Research Article

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QUALITATIVE ANALYSIS OF MAHISHADRAVAKA WITH SPECIAL REFERENCE TO LCMS STUDY

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ABSTRACT

Introduction: Mahishadravaka, a renowned Ayurvedic proprietary medicine, has been historically used for its therapeutic properties. It is the arka preparation (distillation process) of Mahisha bone (bone of a buffalo), along with other herbal medicines. It is traditionally used for Vata vyadhies (diseases caused by Vata). Materials and Method: In this study, we conducted a comprehensive LCMS analysis and vitamin D3 and Calcium analyses of Mahishadravaka. Identification and characterisation of bioactive compounds were also aimed at. Result: Our findings reveal a diverse array of compounds with promising pharmacological activities, including Anti-inflammatory, antioxidant, anti-microbial, and anticancer properties. This article provides an overview of the identified compounds and their reported medicinal benefits, shedding light on the potential mechanisms underlying the therapeutic efficacy of Mahishadravaka. Conclusion: The LCMS of Mahishadravaka unveiled a rich array of bioactive compounds with significant pharmacological potential. The elucidation of the bioactive constituents paves the way for further research and development of novel therapeutic formulations based on Ayurvedic principles. These compounds encompass a broad spectrum of therapeutic activities, validating the traditional use of Mahishadravaka in Ayurvedic medicine. The findings from this study provide a scientific basis for the therapeutic efficacy of Mahishadravaka and underscore its potential for developing novel therapeutic interventions targeting various diseases.

Keywords: Mahishadravaka, LCMS study, Calcium, Vit D3, Bioactive compounds

INTRODUCTION

In Ayurveda, Panchavidhakashaya kalpanas (five basic forms of pharmaceutical preparations) form the backbone of all medicines. Through these forms, medicines are employed to treat various ailments. Swarasa (juice), Kalka (paste), Kwatha (decoction), Seetha (cold infusion) and Phanta (hot infusion) are the five forms of Kashaya kalpanas[1]. According to Ravana, the author of Arka Prakasa, the Panchavidhakashaya kalpanas also includes the Arka[2]. In Arka Prakasa, Panchavidha Kashaya Kalpana is mentioned as Kalka, Churna (powder), Swarasa, Taila (oil), and Arka (distillate). Compared to all Kalpana, Arka Kalpanas are the most potent kalpanas because of their colourlessness, effectiveness in low dosage, palatability, easy intake, especially in paediatrics, ease of formulation and stability[3]. Arka is a liquid preparation obtained by distillation of certain liquids or drugs soaked in water using Arkayantra (distillation apparatus) or any other convenient modern distillation apparatus[4]. This aims to extract active constituents from the content for therapeutic usage.

Keraleeya Ayurveda is rich in many traditional formulations. Mahishadravaka is one of them. At Sitaram Ayurveda (P) Ltd., Mahishadravaka falls under their proprietary medicine category. Mahishadravaka is the Arka preparation of Mahisha bone, along with other herbal medicines. It is traditionally used for Vatavyadhi[5]. Hence, in this study, an attempt was made to prepare Mahishadravaka and analyse its analytical parameters. Mahishadravaka, an Ayurvedic proprietary medicine, has been traditionally used for its potent therapeutic effects on various ailments. However, the molecular basis of its pharmacological actions remained largely unexplored. To address this knowledge gap, we conducted a detailed LCMS study to identify and characterise the bioactive compounds present in Mahishadravaka. Along with the study, Vitamin D analysis by GCMS and calcium analysis by flame photometry were done to get valuable insights into the mechanisms underlying its therapeutic efficacy and offer potential avenues for developing novel therapeutic interventions.

Standardisation of a formulation aims at identifying and determining its quality and purity. Standardisation is the need of the hour in Ayurveda. It is necessary for the globalisation of Ayurveda. Standardisation plays a vital role in deciding the quality and purity of a drug or formulation through different sets of parameters.

MATERIALS AND METHODS

Collection of materials

QA tested and approved herbal ingredients, tabulated in Table 1, collected from the Sitaram Ayurveda (P) Ltd. raw material section and the bone of Mahisha (Buffalo) from the local meat market in Thrissur, respectively.

Pharmaceutical preparation

Dried ingredients for kashayam were cleaned, coarsely powdered, and soaked in water in the drug boiler at 1:16 for 24 hours. The next day, the cleaned buffalo bone was mixed with Kashayam, which was taken in a ratio of 1:10. This combination was subjected to distillation. The distilled liquid was collected in a clean vessel and filtered through a cotton cloth. The filtered liquid was collected in clean bottles and stored.

Table 1: List of ingredients in Mahishadravaka

Plant scientific name	Family	Parts	Herbarium voucher number
Tragia involucrata	Euphorbiaceae	Whole Plant	SAPL/QC/HB/148
Alpinia galanga	Zingiberaceae	Rhizome	SAPL/QC/HB/149
Ricinus communis	Euphorbiaceae	Root	SAPL/QC/HB/107
Adhatoda vasica	Acanthaceae	Root	SAPL/QC/HB/098
Cyperus rotundus	Cyperaceae	Rhizome	SAPL/QC/HB/150
Withania somnifera	Solanaceae	Root	SAPL/QC/HB/043
Cedrus deodara	Pinaceae	Heart Wood	SAPL/QC/CS/047
Acorus calamus	Acoraceae	Root	SAPL/QC/HB/006
Zingiber officinale	Zingiberaceae	Rhizome	SAPL/QC/HB/020
Tinospora cordifolia	Menispermaceae	Stem	SAPL/QC/HB/029
Barleria prionitis	Acanthaceae	Root	SAPL/QC/HB/151
Boerhavia diffusa	Nyctaginaceae	Whole Plant	SAPL/QC/HB/015
Baffalo's bone	Bovidae	As Such	-

Analytical study

Organoleptic analysis
The Mahishadravakawas tested organoleptically for taste, colour, texture and odour.

Physicochemical parameters

Determination of pH: The pH measures the hydrogen ion activity, which is essential from the standpoint of stability or physiological suitability[6]. The pH was measured using a pH meter per the instructions.

Determination of Specific Gravity: The specific gravity of the Mahishadravaka was measured using a Pycnometer as per the standard method[7].

Determination of Refractive Index: The refractive index of the Mahishadravaka was measured using Abbes refractometer[7].

Determination of Acidity: The acid value of a liquid is defined as the number of milligrams of Potassium hydroxide required to neutralise the free acid in 1 gram of the sample. [8]

Determination of Total Solid Substance: Total solids were measured by using a refractometer.

Vitamin D3 by GCMS [9]
Instrument Model -7890 A GC with 5975C with triple axis detector.
Column - DB 5MS 30 m x 0.250mm Diameter x 0.25 Micrometer Thickness.

Analysis was performed by injecting 1µL of the sample in a splitless mode in the Selective Ion Monitoring method. Helium gas (99.9995%) was used as the carrier gas at a 1 ml/min flow rate. The analysis was performed in the EI (electron impact) mode with 70 eV of ionisation energy. The injector temperature was maintained constantly at 280 °C. Carry out the qualitative identification of compounds based on retention time and compare the sample mass spectrum with characteristic ions (3 ions of most incredible relative intensity or any three ions over 30% of relative intensity) in a reference mass spectrum. After identifying the compound, the quantification is carried out by selected ion monitoring (SIM) based on the integrated abundance of the primary characteristic ion.

Calculation

Vitamin D3 mg/kg = Observed Concentration X Purity of Vitamin D3 ×10000

Calcium[10]
The standard stock solution and sample solution are prepared in fresh distilled water. The flame photometer is calibrated by adjusting the air and gas. Then, the flame is allowed to stabilise for about 5 min. Now, the instrument is switched on, and the lids of the filter chamber are opened to insert appropriate colour filters. The galvanometer readings are adjusted to zero by spraying distilled water into the flame. The sensitivity is adjusted by spraying the most concentrated standard working solution into the flame. Now, the full-scale deflection of the galvanometer is recorded. Again, distilled water is sprayed into the flame to attain constant galvanometer readings. Then, the galvanometer is readjusted to zero. Now, each of the standard working solutions is sprayed into the flame three times, and the galvanometer readings are recorded. After each spray, the apparatus must be thoroughly washed. Finally, the sample solution is sprayed into the flame three times, and the galvanometer readings are recorded. After each spray, the apparatus must be thoroughly washed.

Calculate the mean of the galvanometer reading and plot the graph of concentration against the galvanometer reading to find out the concentration of the element in the sample.

Calcium (%) = Systemconcentration x Dilution Factor x 10^{-4}

OBSERVATION AND RESULT

Organoleptic Properties: The Organoleptic characteristics of Mahishadravaka are detailed in Table 2.

Physicochemical parameters: Physicochemical parameters of Mahishadravaka are tabulated in Table 3.

Calcium and Vitamin D3 analysis: The Results of Calcium and Vitamin D3 are tabulated in Table 4.

LCMS study report: The compounds, area, chemical formula and properties identified in the LCMS study are tabulated in Table 5**.**

Table 2: Organoleptic characteristics of Mahishadravaka

Organoleptic Characteristic	Result
Colour	Colourless
Taste	Slightly bitter
Odour	Characteristic
Form	Liquid

Table 3: Physicochemical analysis of Mahishadravaka

Physicochemical parameters	Results (3 Trials)			
	I	II	III	Standard
pH	4.26	4.18	4.30	**4.24**
Specific gravity	0.99	0.98	1.00	**0.99**
Refractive index	1.331	1.332	1.332	**1.331**
Acidity	0.061	0.062	0.062	**0.061**
TSS	0	0	0	**0**

Table 4: Quantity of the calcium and Vitamin D3 in the Mahishadravaka

Parameters	Result
Calcium	40.80 ppm
Vit.D3	91.6 IU/g

Table 5: The compounds identified in the LCMS study

Compounds	Height	Area	Chemical Name	Properties
Fraxin	8212	644717	C16H18O10	Antioxidative stress action, Anti-inflammatory and Antimetastatic[11]
Quercetin-3-Rhamnoside	6577	560238	C21H20O11	Antiinflammation, Antioxidative and neuroprotective [12]
Licoflavanone	14905	557482	C20H20O5	Anti-inflammatory activity [13]
Isovitexin	21481	372552	C21H20O10	Antioxidant, Anti-inflammatory, Anti-neoplastic activities [14]
Protopanaxadiol	3452	319593	C30H52O3	Cardiovascular and central nervous system protection, Anti-diabetic, Anti-neoplastic, and Anti-inflammatory actions [15]
Coptisine	3925	277065	C19H14NO4	Anti-inflammatory[16]
Aucubin	5036	275920	C15H22O9	Anti-inflammatory, Antioxidant, anxiolytic and anti-depressant, Anti-diabetic, Anti-fibrotic, Anti-microbial, Anticancer, anti-hyperlipidemic, gastro, cardio, hepato, retino and neuroprotective [17]
Cyanidin chloride	2983	181446	C15H11ClO6	Antioxidant and Anticarcinogenesis[18]
Isorosmanol	4394	177893	C20H26O5	Antioxidant, neuroprotective and neurotrophic effects [19]
Campesterol	3013	172059	C28H48O	Anti-inflammatory[20]
Fucoxanthin	11433	163556	C42H58O6	Anti-inflammatory and Anticancer effects [21]
Deacetylgedunin	1905	151952	C26H32O6	Antimalarial and Anti-inflammatory[22]
Dephnoretin	5760	147539	C19H12O7	Antioxidant activity [23]
Liquiritin	2142	124177	C21H22O9	Attenuated rheumatoid arthritis via reducing inflammation, suppressing angiogenesis[24]
Rhein	4528	121304	C15H8O6	Antioxidant activity [25]
Fukugetin	2891	120289	C30H20O11	Anti-inflammatory Antibacterial, Antitrypanosomal Antioxidant [26]
Quercitrin	1866	118769	C21H20O11	Antioxidant and Anti-inflammatory[27]
Auraptene	1798	107277	C19H22O3	Antibacterial, Antifungal, Antileishmania and antioxidant activity [28]
Ginkgolide	2319	104928	C20H24O9	Anti-inflammatory [29]
5-Caffeoylquinic acids	6046	100680	C16H18O9	Antioxidant, Anti-inflammatory, Anti-diabetic [30]
Aloesin	1791	100520	C19H22O9	Antioxidant, Anti-inflammatory, and immunomodulatory effects [31]
Loganin	2359	96601	C17H26O10	Antioxidant, neuroprotective properties [32]
Isoliquiritin	3666	80956	C21H22O9	Antioxidant, Anti-inflammatory, and anti-depression activities [33]
Gallic acid	2196	61223	C7H6O5	An antioxidant, an anti-neoplastic agent [34]
Bergenin	2609	59483	C14H16O9	Antihepatotoxic, Antiulcerogenic, AntiHIV, Antifungal, Hepatoprotective, Antiarrhythmic, Neuroprotective, Anti-inflammatory, Immunomodulatory [35]
Polygalasaponin V	4004	57518	C58H94O27	Anti-inflammatory, Antiapoptotic [36]
Sinigrin	2368	53711	C10H17NO9S2	Anticancer, Antibacterial, Antifungal, Antioxidant, Anti-inflammatory, Wound Healing Properties[37]
Cornuside	2981	50019	C24H30O14	Antiapoptotic, Antioxidant, and Anti-inflammatory Properties[38]
Glycyrrhetinic acid	1439	48819	C30H46O4	Antioxidative, Anti-inflammatory and Anti-microbial Activities[39]
Alpha-Viniferin	2934	46931	C42H30O9	Antioxidant, Anti-inflammatory, Anti-diabetic, and Anticancer Properties[40]
Berberine	2180	39464	C20H18NO4	Neuroprotective, Antioxidant, Antiapoptotic and Antiischemic[41]
Azadirachtin	1733	32046	C35H44O16	Antioxidant, Anti-inflammatory [42]
Rubiadin	1318	31402	C15H10O4	Anticancer, Antiosteoporotic, Hepatoprotective, Neuroprotective, Anti-inflammatory, Anti-diabetic, Antioxidant, Antibacterial, Antimalarial, Antifungal, and Antiviral Properties[43]
Phytic acid	1596	30751	C6H18O24P6	Antioxidant [44]
4,5-Dicaffeoyl quinic acid	1282	27660	C25H24O12	Antioxidative, Anti-inflammatory, and Antimelanogenic Properties[45]
Rhoifolin	1354	26639	C27H30O14	Antioxidant, Anti-inflammatory, Anti-microbial, Hepatoprotective and Anticancer Effects [46]
Epicatechin gallate	1210	26447	C22H18O10	Reduce inflammation [47]

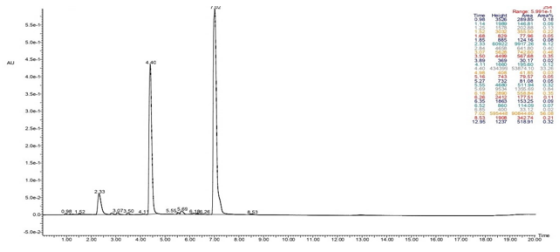

Figure 1: LCMS analysis of Mahishadravaka

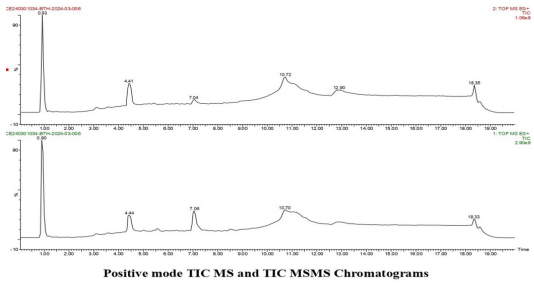

Figure 2: Positive mode of LCMS analysis

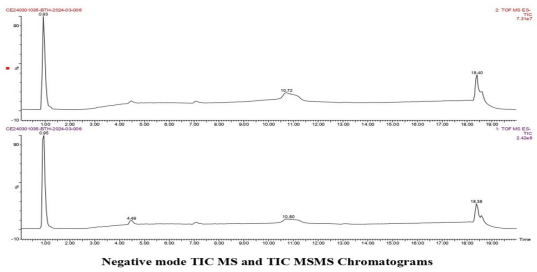

Figure 3: Positive mode of LCMS analysis

DISCUSSION

The disintegration of ingredients and overnight soak made the drug softer, which helped release essential active principles while boiling. When the drug is soaked, the tissue swells up as the drug's cell wall absorbs the liquid. So, it is advisable to soak powdered pharmaceuticals for a while before boiling them, as this increases the drug's water contact duration and makes it easier to extract the active ingredients by distillation.[48] The Arka obtained was a transparent, clear liquid with a distinct smell that satisfied the Uttama Arka lakshana (best distillate features), as stated in Arka Prakasa.

The results of the analytical investigation determine the quality of the final product. The investigation's main objective is to assess Arka's quality in order to ascertain its potential therapeutic benefit. The final Arka product is standardised according to its physical, chemical, and organoleptic qualities. The result was a transparent, clear liquid with the flavour and aroma of the substances employed in the preparation. Mahishadravaka has a pH of 4.24, indicating that it is acidic. The pH level of the drug influences both its effectiveness and absorption. If the drug is overly acidic or alkaline, it will irritate the tissues.

The presence of dissolved substances in the samples impacts the specific gravity value. The specific gravity of Mahishadravaka is 0.99. Here, the active principle isolated from the raw materials is referred to as the solute, and water is the solvent. Mahishadravaka has a refractive index of around 1.331. The mixture's refractive index fluctuates according to the solutes and consistency of the media since distillate Arka is water-like and the solutes and solvents are the same. The solution has a refractive index similar to that of water. The amount of suspended and dissolved solids in water is measured as total solids. In this case, Arka TSS is 0, which included no solid materials. Based on the phytochemical analysis of Mahishadravaka, alkaloids were detected. These alkaloids are responsible for the therapeutic action of Mahishadravaka.

The word Vitamin D' refers to a set of four chemicals that are required for healthy bone formation. There are two types of Vitamin D supplements: Vitamin D3 (also known as cholecalciferol) and Vitamin D2 (also known as Ergocalciferol or pre-vitamin D)[49] Vitamin D3 is naturally produced in the human body and is found in animal foods. Vitamin D3 supplements tend to elevate concentrations of vitamins more and maintain those levels longer than D2 supplements, according to a meta-analysis of randomised controlled trials comparing their effects on blood levels.[50, 51] In addition to being necessary for immune system maintenance, bone and muscular strength, and immune system function, vitamin D3 may also help avoid inflammatory illnesses. Mahishadravaka contains 91.6 IU/g Vitamin D 3, as identified by the GCMS Study.

Mahishadravaka contains approximately 40.80 ppm of calcium. This mineral content is significant, as calcium is crucial in various physiological functions within the human body. Calcium is well known for its role in maintaining strong bones and teeth, but it also supports muscle function, nerve transmission, and hormone secretion[52]. The presence of calcium highlights the therapeutic use of Mahishadravaka.

LCMS study results

The LCMS analysis revealed a rich diversity of bioactive compounds within Mahishadravaka. These compounds belong to various chemical classes, including glycosides, flavonoids, alkaloids, terpenoids, phenolic compounds, and fatty acids. Out of the 37 compounds identified, most possess anti-inflammatory action and antioxidant activity. Notably, many of these compounds have been reported to possess significant pharmacological activities, such as anti-inflammatory, antioxidant, anti-microbial, anticancer, and neuroprotective properties.

The LCMS analysis revealed many bioactive compounds in Mahishadravaka, each with unique pharmacological attributes. Among these, fraxin, a glycoside, exhibited potent antioxidative stress, anti-inflammatory, and anti-metastatic properties.[11] Quercitrin, a bioflavonoid, demonstrated anti-inflammatory, antioxidant, and neuroprotective effects, including the induction of apoptosis in colon cancer cells.[12] Licoflavanone displayed significant anti-inflammatory activity without affecting cell viability, suggesting its safety profile.[13] Isovitexin showcased antioxidant, anti-inflammatory, and anti-neoplastic activities, along with protective effects on various diseases affecting the neurological, psychiatric, cardiovascular, and endocrine systems.[14] Protopanaxadiol-glycoside demonstrated cardiovascular and central nervous system protection and anti-diabetic, anti-neoplastic, and anti-inflammatory actions.[15] Coptisine exhibited potent anti-inflammatory properties,[16] while Aucubin displayed a wide range of pharmacological effects, including anti-inflammatory, antioxidant, anxiolytic, anti-depressant, anti-diabetic, anti-fibrotic, anti-microbial, anticancer, anti-hyperlipidemic, gastroprotective, cardioprotective, hepatic protective, retinoprotective, and neuroprotective activities.[17]

Other notable compounds such as Cyanidin chloride[18], Isorosmanol[19], Campesterol[20], Fucoxanthin[21], Deacetylgedunin[22], Dephnoretin[23], Liquiritin[24], Rhein[25], Fukugetin[26], Quercitrin,[27] Auraptene[28], Ginkgolide A[29], 5-Caffeoylquinic acids[30], Aloesin[31], Loganin[32], Isoliquiritin[33], Gallic acid[34], Bergenin[35], Polygalasaponin[36], Sinigrin[37], Cornuside[38], Glycyrrhetinic acid[39], Alpha-Viniferin[40], Berberine[41], Azadirachtin[42], Rubiadin[43], Phytic acid[44], 4,5-Dicaffeoyl quinicacid[45], and Rhoifolin[46] exhibited diverse pharmacological activities ranging from Anti-inflammatory, antioxidant, anti-microbial, and antiviral properties to wound healing, neuroprotective, anti-diabetic, and anticancer effects the broad spectrum therapeutic potential of Mahishadravaka.

CONCLUSION

The comprehensive analysis of Mahishadravaka reveals the potent and multifaceted therapeutic effects of the formulation. The meticulous preparation process, involving the soaking and disintegration of ingredients, enhances the extraction of active principles essential for therapeutic efficacy. The LCMS study of Mahishadravaka has provided valuable insights into its chemical composition and pharmacological properties. The presence of diverse bioactive compounds with potent therapeutic effects underscores the traditional efficacy of this Ayurvedic formulation. These findings validate the traditional use of Mahishadravaka and open new avenues for developing evidence-based therapeutic interventions rooted in Ayurvedic principles. Further research aimed at elucidating the synergistic interactions among these compounds and their modes of action is warranted to harness the therapeutic potential of Mahishadravaka fully.

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