kashirasukla	*Ipomoea mauritiana*	0.026 g
Kasmari	*Gmelina arborea*	0.200 g	Yasti	*Glycyrrhiza glabara*	0.026 g
Patala	*Stereospermum tetragonum*	0.200 g	Haritaki	*Terminalia chebula*	0.026 g
Syonaka	*Oroxylum indicum*	0.200 g	Amalaki	*Phyllanthus emblica*	0.026 g
Agnimandha	*Premna corymbosa*	0.200 g	Vibhitaki	*Terminalia bellirica*	0.026 g
Salapparni	*Pseudarthria viscida*	0.200 g	Rosa	*Commiphora myrrha*	0.026 g
Prisniparni	*Desmodium gangeticum*	0.200 g	Satahwa	*Anethum graveolens*	0.026 g
Brihati	*Solanum anguivi*	0.200 g	Mashaparni	*Vigna radiata* var. *sublobata*	0.026 g
Nididgdhika	*Solanum surattense*	0.200 g	Mudgaparni	*Dysolobium pilosum*	0.026 g
Gokshura	*Tribulus terrestris*	0.200 g	Ela	*Elettaria cardamomum*	0.026 g
Paya	*Milk*	60.000 ml	Twak	*Cinnamomum verum*	0.026 g
Dwimeda	*Asparagus racemosus*	0.052 g	Patra	*Cinnamomum tamala*	0.026 g
Daru	*Cedrus deodara*	0.026 g	Chandana	*Santalum album*	0.026 g
Manjishtha	*Rubia cordifolia*	0.026 g	Sariba	*Hemidesmus indicus*	0.026 g
Kakolidwaya	*Withania somnifera*	0.052 g	Kushtha	*Saussurea costsus*	0.026 g
Jivakadwaya	*Pueraria tuberosa*	0.052 g	Tagara	*Valeriana jatamansi*	0.026 g

Materials and Methods

Procurement of DM oil formulation and chemicals: DM oil formulation was obtained from M/S Kottakkal Arya Vaidya Sala, an authorized dealer located at Nataraj Theatre Building, 2nd Floor, Station Road, Chembur, Bombay. They are a licensed stockist of Ayurvedic medicine. The analytical grade chemicals and biochemicals used in this experiment were all purchased from HiMedia Research Labs Inc. in Mumbai, India.

Organoleptic and physicochemical assessment: Organoleptic characteristics, such as color, odor and taste as well as physicochemical evaluations of acidity, saponification, iodine value, specific gravity, density, pH and skin irritancy tests were performed in accordance with conventional procedures (11-13).

HPTLC analysis of DM oil extract:

The phytochemicals in DM were quantified by HPTLC utilizing a Hemilton 100 l HPTLC syringe, Camag Linomat V automated spotting equipment, Camag twin trough chamber, Camag TLC Scanner-4 and WINCAT interface software. As the stationary phase, 20 × 10 mm precoated silica gel G60-F254 aluminum sheets (E. Merck, Germany) with a 0.2 mm layer thickness were utilized. Using a 25 ml syringe, the sample was injected at doses of 2, 5 and 10 ml onto the TLC plate. 4. A Twin trough chamber with a mobile phase and a 70 mm developing distance was used to soak the sample-loaded plate. Toluene is made up of the following components: ethanol, 4:4:1, chloroform, 10% methanolic sulfuric acid and using this plate was derivatized. When the clean and produced plates were evaluated under white light for bands at UV 254 nm and UV 366 nm, densitometer scanning was carried out for spectra at 254 nm, 540 nm and 366 nm (14, 15). The phytochemical composition of the prepared extract was examined.

Heavy metals determination:

Mercury (Hg), cadmium (Cd) and arsenic (As) were digested in an aquarigea; for lead (Pb), cadmium (Cd) and arsenic (As), they were digested in a microwave-assisted digestion system (MARS 6, CEM Company, USA) (As). When the samples had been digested, they were filtered, distilled water was used to create a volume of 25 mL and ICP-OES version 5110 was used for the analysis.

Microbial load:

According to the Ayurvedic Pharmacopoeia of India (16), the total number of viable aerobic microorganisms and a test for the presence of certain microorganisms were conducted. It contained the overall number of microorganisms on the plate as well as the presence of particular microbes including *S. aureus, P. vulgaris, P. aeruginosa, E. coli, E. faecalis* and *S. typhi.*

Antibacterial activity

Singh *et al.'s* agar well-diffusion method was utilized to conduct an in vitro bacterial screening of DM and its constituent parts against four bacterial strains. The log phase bacterial cultures (secondary culture) were disseminated on Potato dextrose agar medium plates using a sterile spreader to ensure uniform bacterial growth on test plates. After that, the plates underwent another 18 to 24 h of incubation at 37 ˚C. Inhibitory zones with a (mm) diameter were calculated and their ampicillin activity (50 mg/ml) was compared (1).

Results

Organoleptic Evaluation:

The DM oil formulation employed in this study underwent organoleptic examination and the results showed that it was a reddish-brown liquid with a greasy and fatty character, had an oily bitter taste, was soluble in non-polar solvents and had the distinctive fermentation fragrance of other ayurvedic drugs. The amount of DM components that contain any identifiable foreign material is less than 0.5% (w/w).

Physicochemical Evaluation:

Physical and chemical characteristics, including moisture content, acid value, saponification, weight/ml at 40 °C, density, ester value and pH, were evaluated in order to determine the quality of the DM oil formulation. The outcomes are shown in Table 2. The following values were found for the variables: wt./ml at 40 °C (1.489), density (0.8 kg/m^3), ester value (138.85 gm), pH (6), moisture content (32.42%), acid value (1.4 mg KOH/1 gm oil) and saponification (140.254 mg KOH/gm oil).

Table 2. Results of the physicochemical investigation of Dhanwantharam Mezhupakam (DM)

Evaluation Parameter	Observation
Moisture content	32.42
Acid value	1.4
saponification	140.25
wt/ml at 40 ˚C	1.489
Density	0.8
Ester value	138.85
pH	6

HPTLC Fingerprinting Profile:

The study employed the methanolic extract of DM to produce an ideal fingerprint profile, which was shown as a chromatogram and densitogram (Fig. 1). The mobile phase used provided compact bands for extracts at a range of Rf values. The developed plate after derivatization was scanned at white light, producing a chromatogram revealing the presence of three distinct bands (Fig. 1A). The chromatogram's values were found to be 0.18, 0.29 and 0.65 respectively. A densitogram peak at 540 nm was also produced (Fig. 1B), which included the retention factor values, peak height and peak area.

HPTLC Phytochemical Screening

HPTLC phytochemical study of the Dhanwantharam Mezhupakam methanolic extract is presented in Table 3, which reveals the presence of flavonoids, steroid, triterpenoids, saponin, glycoside and alkaloids. At 366 nm, flavonoids with Rf 0.86 and 0.72 were identified as fluorescent molecules (Fig. 2A). The purple band indicates

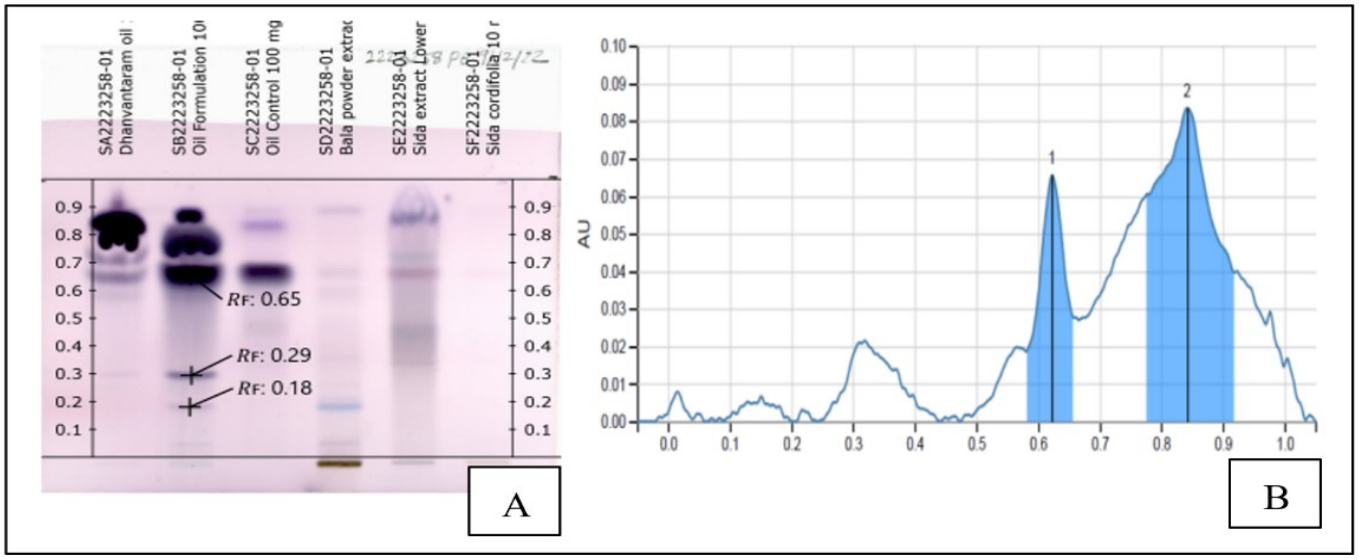

Fig. 1. HPTLC fingerprinting profile of Dhanwantharam Mezhupakam (DM): **[A]** Derivatized fingerprinting plate at R white light showing various bands at Rf value 0.18, 0.29, 0.65. **[B]** Densitometric Analysis of fingerprinting profile.

the presence of glycoside, which can be observed. (Fig. 2B, Fig. 2C) depicts a yellow band at Rf 0.89, which indicates the presence of alkaloids. At Rf 0.94, 0.83 and 0.39 blue bands were observed that corresponded to the saponin (Fig. 2D). Terpenoids were found at Rf 0.84, 0.76, 0.51 and 0.18 (Fig. 2E). The purple band in Fig. 2F shows the presences of steroids at Rf 0.91 0.84. A densitogram at 540 nm was also produced (Fig. 3), which included the retention factor values, peak height and peak area for the present phytochemical compound.

Heavy metals analysis:

The present study revealed no detectable amount of lead < 0.5 ppm, cadmium< 0.5 ppm and mercury <0.5 ppm in this brand except arsenic, which was found to be (As)<6.62* ppm. According to the current ICP-OES investigation, DM was harmless and had no detectable levels of heavy metals. This study amply demonstrated that oil is safe for

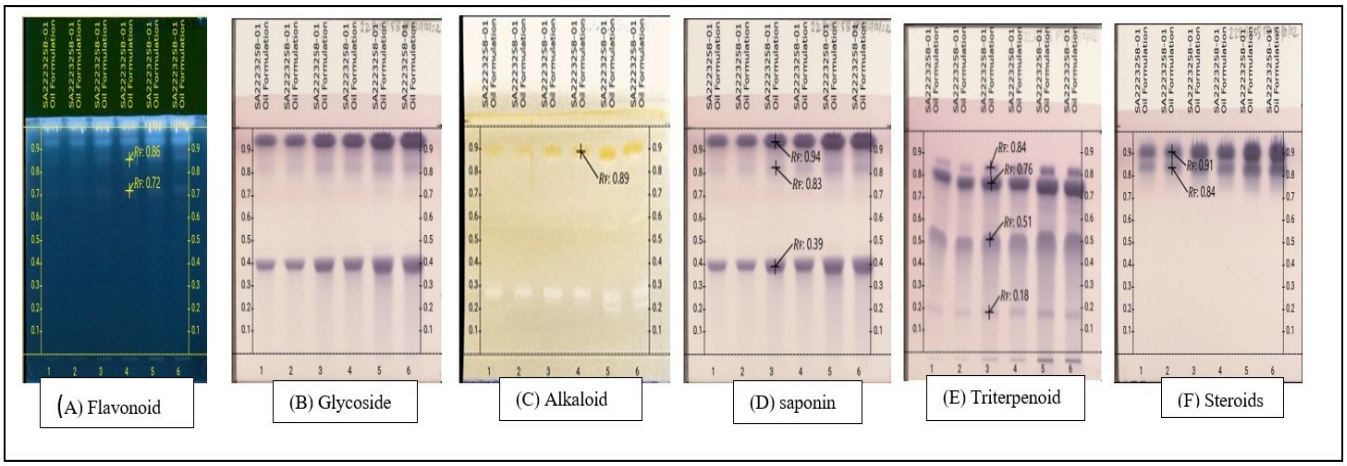

Fig. 2. HPTLC chromatogram of Dhanwantharam Mezhupakam; **[A]** At 366 nm, flavonoids with Rf 0.86 and 0.72 were identified as fluorescent molecules. **[B]** Purple band indicates the prescence of glycoside **[C]** The yellow band at Rf 0.89 depicts the presence of alkaloids. **[D]** At Rf 0.94, 0.83 and 0.39 blue bands were observed that corresponded to the saponin. **[E]** Terpenoids were found at Rf 0.84, 0.76, 0.51 and 0.18. **[F]** The purple band shows the presence of steroids at Rf 0.91, 0.84.

Table 3. HPTLC phytochemical study of the Dhanwantharam Mezhupakam methanolic extract

Name of compounds	Mobile phase	Derivatizing agent	Colour of bands observed	
			White light	UV 366 nm
Flavonoids	Ethyl acetate: Water: Formic acid: Acetic acid (100: 26: 11: 11 v/v/v/v)	NPA reagent	-	Fluorescent bands were observed
Steroid	n-Butanol: Methanol: Water (3:1:1 v/v/v)	ASR	Purple bands	-
Triterpenoids	n-Hexane: Ethyl acetate (1: 1 v/v)	ASR	-	Fluorescent bands were observed
Saponin	Ethyl acetate: Ethanol: Water: Ammonia 25% (65: 25: 9: 1 v/v/v/v)	ASR	Blue bands	-
Glycoside	Ethyl acetate: Methanol: Water (100: 13.5: 10 v/v/v)	ASR	Blue bands	-
Alkaloids	Toluene: Ethyl acetate: Methanol: Ammonia 25% (30: 30: 15: 1 v/v/v/v)	Dragendorff Reagent	Yellow bands	-
Tannin	Toluene: Ethyl acetate: Formic acid (6 : 4 : 0.3 v/v/v)	Alcoholic $FeCl_3$	No dark blue bands	-

(Natural Product A reagent :NPA reagent
Anisaldehyde Sulphuric Acid Reagent: ASR)

people to intake (13).

Analysis of microbial load and antibacterial activity

The microbial examination of DM revealed an overall plate count of 10 c.f.u./g.For the antimicrobial activity test, commercially available DM was tested against 6 distinct bacterial strains, including *S. aureus, P. vulgaris, P. aeruginosa, E. coli, E. faecalis* and *S. typhi* at doses of 200 mg/mL, 400 mg/ml, 600 mg/mL, 800 mg/mL and 1000 mg/mL (17). The zones of inhibition ranged from 12 mm to 17 mm and it was observed that the zone of inhibition increased with the higher formulation concentrations. Dhanwantharam mezhupakam, at a concentration of 1000 mg/ml exhibited significant antibacterial activity *against P. vulgaris, P. aeruginosa, E. coli, E. faecalis* and *S. typhi*. For concentrations of 600 mg/ml and 800 mg/ml, the zone of inhibition ranged from 6 mm to 14 mm. However, only *S. aureus* and *E. faecalis* showed any zone of inhibition at the formulation's 100 mg/ml concentration, while *E. coli* displayed the weakest antibacterial activity. Compared to the formulation tetracycline and streptomycin, conventional medicines, demonstrated higher antibacterial effectiveness. It's worth noting that *E. coli* showed sensitivity to the formulation due to the presence of a zone of inhibition. Fig. 4 illustrates the zones of inhibition of DM against various microorganisms and Table

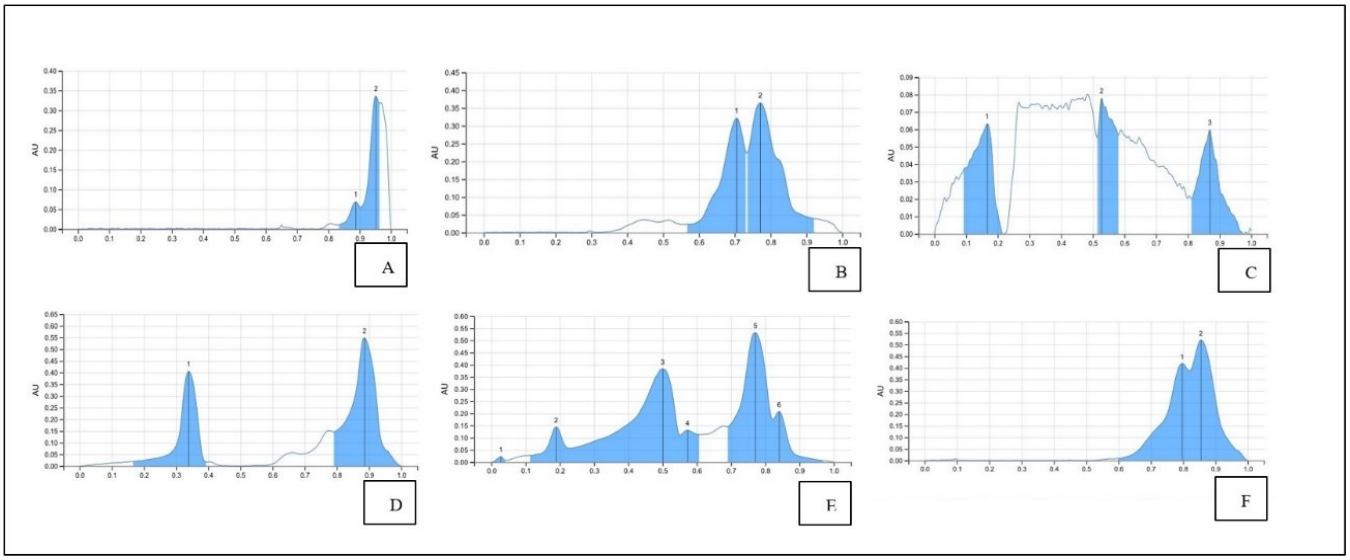

Fig. 3. HPTLC Densitogram of Dhanwantharam Mezhupakam. **[A]** Flavonoid **[B]** Glycoside **[C]** Alkaloid **[D]** Saponins **[E]** Triterpenoid **[F]** Steroid.

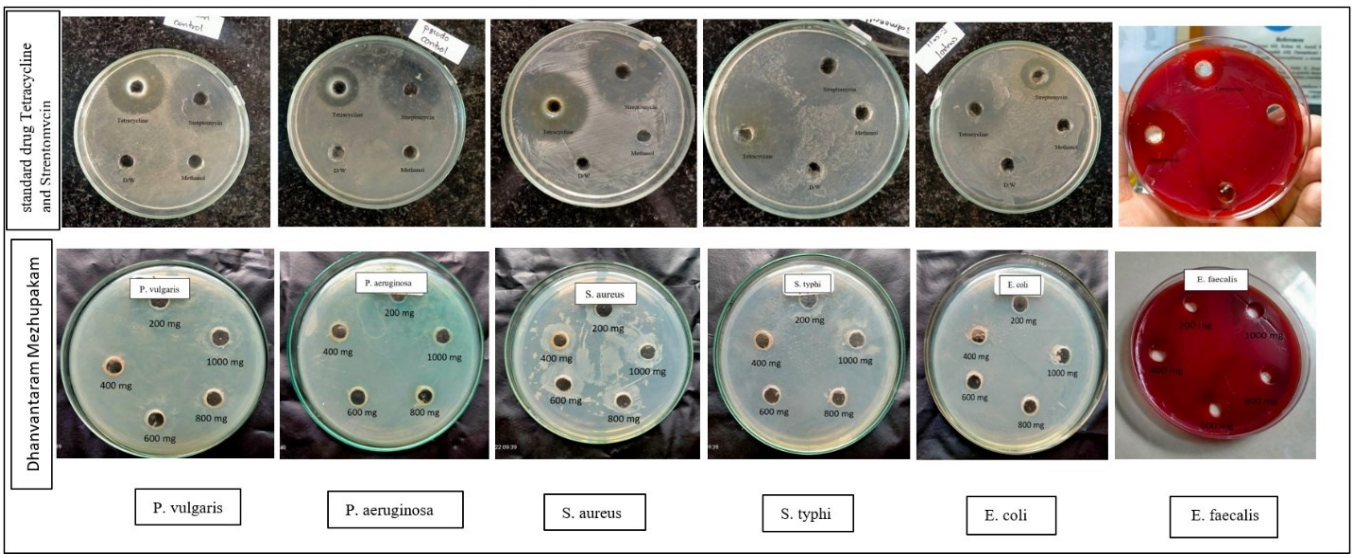

Fig. 4. *In vitro* culture plate (Well-diffusion method) of aqueous extract of Dhanwantharam Mezhupakam (at concentration 200 mg/ml, 400 mg/ml, 600 mg/ml, 800 mg/ml and 1000 mg/ml) and standard drug tetracycline and streptomycin showing zone of inhibition against *S. aureus, P. vulgaris, P. aeruginosa, E. coli, E. faecalis* and *S. typhi.*

Table 4. Represent the zone of inhibition value of antibacterial test for aqueous extract of Dhanwantharam Mezhupakam

Test organism	Zone of Inhibition (mm)				
	200 mg/ml	400 mg/ml	600 mg/ml	800 mg/ml	1000 mg/ml
S. aureus	-	-	-	-	-
P. vulgaris	-	-	-	-	9 mm
P. aeruginosa	-	-	6 mm	8 mm	12 mm
E. coli	5 mm	8 mm	11 mm	14 mm	17 mm
E. faecalis	-	-	-	-	-
S. typhi	-	-	-	9 mm	12 mm

4 provides the results of the antibacterial activities.

Discussion

Herbal medicines are more in demand for basic healthcare globally due to their higher safety margins and cost efficiency. The quality control of herbal medications is fraught with problems. The WHO plays a role in the standardization and quality control of herbal crude drugs/ oils in order to track the physicochemical examination of the oil, which includes elements like choosing, dealing with the crude material, effectiveness, safety and stability evaluation of the finished product, documentation of safety, provision of product information to buyers and advertising for products (18-20). Ayurvedic Pharmacopoeia of India (API) criteria were followed for analysing an Ayurvedic oil formulation, DM, for physicochemical and phytochemical parameters as well as for pollutants such as heavy metals and microbiological load (16). The International Council for Harmonisation (ICH) (21), (WHO) and United States Pharmacopoeia (USP) developed standardization recommendations that were also included (10, 21, 22). The formulation's antimicrobial impact on bacteria was also assessed.

Each Ayurvedic formulation has a characteristic organoleptic property of taste, color, odor and appearance and is expected to show consistency in manufactured product. Any deviation in these aspects gives an indication of quality deviation. An organoleptic analysis of the DM oil formulation used in this study revealed that it was a reddish-brown liquid with a greasy and fatty character, had an oily bitter taste, was soluble in non-polar solvents and had the distinct fermentation odor of other ayurvedic substances. Less than 0.5% (w/w) of the constituents in DM has any detectable foreign material, which was in line with the Indian Ayurveda Pharmacopoeia (API); components' foreign matter levels are 0.5-2%. In-depth research and the standardization of formulation at the industrial level are facilitated by preliminary tests and physicochemical assessment findings. Consequently, Table 2 reports the distinctive organoleptic features.

The physicochemical characteristics are essential for verifying the identification of crude pharmaceuticals and assessing their quality and purity (23). Our In-depth research and the standardization of formulation at the industrial level are facilitated by preliminary tests and physicochemical assessment findings. Rancidity is indicated by an acid value. The production and quality of oil are higher, with a lower acid value. The acid value of DM was discovered to be between 4 and 10. Values of saponification are very important while producing soap. It is crucial that the saponification value be exactly right; if it is too high, the soap may contain too much alkali, and it will react with skin; if it is too low, the fatty acid salts won't be enough to saponify or remove the fat or oil. DM's saponification value was discovered to be 140.25. Density was 0.8 and the Ester value was found to be 138.85. When the influence of temperature on density was estimated using wt/ml at 400 °C, it was found to be 1.489. As the pH of the formulation is a crucial factor since it affects not only the aroma, flavor and color of the oil but also its stability and maturity. At pH 3-5, many harmful microorganisms are unable to reproduce. In DM, a steady pH of 6 provides a natural defense against harmful microorganisms. Neither the sensitivity test nor the irritation test revealed any irritation. Physical evaluation results were provided and it was determined that the formulation's values were all within acceptable ranges. The medicinal effectiveness of formulations made from plants is greatly influenced by the phytochemicals found in them. According to various studies on phytochemical screening in other ayurvedic oils, there are secondary plant metabolites such as phenols, flavonoids, terpenoids, glycosides, alkaloids, tannins, saponins and other alcohols (24). No research had been done to determine the phytoconstituents in DM. Using HPTLC, it is possible to improve the phytochemical fingerprint profiles of methanolic extracts of DM. It was demonstrated clearly that many phytochemicals are present (Fig. 1). Preliminary phytochemical research revealed that the methanolic extract of DM included phenols, alkaloids, flavonoids, triterpenoids, alkaloids, glycosides and steroids. Several mobile phases and derivatizing reagent compositions were used depending on the phytoconstituents (Table 3) and the color changed following derivatization. The sample extract was derivatized and the densitograms were scanned at 540 nm to confirm the presence of alkaloids, glycosides, flavonoids, steroids, triterpenoids and saponin. The extract showed the highest concentration of *Sida cordifolia*, which was visible utilizing the peaks on the densitogram, enhancing its potential for use in medicine. These factors also play a role in the development of pharmacopeia standards, which are essential for both the survival of the outmoded, old medical system and their expanding worldwide relevance.

Heavy metal is a chemically dense metallic element that is relatively dangerous and hazardous above the permissible limits. Permissible limits of heavy metals in Ayurvedic formulations as per Ayurvedic Pharmacopoeia of India (API) are arsenic (3 ppm), mercury (1 ppm), cadmium (0.3 ppm) and lead (10 ppm) (16). It harms the nerve systems, irritates the lungs, eyes and skin and results in skin, lung, liver and nose cancers. Anaemia, renal damage, dermatitis, damage to the brain and nerves and dermatitis are all possible effects of heavy metal poisoning (24, 25). The present study revealed no detectable amount of lead < 0.5 ppm, cadmium< 0.5 ppm and mercury <0.5 ppm in this brand except arsenic, which was found to be (As) <6.62* ppm. According to the current ICP-OES investigation, DM was harmless and had no detectable levels of heavy metals. This study amply demonstrated that oil is safe for intake by people (13). Another investigation on the manufacture of ayurvedic oil (26) discovered that the levels of lead and cadmium were all excessive. In contrast, our research did not identify any lead or cadmium in measurable amounts. Hence, this oil formulation can include trace amounts of heavy metals, with the exception of arsenic, which is not allowed to be

included without losing its therapeutic efficacy. The examination of heavy metals provided significant data on the content of the product, which is worth noticing.

Results also showed that DM possesses strong antibacterial activity against *E. coli* while moderate against *Pseudomonas aeruginosa* and weak against *S. aureus, P. vulgaris, E. faecalis* and *S. typhi.* This early examination has demonstrated that DM is of good quality by proximate studies, physicochemical substance and heavy metal assessment. All of the pharmaceutical parameters that were evaluated fell within the permissible range, making them suitable for use as a standard reference in future research projects and clinical trials. The information could be used to boost DM formulation and oil efficiency for pharmacopeial standards.

Conclusion

Modern scientific quality control procedures have helped to standardize ayurvedic medication. The phytochemical analysis of the DM revealed the distinctive properties of the constituents utilized in the preparation. DM's organoleptic characteristics fell within the accepted range. The current investigation found many phytochemicals in the formulation, which may account for its therapeutic effects. This finding supports its use as a treatment for a variety of nervine and neuroprotective conditions. Additionally, it was noted that the formulation was risk-free and did not contain any measurable quantities of heavy metals. All of the pharmaceutical parameters that were evaluated fell within the permissible range, making them suitable for use as a standard reference in future research projects and clinical trials. The data might potentially be utilized to establish a new set of pharmacopeial standards for DM formulation and oil efficiency.

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Authors' contributions

Conceptualization, HG, KST, MT; methodology, AK, TT; formal analysis, AK, MT, HG, KST; data curation, AK, TT; writing—original draft preparation, MT, HG; writing—review and editing, MM, KST, supervision, MT and HG. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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