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Kushmanda ghrita - A Nano Formulation: Physico-chemical and Pharmaceutical Evaluation

Research Article

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Abstract

Ayurvedic ghee-based formulation *Kushmanda ghrita* (KG) is prescribed for various psychological disorders. Ayurvedic products' quality has been the prime concern over years. There is a lack of literature regarding standardisation of KG and quantification of glabridin, a constituent of licorice, which is part of KG. Ghee is considered an excellent carrier. Hence, this study aimed to prepare, standardise KG, and quantify glabridin using HPLC and to evaluate the particle size of the KG. KG was prepared according to classical texts, subsequently, the KG and Desi cow ghee (DG), which are employed for preparing KG, were subjected to assessment of physicochemical parameters. The amount of glabridin in KG was quantified using HPLC method. The particle size was analysed for the prepared KG. Investigation revealed that there were insignificant differences in physicochemical parameters of KG and DG, and they are within the acceptable limits. Glabridin was extracted by petroleum ether and methanol (70%) solvent system, quantified by HPLC method, and found to be 0.1873±0.003 mg/g of KG. Further, the particle size analysis revealed that the KG sample was showing a varying globule size; the maximum volume occupancy of 43.6%v/v gives a globule size of 260.5nm, and hence it is said to be a Nano formulation. In summary, the quality of KG was assessed in terms of physicochemical parameters. The glabridin was quantified using HPLC by optimising extraction procedure. The particle size of KG was analysed and found to be in Nano region. The outcomes of this study may be helpful in further studies on the molecular mechanism, pharmacodynamics and pharmacokinetic and drug interaction studies of KG.

Keywords: *Ghee-based, Glabridin, HPLC, Kushmanda ghrita, Nano formulation, Physico chemical Analysis*.

Introduction

Kushmanda ghrita (KG) is a polyherbal Ayurvedic formulation mentioned in both *Astanga Hridaya* and *Bhaishajya Ratnavali* for treating various psychological illnesses (1, 2). KG consists of the juice of *Kushmanda* (*Benincasa hispida* (Thunb.) Cogn.), *Yashtimadhu* (*Glycyrrhiza glabra* L.), and go *ghrita* (cow ghee) (3). KG was reported for anti-depressant and anti-psychotic activities in human subjects (4, 5). Glabridin is a major pharmacologically active isoflavan of licorice reported to possess anti-inflammatory, $GABA_A$ potentiating, neuroprotective, and memory-enhancing properties (6, 7). The method of quantification of glabridin is reported for the extracts and formulation (8, 9). There is little data available to estimate glabridin in *ghrita*. As Ayurvedic treatment and products are gaining popularity all over the world, the quality of Ayurvedic products is a concerning issue. Ayurveda refers to ghee- or oil-based preparations as *Sneha kalpana*, which involves boiling ghee or oil with prescribed kasayas (polyherbal decoctions) and a fine paste of botanicals known as *kalkas.* In Ayurveda, ghee is considered *yogavahi* rasayana, which means any drug processed with *ghrita* will possess the added drug's qualities(10), and is evidenced by various modern literature that describes the ghee as a good carrier as a Nano drug delivery system (11). Moreover, keeping *Guggulutikta ghrita* as a model formulation, it has been demonstrated that lipid-based formulations act as Nano drug delivery system (12). Based on above observations, the present study was designed to standardize KG, quantify glabridin present in it and to evaluate the particle size of the prepared KG.

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Materials and methods

Chemicals and reagents

Glabridin reference standard was kindly supplied by Sami Labs Ltd., Bengaluru. HPLC-grade solvents including acetonitrile, methanol, and orthophosphoric acid were obtained from Merck Ltd., Mumbai. *Yashtimadhu* was procured from M/S Amrutakesari Drug Depot, Bangalore, while fresh fruits of *Kushmanda* (*Benincasa hispida*) were sourced from the Yelahanka vegetable market. Botanical materials were

authenticated at the Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore. Desi cow ghee used for the preparation was gifted by Rashtrotthana Goushala, Doddaballapur.

Preparation of *Kushmanda ghrita* (KG)

As per the classical reference from *Chakradatta* (3), KG was prepared using the following proportions: *Kushmanda Swarasa* – 18 L, *Yashtimadhu* – 125 g, and *Go-Ghrita* – 1 L. The ghee was first melted on moderate heat. A paste of *Yashtimadhu* prepared in *Kushmanda* juice was added, followed by the remainder of the juice. The mixture was gently heated for approximately 3 hours daily for three consecutive days. Upon observing *Sneha Siddhi Lakshanas* (specifically, *Madhyama Paka* stage) on the final day (13), the product was filtered warm through a muslin cloth and stored in airtight containers.

Organoleptic and physicochemical Parameters.

Both the prepared KG and base ghee (DG) were evaluated for color, odor, and taste. Additionally, standard physicochemical parameters including refractive index, specific gravity, acid value, iodine value, and saponification value were determined according to the protocols outlined in the *Guidelines for Testing of Ayurveda, Siddha, and Unani Medicines* (14) and the *Ayurvedic Pharmacopoeia of India* (15).

UV Spectroscopic Analysis

Instrumentation

UV absorbance measurements were conducted using a Shimadzu UV-2501 PC UV–VIS spectrophotometer operated via UV Probe software (version 2.51). Spectral scanning was performed across the 200–800 nm wavelength range.

HPLC analysis

The HPLC analysis of KG was carried out keeping Glabridin as a marker compound. The analysis method was done according to the previously reported method with modifications (16).

Instrumentation

A Shimadzu HPLC system fitted with a UV detector and LC Solution software was employed for analysis. Chromatographic separation was achieved on a ZODIAC C18 column (250 × 4.6 mm, 5 µm) at ambient temperature. The mobile phase comprised methanol and water (75:25 v/v), delivered at a flow rate of 1.0 mL/min under isocratic conditions. Each sample run time was 30 minutes with an injection volume of 50 µL, and detection was performed at 230 nm.

Standard Preparation.

A stock solution of glabridin was prepared by dissolving 5.6 mg of the standard in 5 mL of 70% methanol. Serial dilutions were prepared to yield concentrations of 0.224, 0.448, 0.672, 0.896, 1.12, and 1.34 µg/mL. These were used to construct a calibration curve.

Extraction and sample preparation.

Initially, 1 g of KG was dissolved in 50 mL of petroleum ether and extracted with 70% methanol in multiple 25 mL fractions. Each methanolic extract was filtered through a 0.45 µm membrane filter and analyzed by UV and HPLC. The process continued until no further glabridin response was detected. For optimal extraction, 0.6 g of KG was treated with petroleum ether followed by four sequential extractions with 25 mL portions of 70% methanol. The pooled extracts were filtered and analyzed in triplicate.

Determination of particle size and distribution.

Sample was analyzed via Zetasizer Nano ZS (Make: Microtrac, Model: Nanotrac - USA). Stock solution of KG was prepared separately in a concentration of 10 mg/ml in Nano water and sonicated using ultrasonic bath for 5 min, then transferred into the liquid cell and measured for the particle size and zeta potential.

Data collection and analysis

All physicochemical analyses were carried out in triplicate (n = 3), and results were expressed as mean ± SEM. For HPLC quantification, a calibration curve was generated by plotting peak area versus glabridin concentration. The correlation coefficient (R^2) was calculated to assess linearity, with acceptable linearity defined as $R^2 \geq 0.99$. Glabridin content in KG was then quantified based on the linear regression equation derived from the standard curve and reported in mg/g.

Results

The organoleptic observations revealed that DG was yellowish in color, fragrant odor, and characteristic taste, whereas KG was slightly dark yellowish, fragrant (sweetish) odor and taste. The results of the physicochemical parameters such as DG and KG such as refractive index, specific gravity, acid value, iodine value and saponification value are given in Table 1.

Table 1: The Physico-chemical parameters of KG

Sample	Refrac	Specific	Acid value	Iodine	Saponifica
Desigoug	1.461	0.90676	4.452±	30.85	229.4
Kushma	1.460	0.90366	4.087±	33.39	232.9

The method of extraction of glabridin from KG was optimized. Initially around 1g of KG was weighed and dissolved in 50ml of petroleum ether and extracted with 70% methanol (7x25ml) each fraction was scanned at 200 to 600nm range. Two absorption maxima were obtained at 228 and 280 nm, similar to the glabridin standard. (Fig.1a and 1b). In UV analysis 7th fraction showed absorption maxima at 228nm but there was no absorption observed at 280nm. In the HPLC chromatogram, there was no peak detected at that concentration. Then the sample weight was reduced to 600 mg and the same procedure was repeated. The 4th and 5th fractions showed minimal absorption in UV spectra. When injected to HPLC no peak was detected at these fractions. Hence it was decided to use a 600mg

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KG sample and the extraction cycle was limited to 4x25ml, the fractions were pooled and made up to 100 with 70% methanol and proceeded for HPLC estimations.

Figure 1: UV spectra of a) Glabridin, b) Methanol fraction of KG

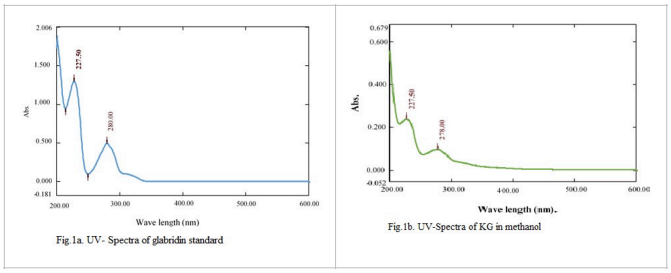


Fig.1a. UV- Spectra of glabridin standard

Fig.1b. UV-Spectra of KG in methanol

Fig. 2 a, b, and c show the HPLC chromatograms of the glabridin standard, KG, and comparison chromatograms. The retention time of standard glabridin was found to be 12.5 min, and a peak appeared in the sample at the same RT.

Figure 2: HPLC Chromatogram of a) Glabridin b) Methanol fraction of KG c) Comparative Chromatogram.

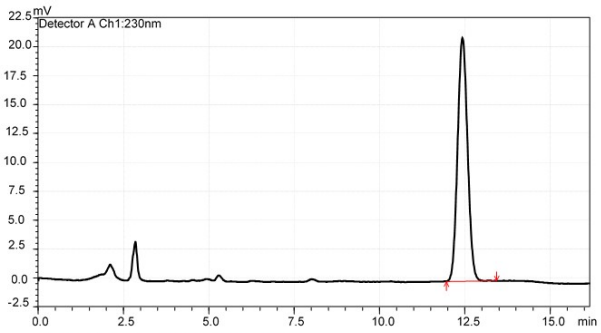


Fig.2a. HPLC chromatogram of glabridin standard

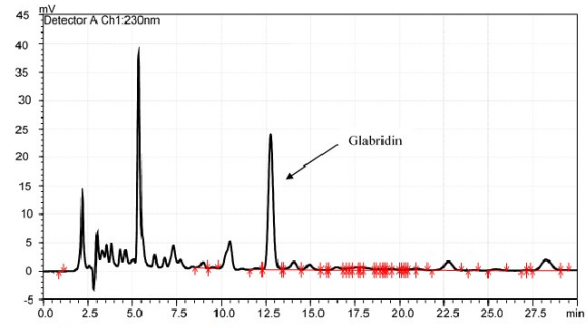


Fig.2b. HPLC chromatogram of KG

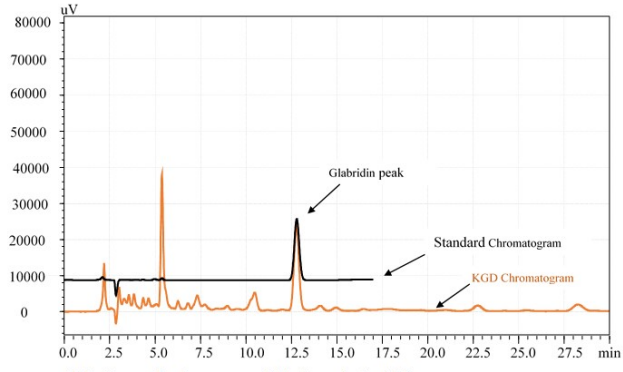


Fig.2c. Comparative chromatogram of glabridin standard and KG

The linearity curve was established using peak response versus concentration. (Fig.3), the r^2 was found to be 0.999. The concentration of the glabridin in the sample was calculated using the equation y = 385071x - 6336.6. The amount of glabridin found to be 0.1873±0.003 mg/g of the KG sample analyzed in triplicate.

Figure 3: Standard Calibration curve for Glabridin

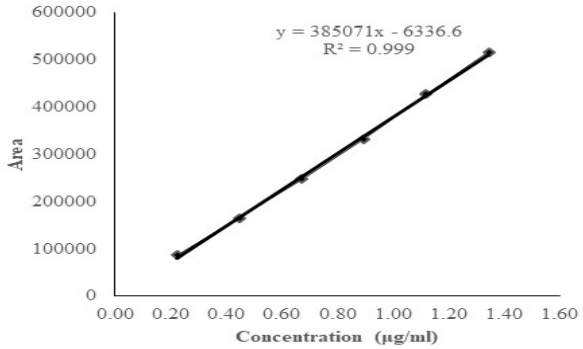


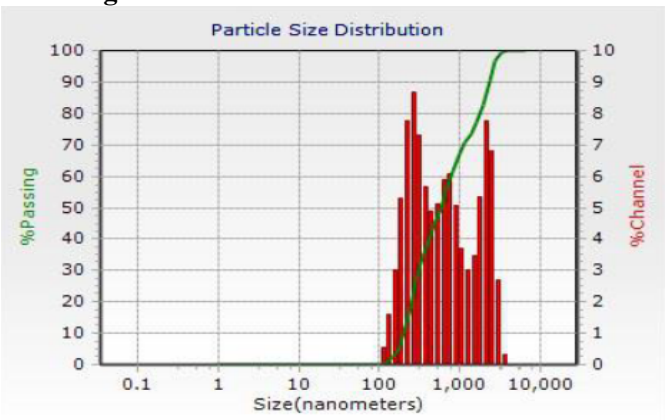
Fig.3. Standard calibration curve for glabridin

The particle size analysis revealed that the KG sample was showing a varying globule size of 2132nm, 737nm & 260.5nm with a volume occupancy of 27.1, 29.3 & 43.6% respectively. (Table.2 and Fig.4). Further for the KG sample the average globule size with 100% volume occupancy shows the globule size of 1.04 µm. However, the maximum volume occupancy of 43.6%v/v gives a globule size of 260.5nm. These findings confirm that KG contains globules within the nanometric and submicron size range.

Table 2: Particle size distribution of KG

Peaks Summary		
Diameter (nm)	Vol%	Width
2132	27.1	991
737	29.3	478
260.5	43.6	183.9

Figure 4: Particle size distribution of KG



Discussion

The consistency of the therapeutic impact depends significantly on the quality of any medication. Several organisations are developing guidelines for alternative forms of Ayurvedic therapy. To produce high-quality Ayurvedic medicine, it is necessary to

adhere to a standard protocol issued by the Central Council of Research in Ayurveda and Siddha. In the present work, organoleptic parameters like color, odor, and taste were observed. There was a slight change in color odor and taste of the KG with that of DG. It is due to the presence of Benincasa juice and licorice which imparted dark yellow color and sweetish odor and taste to the KG. Determination of physico-chemical parameters ensures the quality of raw *ghrita* and prepared *ghrita* and are within the stated limit. Glabridin is a pharmacologically active phytoconstituent of licorice and has anti-inflammatory, neuroprotective, anticholine esterase, and GABA-potentiating activity (6, 7). Hence keeping glabridin as a marker compound KG was analyzed using the RP-HPC method. Previously reported analytical methods for estimating glabridin suggest that methanol is the ideal solvent for the extraction of the same in crude drug and polyherbal formulations (8, 9). Given the miscibility of methanol with petroleum ether, a biphasic extraction method was employed in this study. The *ghrita* sample was initially dissolved in petroleum ether to facilitate phase separation. Glabridin, being more soluble in methanol, was selectively partitioned into 70% methanol through successive extractions. The collected methanolic fractions were then subjected to both UV spectroscopy and HPLC analysis. To ensure maximum extraction efficiency, the procedure was further refined by varying the amount of *ghrita* and adjusting the number of extraction cycles.

While glabridin serves as a potent pharmacological marker and its quantification establishes a valuable baseline for standardization, it represents only one of several bioactive constituents present in KG. The formulation includes *Benincasa hispida* (Kushmanda) and *Glycyrrhiza glabra* (Yashtimadhu), both of which contribute a diverse array of phytoconstituents. In Benincasa hispida, compounds such as triterpenes, steroidal glycosides, flavonoids, and cucurbitacins have been reported and are known for their antioxidant, neuroprotective, and adaptogenic effects (17, 18). *Glycyrrhiza glabra* is not only a source of glabridin but also contains liquiritin, glycyrrhizin, isoliquiritigenin, and flavones, which exhibit anti-inflammatory, hepatoprotective, and CNS-modulatory activities (6, 7, and 19).

To fully understand the therapeutic efficacy and quality of KG, future studies should include advanced phytochemical profiling techniques such as LC-MS/MS, GC-MS, and UPLC-QTOF-MS, which allow for simultaneous identification and quantification of multiple active constituents (20, 21). Such comprehensive analysis can help in identifying synergistic effects among compounds and contribute to the development of multi-marker quality assurance protocols, improving translational and regulatory value of the formulation.

Herbal compounds and plant-derived phytochemicals play a significant role in modern drug discovery; however, their therapeutic utility is often constrained by poor oral bioavailability due to limited solubility and absorption (22–24). Various strategies have been explored to overcome these limitations. Among them, lipid-based formulations have shown considerable promise in enhancing the bioavailability of lipophilic phytoconstituents by facilitating their transport across biological membranes. Within this category, lipid-based nanoparticles—including emulsions, self-emulsifying drug delivery systems, and lipid complexes—offer a versatile platform to improve solubility, stability, and absorption (25, 26). As referred to in Ayurveda ghee- or oil-based preparations are called as Sneha Kalpana, the process of preparation involves mixing the paste of botanicals (*kalka*) and *Swarasa* (juice) or kasayas (polyherbal decoction) with ghee and heating at a moderate temperature with continuous stirring for three consecutive days. During the process, the water is evaporated and the phytoconstituents are incorporated into the vesicular structure and dispersed in the oil phase. Ghee is known as *yogavahi* which acts as a carrier for various phytoconstituents (4). In modern science ghee is considered an excellent carrier due to its bio-compatability, purity, and immunotoxicity properties (26). Recent reports proved that the ghee-based formulations (*ghrita*) behave as Nano drug delivery system (12), furthermore the fatty acids extracted from ghee are more prominent agents for formulating Solid-lipid Nanoparticles (27). A study, using modern techniques such as photon correlation microscopy, optical microscopy, and Environmental scanning electron microscopy, it has proved that ghee-based Ayurvedic formulation *guggulutiktaka ghrita* is in the form of submicron globules and phytoconstituents are incorporated in to vesicular structures and dispersed in oil phase (12). In the present context, the particle size analysis revealed a polymodal distribution with three distinct peaks. This multimodal behavior indicates the presence of heterogeneous populations within the formulation, which is characteristic of traditional lipid-based systems where both micro- and nano-sized particles may coexist due to phase separation, emulsification, or aggregation dynamics. It is important to clarify that although portions of the particle size distribution fall within the nanometer scale (<1000 nm), the term “nano formulation” is used operationally in this study to denote the presence of submicron structures rather than implying strict compliance with regulatory definitions that limit “nanoparticles” to those under 100 nm. More ever according to the Guidelines For Evaluation of Nano pharmaceuticals In India (28), materials with particle sizes up to 1000 nm may also be considered nanomaterials, provided they exhibit size-dependent physical, chemical, or biological activity. In line with this broader and regulatory-backed perspective, the submicron particle range of KG, coupled with its lipid-based delivery potential and evidence of improved dispersion and possible bio enhancement, justifies its classification as a nano formulation under Indian guidelines. This aligns with both traditional Ayurvedic claims of *ghrita* as a "*yogavahi*" (carrier) and modern lipid-based nanotechnology principles.

Conclusion

Kushmanda ghrita was prepared according to classical text, and standardized conferring to Indian Standards for Ayurvedic formulations by which the quality of the prepared *ghrita* was ascertained. The prime active constituent glabridin was quantified using HPLC and may be helpful in further studies on the molecular mechanism, pharmacodynamics and pharmacokinetic and drug interaction studies of KG. The particle size analysis revealed that KG's particle/ globule size is in the Nano metric range, which may improve the bioavailability of phytocnstituents of KG. Additional method development is required to quantify other biologically important phytoconstituents of KG. Further in-depth particle size analysis is necessary to understand the mechanism of formation of nanoparticles/globules.

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Conflicts of Interest statement

Authors declare no conflicts of interest.

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