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## Physicochemical standardisation of the Homoeopathic drug, *Apis mellifica*: A preliminary attempt

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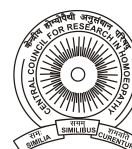
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### Abstract

**Background:** Unlike the mainstream biomedical system, honeybees' therapeutic use has long been well documented in the homoeopathic system. The medicine prepared from the whole honeybee, *Apis mellifica*, is on the Essential Drug List. **Objective:** An in-depth physicochemical standardisation of this homoeopathic drug is attempted. **Material and Methods:** In this study, first the raw drug was authenticated, and then its pharmacopoeial parameters were measured, e.g. moisture content, foreign matter, different ash values, alcohol and water-soluble extractive values. Further, the mother tincture of *Apis mellifica* was prepared from the authentic raw drug. The pharmacopoeial parameters of in-house mother tincture and a commercial mother tincture, e.g. organoleptic parameters, sediment detection, specific gravity measurement, pH measurement, total solids measurement, qualitative ultraviolet-visible spectra determination and high-performance thin layer chromatography were carried out for in-depth standardisation. **Results:** This study provides the standards for the aforementioned pharmacopoeial parameters of *Apis mellifica* using pharmacopoeial procedures. For the first time, the quantitative data obtained here quantifies those pharmacopoeial parameters. The comparative study of the parameters of the in-house mother tincture and commercial sample shows the significance of the reported work for maintaining the high quality of the drugs. **Conclusion:** The data presented here could be used for updating the pharmacopoeial standards and limits for the homoeopathic drug *Apis mellifica* in the future.

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# Physicochemical standardisation of the Homoeopathic drug, *Apis mellifica*: A preliminary attempt

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## Abstract

**Background:** Unlike the mainstream biomedical system, honeybees' therapeutic use has long been well documented in the homoeopathic system. The medicine prepared from the whole honeybee, *Apis mellifica*, is on the Essential Drug List. **Objective:** An in-depth physicochemical standardisation of this homoeopathic drug is attempted. **Material and Methods:** In this study, first the raw drug was authenticated, and then its pharmacopoeial parameters were measured, e.g. moisture content, foreign matter, different ash values, alcohol and water-soluble extractive values. Further, the mother tincture of *Apis mellifica* was prepared from the authentic raw drug. The pharmacopoeial parameters of in-house mother tincture and a commercial mother tincture, e.g. organoleptic parameters, sediment detection, specific gravity measurement, pH measurement, total solids measurement, qualitative ultraviolet-visible spectra determination and high-performance thin layer chromatography were carried out for in-depth standardisation. **Results:** This study provides the standards for the aforementioned pharmacopoeial parameters of *Apis mellifica* using pharmacopoeial procedures. For the first time, the quantitative data obtained here quantifies those pharmacopoeial parameters. The comparative study of the parameters of the in-house mother tincture and commercial sample shows the significance of the reported work for maintaining the high quality of the drugs. **Conclusion:** The data presented here could be used for updating the pharmacopoeial standards and limits for the homoeopathic drug *Apis mellifica* in the future.

**Keywords:** *Apis mellifica*, Homoeopathy, Pharmacopoeia, Physicochemical

## INTRODUCTION

Nature is a treasure trove for medicinally valuable products.<sup>[1,2]</sup> Animal-based natural products have recently drawn the biomedical community's attention.<sup>[3,4]</sup> However, this field of research is still in its early stages. The underdevelopment of animal-based drugs is due to the challenges of sourcing these substances. Unlike plants, animals can resist interactions, making it difficult to collect the desired materials. This is especially true for animals with life-threatening defences. However, modern biomedical research has recently found several medicinal potentials in honeybees.<sup>[5,6]</sup> Homoeopathic materia medica incorporates several animal-based drugs, including venoms as medicines, which have been in use for a long time, and the research findings support their application.<sup>[7]</sup> Among them, honeybee venom is important and unique. Unlike other venoms, honeybee's venom is rarely life-threatening for humans in small doses and, thus, has been well studied.<sup>[8]</sup> Honeybee venom has been widely mentioned in various homoeopathic literatures.

Rev. Brauns, a clergyman from Thuringia, first reported the medicinal properties of pure honeybee venom.<sup>[7-9]</sup> Persisting ailments in horses were successfully treated with this drug.<sup>[9]</sup> In 1850, E. E. Marcy reported using dried and powdered bees in *theory and practice*, which was strong evidence of the medicinal properties of the venoms.<sup>[9]</sup> The aforementioned clinical findings were collectively published as a monograph in the American drug provings.<sup>[9]</sup> This monograph provided comprehensive scientific findings and medicinal usage of the drug *Apis mellifica*.<sup>[7]</sup> Dr. Wolf and Dr. William Boericke reported the polychrest nature of *Apis mellifica*.<sup>[9,10]</sup> Dr. Boericke noted that *Apis mellifica*

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could be used for numerous ailments of the body and mind. Overall, the physiological effects of the venom are chiefly related to inflammation, both external and internal.<sup>[10]</sup> In the Homoeopathic Pharmacopoeia of India (HPI), the history and authority section for this drug is attributed to Allen's Encyclop. Mat. Med. Vol. I, 400.<sup>[11]</sup> The anti-nociceptive effects of honeybee venom have been demonstrated in different pain models, supporting the homoeopathic literature for its use as a broad-spectrum anti-inflammatory drug.<sup>[12]</sup> A pre-clinical study shows the effectiveness of bee venom in treating benign prostatic hyperplasia.<sup>[13]</sup> Similar clinical effects of *Apis mellifica* are reported in homoeopathic literature as well.<sup>[14]</sup> This indicates that even though whole honeybees are used for the preparation of the homoeopathic medicine *Apis mellifica*, the bee's venom is probably the key to its therapeutic effects.

*Apis mellifica* is included in the Ministry of AYUSH's Essential Drug List.<sup>[15]</sup> However, its physicochemical and pharmacopoeial parameters are not yet available in the HPI that are required for ensuring the manufactured drugs' quality. Therefore, we sought to undertake this physicochemical standardisation study. Our principal aim was to generate certain simple physicochemical parameters as per pharmacopoeial standards. We needed to fulfil three important criteria to achieve our goal: first, the use of taxonomically authenticated, genuine raw drug for mother tincture preparation; secondly, adherence to the HPI procedure for mother tincture preparation; and lastly, evaluation of existing pharmacopoeial physicochemical parameters for both the raw drug and mother tincture. With the fulfilment of these conditions, the present work would help to establish the drug's pharmacopoeial standards.

## MATERIALS AND METHODS

### Materials

The reagents, chemicals and solvents were of analytical grade (Analytical Reagent: AR) and purchased from Sigma-Aldrich. All the chemicals' material safety data sheet (MSDS) documents were minutely evaluated before their use.

The glassware was cleaned in a base bath, followed by a thorough cleaning with distilled water and drying at 150°C after an acetone wash for at least 6 h.

*Apis mellifera* is the zoological name of the bee species required to prepare the homoeopathic medicine *Apis mellifica*.<sup>[16]</sup> For preparing the mother tincture as per the HPI method, live *Apis mellifera* were collected from apiaries (M/s. Moulali Honey Bee Keeping, Araku Vally, AP, India, Latitude: 18° 19' 59.88" N, Longitude: 82° 52' 0.12" E, Altitude: 910 m). The collected honeybees were duly identified and authenticated as *Apis mellifera* by a scientist from the Zoological Survey of India, Kolkata. The whole body of the bee was treated as the raw drug for a physicochemical study. The handling of live bees was done by a zoology researcher.

A mother tincture sample from a Good Manufacturing Practices-certified commercial manufacturer was used for the comparison with the mother tincture prepared under study.

### Methods

The bees were separated and procured directly from the beehives. The raw drug sample was checked for visible signs of mold growth, sliminess, insect contamination, animal excreta or other noxious foreign matter.

### Foreign matter

Around 10g of the whole bee sample was examined thoroughly under sunlight with a 10x magnifying glass for removal of the foreign matter. Sieving for dust removal could not be done as the bees were taken as a whole. This method followed the procedure described in the Ayurvedic Pharmacopoeia of India.<sup>[17]</sup>

The foreign matter was determined as follows:

$$\text{Weight of the foreign matter, } W_{FM} = (W_{\text{empty}} + W_{\text{uncleaned bee}}) - (W_{\text{empty}} + W_{\text{cleaned bees}})$$

$$\% \text{ of Foreign Matter} = (W_{FM} / W_{\text{uncleaned bee}}) * 100 = \% \text{ w/w}$$

where,  $W_{\text{empty}}$  was the weight of the empty evaporating dish,  $W_{\text{uncleaned bee}}$  was the weight of the uncleaned bees and  $W_{\text{cleaned bees}}$  was the weight of the cleaned bees.

### Loss on drying (LOD)

Since there was no specific method available for calculating the LOD of animal products, the standard method for vegetable products described in HPI was adopted since both are majorly organic substances.<sup>[18]</sup> About 2 g of the raw drug was kept on a clean and accurately weighed evaporating dish in a well-ventilated hot air oven for 3 h at 105°C. The raw drug fragments were arranged in a monolayer. The raw drug was removed from the oven and allowed to cool to room temperature by keeping it in a desiccator for 20 min before weighing. The heating and subsequent weighing processes were repeated until the difference between two consecutive weightings was no more than 0.25%. The hot air oven (Model: BPI-9, Manufacturer: Ambassador) and weighing balance (Model: BSA224S,-CW Manufacturer: Sartorius) were used for this experiment. The experiment was done twice, and the average value was reported.

The LOD (*moisture content*) in % w/w was determined in the following manner:

$$\text{Weight of Empty dish} = W_{\text{empty}}$$

$$\begin{aligned} & - \text{Weight of the dish with air-dried raw drug} = W_{\text{+wet}} \\ & - \text{Weight of the air-dried raw drug} = W_{\text{+wet}} - W_{\text{empty}} = W_{\text{wet}} \end{aligned}$$

$$\text{Weight of the oven-dried (105°C) raw drug with dish} = W_{\text{+dry}}$$

$$\text{Weight of the oven-dried (105°C) raw drug} = W_{\text{+dry}} - W_{\text{empty}} = W_{\text{dry}}$$

$$\text{LOD \% w/w} = \left[ \frac{(W_{\text{wet}} - W_{\text{dry}})}{W_{\text{wet}}} \right] * 100$$

### Preparation of mother tincture

The mother tincture was prepared as per the procedure described in HPI.<sup>[11]</sup> The required live bees were placed in a

clean, wide-mouthed, closed glass container. Then, they were irritated by shaking while keeping the lid closed. Then, based on the dry drug weight, the appropriate volume of the required solvent system (i.e. menstrum) was poured in, and the whole was allowed to macerate for 10 days. For each 100 g of the dry weight of the raw drug, 225 mL of glycerin, 425 mL of strong alcohol and 375 mL of water (including the water originating from the moisture present in the whole bees) were required. The entire mixture was shaken twice a day. The resulting tincture was poured off and filtered. The bees were not pressed.

## Extractive values

### Alcohol soluble extractive

As per the HPI method,<sup>[19]</sup> approximately 2.5 g of precisely measured live bees were used (considering moisture content) and agitated. To this, 50 mL of alcohol was added and allowed to sit for 24h at ambient temperature in a sealed environment in an amber-colored bottle. The container containing this mixture was shaken frequently during the initial 6 h period and allowed to stand for 18 h more (total 24 h). After filtering it with quantitative filter paper, 10 mL was taken in a flat-bottomed evaporating dish and evaporated in a water bath to remove most of the alcohol. Then, the evaporating dish containing the residual substance was heated to 105°C in an adequately ventilated oven. This procedure was repeated until a constant weight was reached through the evaporation of the residual solvent. The described experiment was conducted on two separate occasions. Each time, the alcohol-soluble extractive was calculated. The average of these two experiments was calculated and reported. The alcohol extract value was calculated in the following manner:

Drug weight =  $W_{dry}$  (moisture content was subtracted to calculate the drug weight)

Empty Beaker weight =  $w_{empty}$

Beaker + dried Extract weight =  $w_{+drug}$

$$\text{EtOH extract} = \frac{(W_{+drug} - W_{empty})}{W_{drug} / 5} * 100 \quad \%w/w$$

**Water-Soluble Extractive:** In closely associated experiments, water-soluble extract was evaluated using 50 mL of chloroform-water instead of ethanol as per the HPI method.<sup>[20]</sup> For the alcohol and water-soluble extractive values, a hot air oven, a water bath (Model: BPI-22, Manufacturer: Ambassador), and a weighing balance were used.

## Ash values

### Determination of total ash value

Approximately 2.0 g of whole honeybees were placed (considering moisture content) in a thermally resistant, previously weighed silica crucible. The crucible and its contents were heated at 600°C for 30 min in the presence of air. Subsequently, the crucible was allowed to cool in a desiccator for 30 min to reach the ambient temperature before weighing. This heating cycle was repeated until a consistent

weight was achieved. This was as per the method delineated in the Ayurvedic Pharmacopoeia of India.<sup>[21]</sup>

The calculation of the total ash in % w/w was determined in the following manner:

Empty Crucible Weight =  $W_{empty}$

Drug weight =  $W_{drug}$  (moisture content was subtracted to calculate the drug weight)

Crucible +ash weight =  $W_{+ash}$

Total ash =  $W_{+ash} - W_{empty} = W_{ash}$

$$\% \text{ Total ash} = \frac{W_{ash}}{W_{drug}} * 100 \quad \%w/w$$

The above experiment was performed twice, and the average value was reported.

### Determination of acid-insoluble ash value

The residual ash resulting from the procedure for LOD calculation was treated with 25 ml of dilute hydrochloric acid. The insoluble residue was quantitatively collected using a quantitative ash-less filter paper. The ash collected over the filter paper was rinsed with hot water until the runoff water became neutral. Subsequently, the acid-insoluble ash with ash-less filter paper was ignited at 450°C for 15 min under aerial conditions. The crucible was then allowed to cool in a desiccator for 30 min to reach the ambient temperature before weighing. This heating and subsequent weighing were repeated until a constant weight was achieved. This experiment was conducted twice; the resulting average value has been reported here. This method adheres to the method delineated in the Ayurvedic Pharmacopoeia of India.<sup>[22]</sup> The calculation of the acid-insoluble ash in % w/w was done as follows:

Empty Crucible weight =  $W_{empty}$

Drug weight =  $E_{drug}$  (moisture content was subtracted to calculate the drug weight)

Crucible +acid insoluble ash weight =  $W_{+acid(i) ash}$

Acid insoluble ash =  $W_{+acid(i) ash} - W_{empty}$

$$\% \text{ acid insoluble ash} = \left[ \frac{\{W_{+acid(i) ash} - W_{empty}\}}{W_{drug}} \right] * 100 \quad \%w/w$$

### Determination of water-soluble ash value

The remaining ash from the preceding experiment was boiled with 25 ml of water for 5 min. The resultant insoluble residue was collected onto an ash-less filter paper and thoroughly washed using 100 mL of boiling water. The ash-less filter paper containing the residue was ignited at 450°C for 15 min under atmospheric conditions. Afterwards, the crucible was cooled in a desiccator for 30 min to reach the ambient temperature before weighing. This heating and subsequent weighing were repeated until a constant weight was achieved. The weight of the insoluble residue was subtracted from the total ash weight. The resulting difference in weight corresponded to the water-soluble ash. This entire experiment was conducted



twice; the resulting average value has been reported here. This method adheres to the method delineated in the Ayurvedic Pharmacopoeia of India.<sup>[23]</sup> The calculation of the water-soluble ash in % w/w was calculated in the following manner:

$$\text{Empty Crucible weight} = W_{\text{empty}}$$

Drug weight =  $W_{\text{drug}}$  (moisture content was subtracted to calculate the drug weight)

$$\text{Crucible +water insoluble ash weight} = W_{\text{+water(i) ash}}$$

$$\text{Water insoluble ash} = W_{\text{+water(i) ash}} - W_{\text{empty}}$$

$$\text{Total ash} = W_{\text{ash}}$$

$$\text{Water-soluble ash} = \text{Total ash} - \text{Water-insoluble ash} = W_{\text{ash}} -$$

$$W_{\text{+water(i) ash}} = W_{\text{water (s)}} \quad \% \text{ Water soluble ash} =$$

$$\left[ \frac{W_{\text{water(s)}}}{W_{\text{drug}}} \right] * 100 = \% \text{w/w}$$

Overall, for ash value determination, the muffle furnace (Model: BPI-18, Manufacturer: Ambassador) and weighing balance were used for these experiments.

### Determination of Total solids

A precisely measured 10 mL of mother tincture was taken in a flat-bottomed evaporating flask and heated in a water bath to remove the alcohol. Subsequently, the water content was expelled by heating it inside a well-ventilated oven at 105°C. The sample was cooled in a desiccator for 15 min to reach the ambient temperature before weighing. The heating and subsequent weighing were continued until a constant weight was reached. This method followed the procedure described in HPI.<sup>[24]</sup> The hot air oven, water bath and weighing balance were used for this experiment. The above experiment was performed twice, and the average value was reported.

$$\text{Empty Beaker weight} = W_{\text{empty}}$$

$$\text{Beaker +dried Extract weight} = W_{\text{+dried drug}}$$

$$\text{Total solids} = W_{\text{+dried drug}} - W_{\text{empty}}$$

$$\% \text{ Total solids} = \left[ \frac{W_{\text{+dried drug}} - W_{\text{empty}}}{\text{drug volume}} \right] * 100 = \% \text{w/w}$$

Specifically, in this case:

$$\% \text{Total solids} = \left[ \frac{(W_{\text{+dried drug}} - W_{\text{empty}})}{10} \right] * 100 = \% \text{ w/w}$$

### Weight per mL

10 mL of the mother tincture was accurately measured using a burette. Then, the mother tincture was weighed. The weight was divided by 10 to calculate the weight per mL. A weighing balance was used for this experiment.

### pH

The pH was determined by a digital pH meter at 25°C (Model: 707 pH Meter, Manufacturer: Design Electronics Services).

The pH meter was calibrated each time with buffer solutions with pH 4.0 and 9.2. The pH of each mother tincture sample was recorded thrice, and an average was reported.

### $\lambda_{\text{max}}$

The solvent system that was used to make the mother tincture was used as the blank. This blank was used for the baseline. The  $\lambda_{\text{max}}$  values of the mother tinctures were measured by diluting them 100 times. The diluting solvent was the same as the mother tincture's solvent system. An ultraviolet-visible (UV-Vis) spectrophotometer (Model: Lambda 25, Manufacturer: Perkin Elmer) was used for this experiment.

### Thin layer chromatography

20 mL of the mother tincture was taken in a 50 mL beaker and heated in a water bath to remove the alcohol from the mother tincture. The resultant residue was then extracted using three separate portions of 20 mL of chloroform. These fractions of the chloroform extract were combined and subsequently condensed to approximately 2 mL through heating in a water bath. This concentrated chloroform extract was subjected to high-performance thin layer chromatography (HPTLC) on a Merck-manufactured pre-coated silica gel aluminium plate: (60F-<sub>254</sub> variant) with a thickness of 0.25 mm. The employed solvent system consisted of a 90:10 (v/v) ratio of chloroform to methanol.

### Application and development

On a TLC plate, 10  $\mu\text{L}$  of this concentrated chloroform extract was applied as a 10 mm band. The TLC spots were developed at 8 cm. The TLC plates were air-dried. The spots were recorded under 254 nm, 365 nm light and with an anisaldehyde-sulphuric acid stain (followed by heating) under visible light. (This is the presently approved pharmacopoeial procedure for TLC/HPTLC analysis of the mother tinctures).

## RESULTS

### Biological description

The honey bee's body was 15–20 mm long, red or brown with black bands and orange-yellow rings on the abdomen. The taxonomical hierarchy of *Apis mellifera* is Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Order: Hymenoptera, Family: Apidae, Genus: *Apis*, Species: *Apis mellifera*. The queen bee has an elongated abdomen with a triangular tip, armed with a sting, wings that do not cover the abdomen entirely, and small eyes. The drones are males, very stout in appearance, and their wings cover the entire abdomen. The tip of the abdomen is almost rectangular. There is no sting, the eyes are large and meet in the centre of the head and the mouth parts are nearly aborted. The workers are sterile females. They are the smallest and most numerous members of the colony. The abdomen is short, covered by wings, pointed at the tip and armed with a sting. Abdominal segments 4-8 are provided with a pair of wax glands. Metathoracic legs bear corbicula (pollen baskets) and mouth parts are modified for chewing and lapping. The antennae are typically geniculate

(elbow-shaped). A photo of the dried specimen collected is given in Figure 1.

### Physicochemical study

The experimentally obtained pharmacopeial and physicochemical parameters of the raw drug are tabulated in Table 1. The moisture content (LOD) was quite high, at 78.63%. The ash values here are very low [Table 1], indicating low metal contents in the bee. Furthermore, it is to be noted that total ash and water-soluble ash are similar, and these values are considerably higher than acid-insoluble ash values. As the alcohol and water-soluble extractive values were determined without pressing the bees, those values were quite low, ranging between 1% and 3% [Table 1].

The physicochemical parameters of the in-house and commercial mother tinctures were compared [Table 2].

The organoleptic parameters, appearance, colour and odour of the in-house and commercial mother tinctures differed considerably [Table 2, organoleptic parameters and Figure 2]. The density (weight/mL) of the in-house mother tincture was >1.03 g/mL, while the commercial one had only 0.81 g/mL [Table 2].

Most importantly, the total solids of the in-house mother tincture (1.03 % w/v) were much higher than the total solids

of the commercial mother tincture (0.76 % w/v), indicating greater extraction in the in-house mother tincture [Table 2]. Both the mother tincture samples had a pH < 7. The UV-Vis absorption spectroscopy showed that  $\lambda_{\max}$  values of the in-house mother tincture (205 nm, 249 nm and 600 nm) and the commercial mother tincture (205 nm, 259 nm and 600 nm) were similar yet not the same. Furthermore, the overall absorption spectral patterns were similar [ $\lambda_{\max}$  values of Table 2 and Figure 3]. The  $R_f$  values of the simultaneous high-performance thin layer chromatography of the chloroform extracts of the in-house mother tincture (AA) and commercial mother tincture (AC) are given in Table 3. The chromatograms of AA and AC under 254 nm, 365 nm and with anisaldehyde-sulphuric acid stain (under white light) are shown in Figure 4. The densitograms of AA and AC under 365 nm are given in Figure 5.

## DISCUSSION

### Physicochemical study on the raw drug

The moisture content of the raw drug, i.e. whole honeybees, was around 75%. The extremely low ash values indicate very low metal content in the honeybees. Furthermore, the water-soluble ash value is considerably higher than the acid-insoluble ash. This shows that the alkali and light metals contributed majorly to the total metal contents of the honeybees.



**Figure 1:** (a) Dried collected *Apis Mellifera*. (b) Collected *Apis Mellifera* In 70% Alcohol



**Figure 2:** Colour of the in-house (a) and commercial (c) samples

**Table 1: Physicochemical parameters of the raw drug (i.e. Whole Honeybees)**

| Parameters                      | Value      |
|---------------------------------|------------|
| Foreign matter                  | 0.1% w/w   |
| Moisture content (LOD at 105°C) | 78.63% w/w |
| Total ash                       | 1.45% w/w  |
| Acid insoluble ash              | 0.38% w/w  |
| Water soluble ash               | 0.84% w/w  |
| <i>Extractive values</i>        |            |
| Alcohol                         | 1.14% w/w  |
| Water                           | 2.79% w/w  |

LOD: Loss on drying

**Table 2. Physicochemical Parameters of Mother Tincture**

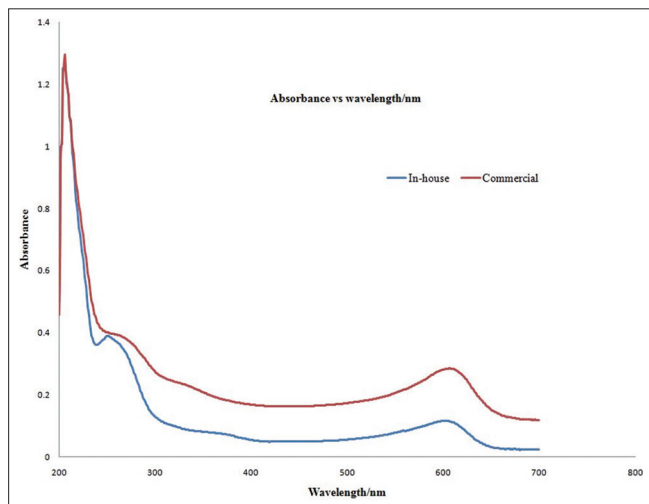
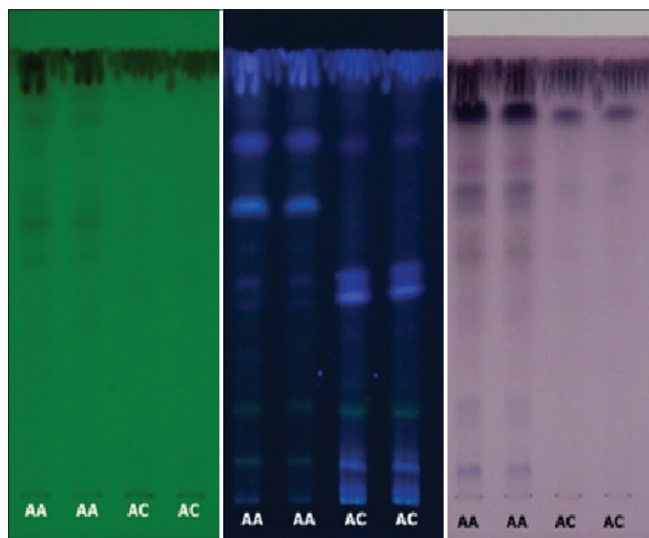
| Parameter                      | In-house                  | Commercial                |
|--------------------------------|---------------------------|---------------------------|
| <i>Organoleptic parameters</i> |                           |                           |
| Appearance                     | Clear, non-viscous        | Clear, non-viscous        |
| Colour                         | Colourless                | Sunset Yellow             |
| Odour                          | Sweet pleasant            | Rancid Sweet              |
| Sediments                      | Absent                    | Absent                    |
| Weight per mL                  | 1.03 g                    | 0.81 g                    |
| Total solids                   | 1.03% w/v                 | 0.76% w/v                 |
| Alcohol (Ethanol) content      | 41% v/v                   | 42% v/v*                  |
| pH                             | 5.46                      | 5.11                      |
| $\lambda_{\max}$               | 205 nm, 249 nm and 600 nm | 205 nm, 259 nm and 600 nm |

\*Reported in the label

**Table 3: R<sub>f</sub> values of the in-house and commercial mother tinctures in HPTLC analysis**

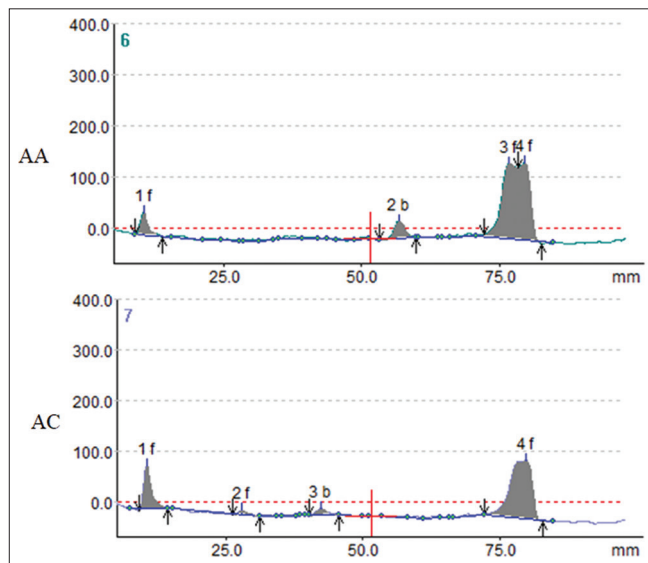
| Light/Stain Used                    | In-House   | Commercial   |
|-------------------------------------|--|--|
| 254 UV                              | 0.54, 0.63, 0.87 (all black)   |  |
| 365 UV                              | 0.07 (pale yellow), 0.20 (pale yellow), 0.46 (blue), 0.50 (blue), 0.66 (blue), 0.83 (blue)     | 0.09 (blue), 0.20 (pale yellow), 0.49 (dark blue), 0.51 (dark blue), 0.83 (blue) |
| Anisaldehyde – Sulphuric acid stain | 0.06 (purple), 0.21 (purple), 0.57 (purple), 0.71 (purple), 0.79 (Reddish pink), 0.91 (purple) | 0.71 (purple), 0.91 (purple)   |

HPTLC: High-performance thin-layer chromatography

**Figure 3:** Ultraviolet-visible spectra of in-house and commercial mother tinctures of *Apis mellifica***Figure 4:** High-performance thin layer chromatography of in-house (AA) and commercial (AC) sample. Left to right: 254 nm, 365 nm and anisaldehyde-sulphuric acid stain.

### Physicochemical study on the in-house and commercial mother tinctures

The organoleptic profile of the mother tinctures indicates that the commercial sample differed considerably from the authentic in-house sample [Table 2 and Figure 3]. The in-house mother tincture was colourless. This was expected as the honeybees

**Figure 5:** Overlay densitogram of AA and AC 10% methanol/chloroform at UV 365 NM

were not pressed during mother tincture preparation. However, the commercial mother tincture was unexpectedly yellow. Interestingly, the UV-Vis absorption spectra of the samples were quite similar [Figure 2 and  $\lambda_{\max}$  in Table 2]. This indicates that organoleptic parameters may not be very conclusive. The explanation of such colour needs further in-depth study in the future. The density of the commercial mother tincture was merely 0.81 g/mL (<1 g/mL). This observation was unexpected as the sum of the glycerine (density: 1.26 g/mL) and water (density: 1.00 g/mL) content is ~60% in the mother tincture as per HPI. Furthermore, the in-house mother tincture's total solid value was much higher than the commercial mother tincture, indicating higher extraction in the in-house mother tincture [Table 2, total solids]. The pH of the mother tincture samples indicates that the mother tinctures under study were only slightly acidic [Table 2, pH]. The qualitative HPTLC fingerprinting indicated that although the patterns were somewhat similar, the relative amounts of the chemicals in those samples differed considerably [Figures 4, 5 and Table 3]. The comparative qualitative HPTLC study indicates that the HPTLC fingerprinting of the commercial sample only partially matches the authentic in-house sample. Even with this qualitative study, it was possible to locate the chemical differences between these two samples. The exploration of the origin of such differences and their consequences requires further in-depth analysis employing



modern analytical techniques. We generally have employed certain simple physicochemical pharmacopoeial standards for physicochemical standardisation. However, this work is by no means complete but still has relevance, considering the dearth of physicochemical data present in the existing HPI monograph of the drug under study. We hope further employment of modern analytical techniques for standardisation may be integrated into internationally harmonised pharmacopoeial documents as per the direction of the regulatory bodies. Besides, it is suggested that while incorporating the new methods, multiple raw drug samples may be used to generate robust data.

Furthermore, our comparative study on the taxonomically authentic in-house and commercial mother tinctures shows this study's importance. This study can further be improved by employing cutting-edge analytical techniques, high-performance liquid chromatography coupled with mass spectrometry, nuclear magnetic resonance, Fourier-transform infrared spectroscopy, etc. in the future. Although these methods are presently beyond the scope of the pharmacopoeial standards, there is a scope for exploring these in the future. Such analyses will improve the quality control and quality assurance of the homeopathic drug under study. However, incorporating such modern analytical techniques into regulation may also be judged through pragmatism. Finally, we believe our work would eventually lay down the pharmacopoeial standards, and this work may be regarded as the first stepping stone towards preparing the high-quality homeopathic medicine *Apis mellifica*.

In the present study, live bees were used for the experiments, however, since animal ethics committee approval is not required for invertebrates as per CPCSEA guidelines 2018, the same was not sought.<sup>[25]</sup>

## CONCLUSION

The present study has generated and reported the pharmacopoeial physicochemical parameters of the homeopathic drug *Apis mellifica* for the first time. We believe these physicochemical data would benefit the manufacturers in ensuring quality control and quality assurance.

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## Authors' Contributions

**BB:** Concepts, Design, Definition of intellectual content, Literature search, Experimental studies (physicochemical work, Chemistry related Data acquisition), Data analysis, Manuscript preparation, editing, and revision. **SM:** Literature search, Chemistry Data analysis, and Manuscript editing, review, and revision. **ENS:** Experimental studies (Live honeybee collection,

spot taxonomic identification, and live honeybee handling), Morphological study of the honeybees. **GVNK:** Manuscript review, editing, and revision. **SP:** Manuscript review.

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## Conflicts of interest

None declared.

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## Normalisation physico-chimique du médicament homéopathique, *Apis mellifica* : une tentative préliminaire

**Contexte:** Contrairement au système biomédical traditionnel, l'utilisation thérapeutique des abeilles domestiques a longtemps été bien documentée dans le système homéopathique. Le médicament préparé à partir de l'abeille entière, *Apis mellifica*, est sur la liste des médicaments essentiels

**Objectif:** Une standardisation physico-chimique approfondie de ce médicament homéopathique est tentée.

**Matériels et méthodes:** Dans cette étude, le médicament brut a d'abord été authentifié, puis ses paramètres pharmacopées ont été mesurés, par exemple la teneur en humidité, les matières étrangères, les différentes valeurs de cendres, l'alcool et les valeurs d'extraction solubles dans l'eau. De plus, la teinture mère d'*Apis mellifica* a été préparée à partir de la drogue brute authentique. Les paramètres pharmacopées d'une teinture mère maison et d'une teinture mère commerciale, par exemple, paramètres organoleptiques, détection des sédiments, mesure de la densité spécifique ; La mesure du pH, la mesure des solides totaux, la détermination qualitative des spectres UV-Vis et la chromatographie sur couche mince haute performance ont été réalisées pour une standardisation approfondie.

**Résultats:** Cette étude fournit les normes pour les paramètres pharmacopées susmentionnés d'*Apis mellifica* en utilisant des procédures pharmacopées. Pour la première fois, les données quantitatives obtenues ici quantifient ces paramètres pharmacopées. L'étude comparative des paramètres de la teinture mère interne et de l'échantillon commercial montre l'importance du travail rapporté pour maintenir la haute qualité des médicaments.

**Conclusion:** Les données présentées ici pourraient être utilisées à l'avenir pour mettre à jour les normes et limites de la pharmacopée du médicament homéopathique *Apis mellifica*

## Physikalisch-chemische Standardisierung des homöopathischen Arzneimittels *Apis mellifica*: Ein vorläufiger Versuch

**Hintergrund:** Im Gegensatz zum biomedizinischen System ist die therapeutische Verwendung von Honigbienen im homöopathischen System seit langem gut dokumentiert. Das aus der ganzen Honigbiene, *Apis mellifica*, hergestellte Arzneimittel steht auf der Liste der unentbehrlichen Arzneimittel. **Zielsetzung:** Es wird eine eingehende physikochemische Standardisierung dieses homöopathischen Arzneimittels angestrebt. **Materialien und Methoden:** In dieser Studie wurde zunächst die Rohdroge authentifiziert, und dann wurden ihre pharmakopöischen Parameter gemessen, z. B. Feuchtigkeitsgehalt, Fremdstoffe, verschiedene Aschewerte, Alkohol und wasserlösliche Extraktivwerte. Aus der authentischen Rohdroge wurde dann die Urtinktur von *Apis mellifica* hergestellt. Die pharmakopöischen Parameter der hauseigenen Urtinktur und einer kommerziellen Urtinktur, z. B. organoleptische Parameter, Sedimentnachweis, Messung des spezifischen Gewichts, pH-Messung, Messung des Gesamtfeststoffgehalts, qualitative UV-Vis-Spektrenbestimmung und Hochleistungs-Dünnschichtchromatographie wurden für eine eingehende Standardisierung durchgeführt. **Ergebnisse:** Diese Studie liefert die Standards für die oben genannten pharmakopöischen Parameter von *Apis mellifica* unter Verwendung pharmakopöischer Verfahren. Die hier gewonnenen quantitativen Daten quantifizieren erstmals diese pharmakopöischen Parameter. Die vergleichende Studie der Parameter der hauseigenen Urtinktur und der kommerziellen Probe zeigt die Bedeutung der hier vorgestellten Arbeit für die Aufrechterhaltung der hohen Qualität der Drogen. **Schlussfolgerung:** Die hier vorgestellten Daten könnten in Zukunft für die Aktualisierung der pharmakopöischen Standards und Grenzwerte für das homöopathische Arzneimittel *Apis mellifica* verwendet werden.

## होम्योपैथी दवा, एपिस मेलिफिका का भौतिक रासायनिक मानकीकरण: एक प्रारंभिक प्रयास

**पृष्ठभूमि:** जैव चिकित्सा प्रणाली की मुख्यधारा के विपरीत, मधुमक्खियों का चिकित्सीय उपयोग होम्योपैथी प्रणाली में लंबे समय से अच्छी तरह से प्रलेखित है। संपूर्ण मधुमक्खी से तैयार दवा, एपिस मेलिफिका, आवश्यक दवा सूची में है।

**उद्देश्य:** इस होम्योपैथी दवा के गहन भौतिक-रासायनिक मानकीकरण का प्रयास किया गया है।

**सामग्री और तरीके:** इस अध्ययन में, पहले प्राकृतिक दवा पदार्थ को प्रमाणित किया गया, और फिर इसके फार्माकोपियल मापदंडों को मापा गया, जैसे नमी की मात्रा, भिन्न पदार्थ, विभिन्न राख मूल्य, अल्कोहल और पानी में घुलनशील अर्क मूल्य। इसके बाद, एपिस मेलिफिका का मदर टिचर प्रामाणिक कच्ची दवा से तैयार किया गया था। इन-हाउस मदर टिचर और एक कॉमर्शियल मदर टिचर के फार्माकोपियल पैरामीटर, जैसे, ऑर्गेनोलेप्टिक पैरामीटर, सेडीमेंट का पता लगाना, विशिष्ट गुरुत्वाकर्षण माप; पीएच माप, कुल ठोस माप, गुणात्मक यूवी-विस स्पेक्ट्रा निर्धारण और उच्च प्रदर्शन थिन लेयर क्रोमैटोग्राफी के लिए गहन मानकीकरण किए गए।

**परिणाम:** यह अध्ययन फार्माकोपियल प्रक्रियाओं द्वारा एपिस मेलिफिका के उपरोक्त फार्माकोपियल मापदंडों के लिए मानक प्रदान करता है। पहली बार, यहां प्राप्त मात्रात्मक डेटा द्वारा उन फार्माकोपियल मापदंडों को मापा गया है। इन-हाउस मदर टिचर और कॉमर्शियल नमूने के मापदंडों का तुलनात्मक अध्ययन रिपोर्ट किए गए कार्य के महत्व को दर्शाता है। जिससे दवाओं की उच्च गुणवत्ता बनी रह सकती है।

**निष्कर्ष:** यहां प्रस्तुत डेटा का उपयोग भविष्य में होम्योपैथिक दवा एपिस मेलिफिका के फार्माकोपियल मानकों और सीमाओं को अद्यतन करने के लिए किया जा सकता है।

## Normalización fisicoquímica del medicamento homeopático Apis mellifica: Un intento preliminar

**Antecedentes:** A diferencia del sistema biomédico dominante, el uso terapéutico de las abejas melíferas está bien documentado desde hace mucho tiempo en el sistema homeopático. El medicamento preparado a partir de la abeja melífera entera, Apis mellifica, figura en la Lista de Medicamentos Esenciales. **Objetivo:** Se intenta una estandarización fisicoquímica en profundidad de este medicamento homeopático. **Materiales y métodos:** En este estudio, primero se autenticó la droga cruda y luego se midieron sus parámetros farmacopeicos, por ejemplo, contenido de humedad, materia extraña, diferentes valores de cenizas, alcohol y valores extractivos hidrosolubles. Además, se preparó la tintura madre de Apis mellifica a partir de la auténtica droga cruda. Se llevaron a cabo los parámetros farmacopeicos de la tintura madre interna y de una tintura madre comercial, por ejemplo, parámetros organolépticos, detección de sedimentos, medición de la gravedad específica; medición del pH, medición de sólidos totales, determinación cualitativa del espectro UV-Vis y cromatografía de capa fina de alto rendimiento para una estandarización en profundidad. **Resultados:** Este estudio proporciona los estándares para los parámetros farmacopeicos mencionados de Apis mellifica utilizando procedimientos farmacopeicos. Por primera vez, los datos cuantitativos aquí obtenidos cuantifican dichos parámetros farmacopeicos. El estudio comparativo de los parámetros de la tintura madre propia y de la muestra comercial muestra la importancia del trabajo descrito para mantener la alta calidad de los medicamentos. **Conclusiones:** Los datos aquí presentados podrían servir para actualizar en el futuro las normas y límites farmacopeicos del medicamento homeopático Apis mellifica.

### 势法物Apis mellicica的理化 准化：初步

**背景:** 与主流生物医学系不同，蜜蜂的治用途 期以来在 势法系 中得到了充分的 明。由整只蜜蜂Apis mellicica制成的 物被列入基本 物清单。

**目的:** 对 势法物 行深入的理化 准化研究。**材料和方法:** 本研究首先对原料 行 定，然后 定其 典参数，如水分含量、异物、不同灰分值、酒精和水溶性提取物值。此外，Apis mellicica的母酊是由正宗的原料 制的。内部母酊和商 母酊的 典参数，例如感官参数、沉淀物 、比重 量； 行了pH 量、 固体 量、定性紫外-可光 定和高效薄 色法，以 行深入的 准化。**果:** 本研究采用 典程序 上述香蜂属 典参数提供了 准。这里获得的定量数据首次量化了这些 典参数。对内部母体酊 和商 样品的参数 行的比 研究表明，所报告的工作对保持 物的高 量具有重要意义。 : 本文提供的数据可用于更新 势法物Apis mellicica的 典 准和限量