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# Self-assembled Patterns Formed in Evaporating Droplets to Analyze Bi-component Homeopathic Preparations in the Low Dilution Range

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## Abstract

**Background** Homeopathic complex remedies, composed of several homeopathic medicines in the low potency range, are frequently used in the treatment of a number of common disorders. At the same time, they represent an almost unexplored area of research. Are complex remedies just additive mixtures of the components, or are there interactions between the latter leading to new properties of the complex?

**Methods** In the present study, we analyzed as an example the simple bi-component complex, *Luffa* 4x – *Mercurius bijodatus* 9x, by means of patterns from evaporated droplets and tested what influences the complex's single compounds have upon the patterns and if there are any interactions. For this purpose, we compared in a series of five experiments patterns from evaporated droplets of the complex, *Luffa* 4x – *Mercurius bijodatus* 9x, and three comparison samples in which one or both of the complex's compounds were replaced by potentized solute. The patterns were photographed and evaluated for their gray-level distribution and texture using the software *ImageJ*. The experimental set-up's stability was tested by means of systematic control experiments.

**Results** We found that *Mercurius bijodatus* 9x significantly influenced the patterns of *Luffa* 4x, increasing their homogeneity; at the same time, the patterns of *Mercurius bijodatus* 9x combined with solvent were more heterogeneous than those obtained from a control consisting of two pure solvents.

**Conclusion** In this phenomenological assay, the complex *Luffa* 4x – *Mercurius bijodatus* 9x does not correspond to a simple addition of the components. The exact nature of the underlying interaction needs to be elucidated in further investigations.

**Keywords**
- homeopathic complex remedies
- self-assembled patterns
- droplet evaporation
- texture analysis

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## Introduction

### Background

Due to its multiple application possibilities, the evaporation of droplets constitutes a widely studied field of science. The main characteristic of this method is the formation of self-assembled structures in course of the phase transition process.[1] The formation of these structures has been shown to be useful for many technological developments such as smart surfaces, novel materials, coatings, inkjet printing and microelectronics.[1–3] Another area of application is to characterize phenomenologically a given sample based on the patterns that develop in evaporating droplets. These patterns constitute self-assembled structures, and it was hypothesized that "holistic" properties of a given sample can be captured by this method, referred to as the Droplet Evaporation Method (DEM).[4–6] Thus, this approach stands quite distinct from common chemical analysis methods that normally aim at breaking down the sample into its components, and additionally try to identify and quantify the single compounds. In contrast to this classical analytical approach, DEM aims at the evaluation of the sample's ability to self-assemble into ordered structures; besides composition, the method may therefore also capture a sample's other *dimensions*, which can be hypothesized to be related to vitality and/or health. In fact, as a scientific tool, DEM is applied mainly in medicine as a diagnostic test[7] and in homeopathy for basic research,[8,9] since both areas require the characterization of complex samples.

The present study continues the research conducted by our team in the area of homeopathic preparations in the low potency range. In previous studies, we performed the following: (1) screened homeopathic low potencies of substances of mineral, plant and animal origin; (2) tested the potential of DEM to differentiate between low potencies of different origin[10]; and (3) analyzed potencies produced by the application of different numbers of succussion strokes.[9] In the present study, for the first time, we apply DEM to analyze multi-component homeopathic preparations composed of different potencies in the low dilution range.

One of the reasons for prescribing homeopathic complex remedies is to shorten the search for the right remedy, following the Similia principle. Attempts to do so were first performed approximately 20 years after applications of the Similia principle in regard to single-component remedies.[11] A paragraph regarding the use of combinations of two remedies was added to the *Organon of Medicine* sixth edition in 1865.[12] Nowadays, though less frequently prescribed compared with single remedies (in Germany approximately 34–40% of homeopaths profess to prescribing complex remedies[13]), homeopathic complex remedies constitute a well-established branch of homeopathy. Complex remedies mainly serve in the treatment of common disorders such as respiratory tract infections.[14,15] Many of these products are not prescribed by doctors or therapists but sold as over-the-counter products. Most of the compositions available are based on concepts and empirical evidence gathered decades ago, and some even go back to the second half of the 19$^{th}$ century. Most complex remedies were first introduced by therapists, but afterward traditions and knowledge were passed on to manufacturers. This is the reason that nowadays each manufacturer has its own therapy system and related knowledge. There is little basic research regarding homeopathic complex remedies, their mode of action and/or the interactions between their single components.[8,10]

The aim of the present study was to analyze the influence of both single compounds of the remedy complex, *Luffa* 4x – *Mercurius bijodatus* 9x, as well as their combination, upon the patterns from desiccated droplets. In the choice of the compounds we were guided by the experience collected during our previous study on low potencies.[10] First, since during droplet desiccation the pattern-forming mechanisms differ for mineral and plant-based homeopathic preparations (in mineral potencies they are predominantly driven by the intra-molecular forces, and in the plant potencies by diffusion-limited aggregation), we have chosen two components of different origins (*Mercurius bijodatus* and *Luffa* are of mineral and plant origin respectively). Second, since in DEM differences due to the origin of the potentized substance are visible until approximately the four-fold decimal dilution, we chose one potency level that is still within this range (*Luffa* 4x) and one where it is beyond it (*Mercurius bijodatus* 9x). Third, we wanted to study a combination that is actually being used: *Luffa* 4x and *Mercurius bijodatus* 9x are both present in the complex *Sinusitis Hevert SL*.[16] The mixing ratio and potency levels of the two compounds therefore correspond to those used in that commercially available drug complex.

## Methods

### Experimental Layout

Since the influence of the *Luffa* 4x compound was expected to be dominant, the experimental set-up was designed to focus on the influence of *Mercurius bijodatus* 9x which, due to its higher dilution, was expected to be minor.

In each main experiment, the combination *Luffa* 4x – *Mercurius bijodatus* 9x (LM) was compared against three comparison (C) samples, in which one or both components were replaced with potentized solute exactly corresponding to the replaced component: *Luffa* 4x – ethanol 43% 3x ($LC_M$); ethanol 62% 3x – *Mercurius bijodatus* 9x ($C_LM$); and ethanol 62% 3x – ethanol 43% 3x ($C_LC_M$).

The experimentation consisted of five main and five systematic control experiments. In each main experiment, droplets of samples LM, $LC_M$, $C_LM$ and $C_LC_M$ were evaporated on slides (six slides per sample, 24 slides per experiment) placed in two evaporation chambers (slides with LM and $LC_M$ droplets in the upper chamber, and slides with $C_LM$ and $C_LC_M$ droplets in the lower chamber) following a quasi-randomization design.

### Systematic Control Experiments

Systematic control experiments serve to assess the experimental system's robustness. Each main experiment had its corresponding systematic control experiment in which

 

droplets of samples $LC_M$ and $C_LC_M$ were evaporated on slides (12 slides per sample, 24 slides per experiment) placed in the two evaporation chambers (slides with $LC_M$ droplets in the upper chamber, and slides with $C_LC_M$ droplets in the lower chamber). In the systematic control experiments, depending on the allocating space, the slides with $LC_M$ were treated and evaluated as control-LM or control-$LC_M$ and the slides with $C_LC_M$, as control-$C_L$M and control-$C_LC_M$. Statistically non-significant results between the samples control-LM and control-$LC_M$ as well as control-$C_L$M and control-$C_LC_M$ in the systematic control experiments indicate a robust experimental system.

## Manufacturing of Pharmaceutical Preparations

The potencies *Luffa* 1x and *Mercurius bijodatus* 6x were manufactured by Hevert-Arzneimittel GmbH & Co. KG (Nussbaum, Germany) according to the European Pharmacopoeia for homoeopathic preparations (Pharm. Eur.).[17] In particular, *Luffa* 1x was prepared in ethanol 62% (w/w) (following the method 1.1.8) and *Mercurius bijodatus* 6x in ethanol 43% (w/w) (following the method 3.1.1). The two potencies, as well as the two ethanolic solutions used as solvents, were sent by post to the laboratories of the Society for Cancer Research (Arlesheim, Switzerland) for investigation.

## Preparation of Potencies, Controls, and Their Binary Combinations

The potencies and controls were prepared freshly on each experimental day. *Luffa* 4x, *Mercurius bijodatus* 9x, ethanol 62% 3x, and ethanol 43% 3x were prepared for each main experiment (►**Fig. 1**), whereas *Luffa* 4x, ethanol 62% 3x, and ethanol 43% 3x were prepared for each systematic control experiment.

For each potentization step, 0.8 g of a pharmaceutical preparation was weighed and placed in a sterile glass cylinder (SBR-ET, Mix Cyl. 10 mL, B; Brand GmbH & Co. KG, Wertheim, Germany) with stopper (untargeted volume 13 mL); subsequently 7.2 mL purified water according to Pharm. Eur. 9.412 ("purified water in bulk", X-SEPTRON LINE 10 VAL, BWT AQUA AG, Aesch, Switzerland) was added to reach a dilution of 1:9. The cylinder was closed tightly; 10 succussion strokes were applied by hand. The movement to achieve succussion was performed in the air, without hitting against a firm base.

To maintain the ratio between the potencies *Luffa* 4x and *Mercurius bijodatus* 9x just as it is in the homeopathic remedy *Sinusitis Hevert SL*,[16] the potencies or their corresponding control samples were combined in the ratio six parts to seven parts. The combination of the two compounds was done after the potentization of each compound to its final potency. The combination was then mixed by hand and left for approximately 30 minutes. In the main experiments, the samples were blinded within the sample pairs (LM and $LC_M$; $C_L$M and $C_LC_M$).

## Droplet Evaporation Method

Microscope slides (76 × 26 mm, pre-cleaned, cut edges; Thermo Scientific, Gerhard Menzel B.V. & Co. KG, Braunschweig, Germany) were de-greased by washing them with dishwasher liquid, then thoroughly rinsed with hot tap water, and placed in four consecutive purified water baths. Each slide was wiped dry with a laboratory wiper (KIMTECH science, Kimberly-Clark Professional, Roswell, Canada) just before droplet deposition. 2.6 µL droplets of the tested samples were deposited on the slides in two parallel rows, seven droplets per row, using a micropipette of 20 µL capacity (Eppendorf Research Plus, Eppendorf, Hamburg, Germany). Evaporation took place in an incubator (KBF 720, cooled incubator with controlled humidity system, WTB Binder Labortechnik GmbH, Tuttlingen, Germany) with two inner plexi-glass chambers, each covered with a semi-permeable foam and placed on a vibration-absorbing base. The microscope slides with droplets were placed in the inner-chambers and left for evaporation in 26°C and 44% rH for 1 hour. The slide distribution inside the chambers followed a quasi-randomization design to provide a uniform arrangement of the samples within the rows.

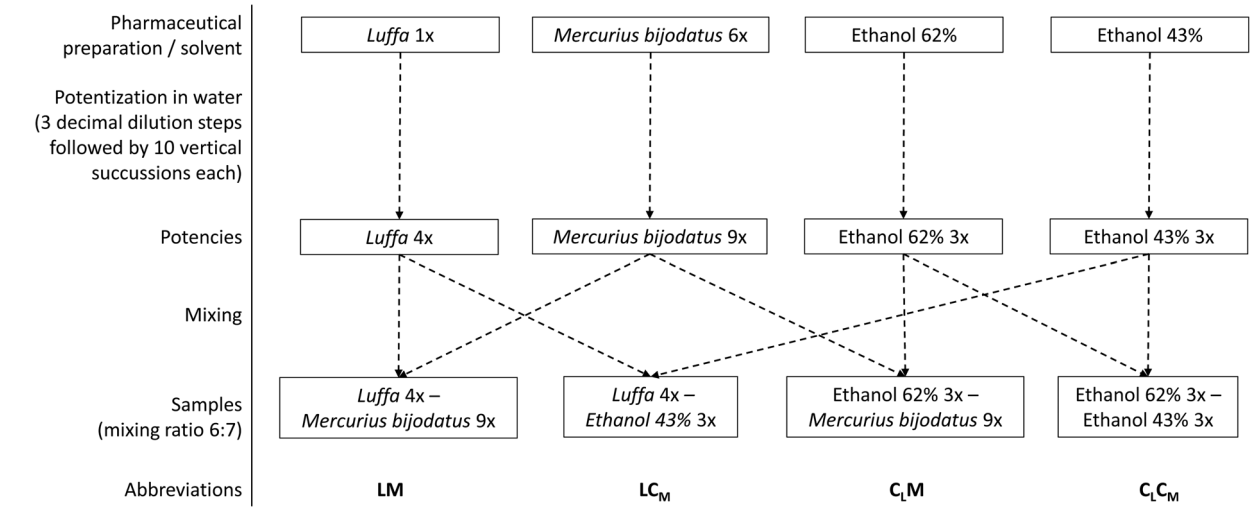


**Fig. 1** Preparation process of single potencies and their combinations (samples) for the experiment.

 

### Acquisition of Patterns

The droplet residues were examined and photographed in dark field at magnification 100× by use of an optical microscope (Zeiss Laboratory.A1; Carl Zeiss Microscopy GmbH, Jena, Germany) with an attached camera (Moticam 5.0 MP; CMOS; Motic Electric Group Co., Ltd, Xiamen, China). Droplets with disturbed crystallization due to presence of contaminating particles or due to edge effects on the slide (ca. 10%) were not considered and not photographed. Per experiment, a maximum of 336 patterns were obtained (two inner-chambers filled with 12 slides each; 14 droplets per slide). Images were saved in jpeg format (2592 × 1944 pixels).

### Computerized Pattern Evaluation

Image analysis was performed with the software *ImageJ* (v. 1.50b)[18] with the plug-in GLCM Texture.[19] All images were subjected to background subtraction by means of the sliding paraboloid with rolling ball radius set at 50 pixels, ensuring the same background throughout the image database. Consecutively the images were analyzed (1) for their gray-level distribution (GLD), and (2) after conversion into 8-bit type, by running the GLCM algorithm (considering distances between pixel pairs of four pixels and angles of 90 degrees) for determination of texture analysis variables (the parameters angular second moment, correlation, contrast, inverse difference moment and entropy).

### Statistical Analysis

The data deriving from the computerized image analysis were analyzed by means of two-way analysis of variance (CoStat, v. 6.311) (CoHort Software, Monterey, USA) at $\alpha = 0.05$, with independent factors *sample* and *day*. An interaction term between the independent factors was included in the statistical model to assess stability and reproducibility. Distribution of data was checked by visual inspection. Slight deviations from Normality were irrelevant due to the central limit theorem. Data sets with larger deviations from Normality were logarithmically transformed (log10). Global dataset statistical significance was determined with *F*-tests. Pairwise mean comparison was performed two-tailed, using the protected Fisher's Least-Significant-Difference test (pairwise comparisons were evaluated only if the global *F*-test was significant at $p < 0.05$). This procedure safeguards against type I as well as type II errors, and thus provides a suitable balance between false-positive and false-negative conclusions.[20] Results of transformed datasets were back-transformed for presentation. For graphical representation, data were standardized as follows: $z = (\text{value} - \text{mean}) / \text{standard deviation}$.

## Results

### Visual Pattern Assessment

Patterns from desiccated droplets of the two sample pairs containing *Luffa* 4x (LM and $LC_M$) showed in their structures a clearly visible dominance of the *Luffa* component that, being present in the fourth decimal dilution, contained still enough matter to form *Luffa*-typical structures (►**Fig. 2A, B**). In contrast, the two other sample pairs, where the dominant

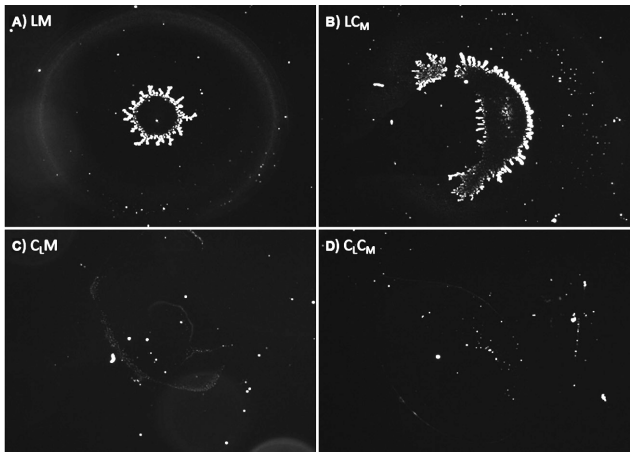


**Fig. 2** Examples of patterns from evaporated droplets of mixtures (ratio 6:7) of: *Luffa* 4x and *Mercurius bijodatus* 9x (LM) (**A**); *Luffa* 4x and ethanol 43% 3x ($LC_M$) (**B**); ethanol 62% 3x and *Mercurius bijodatus* 9x ($C_LM$) (**C**); and ethanol 62% 3x and ethanol 43% 3x ($C_LC_M$) (**D**). Pattern examples were selected based on entropy values that were near the mean entropy values of the samples for experimental day 2.

*Luffa* component was replaced with its solvent control ($C_LM$ and $C_LC_M$), formed poorly structured patterns with few visible forms (►**Fig. 2C, D**).

Concerning the influence of *Mercurius bijodatus* 9x on the pattern of the whole complex (difference between LM and $LC_M$), there was visual evidence that it slightly reduced the pattern's size and its form differentiation (►**Fig. 2A**) in comparison with the complex pairing in which it was replaced with its control imitating the solvent (►**Fig. 2B**). By contrast, in the pairs without the *Luffa* 4x compound ($C_LM$ and $C_LC_M$) *Mercurius bijodatus* 9x seemed to minimally enhance the few structures in comparison to the pair where it was replaced with its control. However, this visual assessment is only tentative, since the total pattern amount was too large to perform a structured visual evaluation (1,331 images from the main experiments).

### Computerized Pattern Evaluation

►**Table 1** shows the results of two-way analyses of variance, with independent factors *sample* and *day*, performed on datasets from the 5-day repetitions of main and systematic control experiments comparing the sample pairs (1) LM and $LC_M$ and (2) $C_LM$ and $C_LC_M$, together with the results of the corresponding systematic control experiments for each of the analyzed pattern-evaluation parameters. We considered as reliable only results that showed a statistically significant influence of the factor *sample* in the main experiments, a greater *F*-value for the factor *sample* than that for the interaction between the factors *sample* and *day*, and a non-significant influence of the factor *sample* in the systematic control experiments.

It can be seen that these statistical conditions were met in all experiments concerning the difference between the pairs LM and $LC_M$, whereas for the sample pairs $C_LM$ and $C_LC_M$ for the parameter *angular second moment* (ASM), the *F*-value for the interaction of *sample* and *day* in the main experiments was greater than that of the factor *sample*.

 

**Table 1** Results of pattern evaluation of the sample pairs *Luffa* 4x – *Mercurius bijodatus* 9x (LM), *Luffa* 4x and ethanol 43% 3x ($LC_M$), ethanol 62% 3x and *Mercurius bijodatus* 9x ($C_LM$), and ethanol 62% 3x and ethanol 43% 3x ($C_LC_M$) and the corresponding systematic control experiments. On left: mean values and *n* of sample pairs to be compared; mean values with different letter codes (a, b) are significantly different ($p < 0.05$). On right: *F*-test statistics of the corresponding two-way analysis of variance for the factors *sample* and *day*.

| Parameter | Sample | Main experiments | | | Systematic control experiments | | | Factor | Main experiments | | Systematic control experiments | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *n* | Mean | | *n* | Mean | | | *F* | *p* | *F* | *p* |
| GLD | LM | 347 | **3.85** | **b** | 355 | **4.58** | **a** | Sample | 15.19 | 0.0001*** | 2.48 | 0.1157 |
| | $LC_M$ | 322 | **4.56** | **a** | 358 | **4.89** | **a** | Day | 102.77 | 0.0000*** | 131.79 | 0.0000*** |
| | | | | | | | | Interaction | 9.25 | 0.0000*** | 1.24 | 0.2927 |
| | $C_LM$ | 335 | **1.39** | **a** | 341 | **2.71** | **a** | Sample | 11.96 | 0.0006*** | 3.17 | 0.0745 |
| | $C_LC_M$ | 327 | **1.17** | **b** | 321 | **2.96** | **a** | Day | 53.12 | 0.0000*** | 57.24 | 0.0000*** |
| | | | | | | | | Interaction | 5.00 | 0.0006*** | 1.24 | 0.2827 |
| Contrast | LM | 347 | **145.93** | **b** | 355 | **138.17** | **a** | Sample | 15.98 | 0.0001*** | 1.89 | 0.1691 |
| | $LC_M$ | 322 | **181.16** | **a** | 358 | **148.43** | **a** | Day | 46.51 | 0.0000*** | 83.30 | 0.0000*** |
| | | | | | | | | Interaction | 10.78 | 0.0000*** | 0.58 | 0.6733 |
| | $C_LM$ | 335 | **48.90** | **a** | 341 | **63.23** | **a** | Sample | 14.90 | 0.0001*** | 1.94 | 0.1641 |
| | $C_LC_M$ | 327 | **36.60** | **b** | 321 | **70.90** | **a** | Day | 13.61 | 0.0000*** | 15.01 | 0.0000*** |
| | | | | | | | | Interaction | 3.66 | 0.0059** | 1.97 | 0.0976 |
| Entropy | LM | 347 | **2.40** | **b** | 355 | **3.11** | **a** | Sample | 15.78 | 0.0001*** | 0.95 | 0.3304 |
| | $LC_M$ | 322 | **2.61** | **a** | 358 | **3.17** | **a** | Day | 235.80 | 0.0000*** | 58.15 | 0.0000*** |
| | | | | | | | | Interaction | 3.37 | 0.0096** | 0.66 | 0.6171 |
| | $C_LM$ | 335 | **1.73** | **a** | 341 | **2.53** | **a** | Sample | 11.08 | 0.0009*** | 2.92 | 0.0878 |
| | $C_LC_M$ | 327 | **1.62** | **b** | 321 | **2.62** | **a** | Day | 188.29 | 0.0000*** | 65.20 | 0.0000*** |
| | | | | | | | | Interaction | 7.61 | 0.0000*** | 0.95 | 0.4367 |
| IDM | LM | 347 | **0.79** | **a** | 355 | **0.73** | **a** | Sample | 10.31 | 0.0014** | 0.55 | 0.4586 |
| | $LC_M$ | 322 | **0.77** | **b** | 358 | **0.72** | **a** | Day | 188.49 | 0.0000*** | 24.57 | 0.0000*** |
| | | | | | | | | Interaction | 2.03 | 0.0890 | 0.79 | 0.5293 |
| | $C_LM$ | 335 | **0.85** | **b** | 341 | **0.79** | **a** | Sample | 10.80 | 0.0011** | 2.04 | 0.1532 |
| | $C_LC_M$ | 327 | **0.87** | **a** | 321 | **0.78** | **a** | Day | 188.76 | 0.0000*** | 45.08 | 0.0000*** |
| | | | | | | | | Interaction | 7.87 | 0.0000*** | 1.11 | 0.3491 |
| ASM | LM | 347 | **0.33** | **a** | 355 | **0.20** | **a** | Sample | 5.47 | 0.0196* | 0.67 | 0.4135 |
| | $LC_M$ | 322 | **0.32** | **b** | 358 | **0.20** | **a** | Day | 932.77 | 0.0000*** | 26.76 | 0.0000*** |
| | | | | | | | | Interaction | 3.29 | 0.0110* | 0.97 | 0.4224 |
| | $C_LM$ | 335 | **0.43** | **b** | 341 | **0.28** | **a** | Sample | 8.59 | 0.0035** | 1.51 | 0.2194 |
| | $C_LC_M$ | 327 | **0.45** | **a** | 321 | **0.27** | **a** | Day | 565.42 | 0.0000*** | 102.85 | 0.0000*** |
| | | | | | | | | Interaction | 9.62 | 0.0000*** | 2.23 | 0.0638 |

Abbreviations: ASM, angular second moment; GLD, gray-level distribution; IDM, inverse difference moment; Interaction, interaction between the factors *sample* and *day; n*, number of patterns.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

None of the systematic control experiments yielded significant effects, either for the factor *sample* or for the interaction of *sample* influence and experimental *day*, for any evaluation parameter. We thus conclude that the experimental system was stable and did not produce false-positive results.

►**Fig. 3** depicts the mean values of the pattern-evaluation parameters for the pairs LM and $LC_M$ as well as for $C_LM$ and $C_LC_M$ and their corresponding control samples calculated from standardized datasets. The patterns of the pair LM had a reduced GLD, *contrast* and *entropy* compared with the control pair $LC_M$, whereas the parameters ASM and *inverse*

 

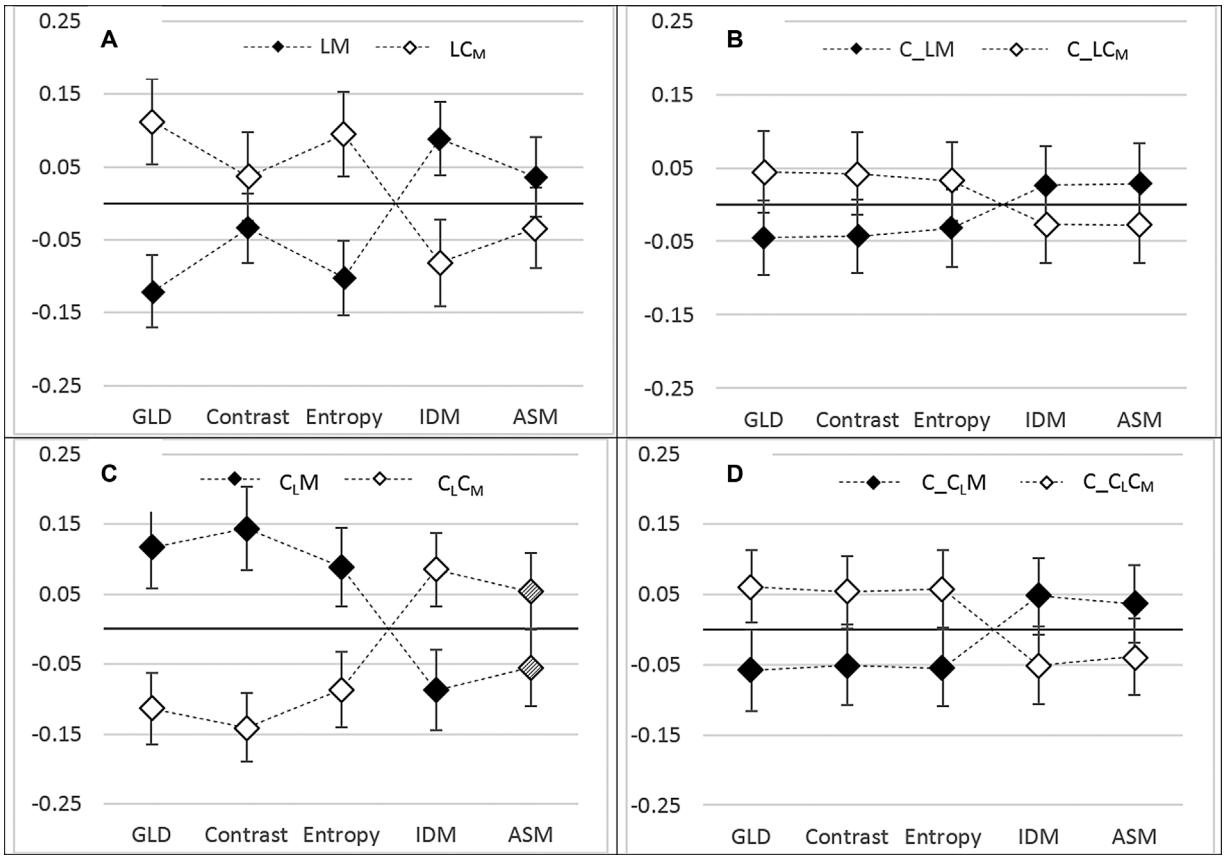


**Fig. 3** Graphical representation of the mean pattern-evaluation parameter values of patterns from desiccated droplets of: (**A**) *Luffa* 4x – *Mercurius bijodatus* 9x (LM) complex and the control sample *Luffa* 4x – ethanol 43% 3x ($LC_M$); (**B**) the corresponding systematic control experiment; (**C**) ethanol 62% 3x – *Mercurius bijodatus* 9x ($C_LM$) and ethanol 62% 3x – ethanol 43% 3x ($C_LC_M$) samples; and (**D**) the corresponding systematic control experiment. The values of the pattern-evaluation parameters were standardized to the experimental mean. ASM results in (**C**) did not meet the criteria for reliability. Error bars indicate standard error of the mean. Abbreviation: ASM, angular second moment; GLD, gray-level distribution; IDM, inverse difference moment.

*difference moment* (IDM) were increased compared with $LC_M$. In the case of the pairs $C_LM$ and $C_LC_M$, the values of GLD, *contrast* and *entropy* were greater in $C_LM$, whereas the IDM was smaller in comparison with $C_LC_M$. For the latter pairs the results of ASM were not considered, since they did not meet the criteria for reliable results.

## Discussion

Our results suggest that *Mercurius bijodatus* 9x (containing parts per billion of the starting material, and thus 1,000 times less than the impurity content of purified water, which is in the parts per million range), when added to *Luffa* 4x, can significantly influence the patterns obtained from evaporated droplets versus control.

*Mercurius bijodatus* 9x increased the homogeneity of the patterns of *Luffa* 4x (decrease in GLD, *entropy* and *contrast*; increase in ASM and IDM). However, the patterns of *Mercurius bijodatus* 9x combined with solvent were more heterogeneous compared with the solvent control (increase in GLD, *entropy* and *contrast*; decrease in IDM). This corresponds to a complete inversion in all outcome parameters measured. Thus, in this phenomenological assay, the complex *Luffa* 4x – *Mercurius bijodatus* 9x does not correspond to a simple addition of the components since the effect of *Mercurius bijodatus* 9x was inverted in all parameters. We thus conclude that some interaction between *Luffa* 4x and *Mercurius bijodatus* 9x occurred. The exact nature of the proposed interaction is unknown at the present stage of investigation, however, and needs to be elucidated in further investigations.

*Luffa* 4x created patterns formed out of thick dendrites that surrounded an often structure-free central part of the structure (►**Fig. 2A, B**) and that resembled the patterns of *Luffa* obtained in our previous experiments.[9,10] We thus conclude that they were typical *Luffa* 4x patterns. The addition of *Mercurius bijodatus* 9x did not change the overall character of the patterns but reduced its size and heterogeneity.

We observed a statistical interaction between treatment and experimental day for all but one outcome parameter. This means that there was some variability in the effects over the five experiments conducted. Since the *F*-values of the main effect were larger than the *F*-values of the interaction in all but one case, we judge this interaction as not critical for the main conclusions of the present experiments. It means, however, that the effects were modulated by still-unknown factors correlated to experimental day that need to be elucidated. Since we further observed neither significant sample nor significant sample/experimental day effects,

 

we conclude that the experimental system was stable and that the results observed were not due to unidentified systematic errors.

Potencies containing even less starting material than *Mercurius bijodatus* 9x and lying in the high-potency range have also been shown to influence crystalline patterns obtained from evaporated droplets[21] or from copper chloride biocrystallization[22] – however, only in the case of samples analyzed from biological models. The model proposed here is a physical one, and most probably measures purely physical phenomena taking place between the components of the complex's pair in solution and during phase-transition. On the other hand, *Luffa* 4x is *de facto* a biological substance and we cannot fully exclude the possibility that the model's outcome reflects some influence of *Mercurius bijodatus* 9x upon the potentized plant extract. In this case our experimental set-up would act as a biological model. Experiments testing this hypothesis, conducted for instance on potencies of mineral origin only, could be performed in the future.

Present knowledge from basic research into homeopathic complex remedies is rather limited. Though there are clinical studies in this field, which have tested the effectiveness of homeopathic complex remedies,[23–25] we are not aware of sustained basic research activities providing insight into homeopathic complex remedies' mechanisms of action, possible interactions between their components, or the function of different components and their ratios. Homeopathic complex remedies, despite their long tradition, seem to represent a new field of basic research, which has many unanswered questions regarding for instance the correct study methods to apply. One such question is related to the control samples that the homeopathic complex remedy might be compared with. In the present study, we have chosen control samples in which one or two components of a bi-component complex were replaced by a control sample imitating the solvent of the replaced component. These samples were optimal from a methodological point of view, since they allowed an exact estimation of the influence of the *Mercurius bijodatus* 9x component on the pattern; on the other hand, such a procedure increases exponentially the number of control samples needed with each additional component of a multi-component potency complex.

In the present experimentation we restricted the research question to the influence of *Mercurius bijodatus* 9x on the patterns; another possible question would concern the influence of the *Luffa* 4x compound. Our decision was based first on the fact that the influence of *Luffa* 4x on the pattern would be obvious because of the amount of starting material contained in a fourth decimal potency, and thus the influence of *Mercurius bijodatus* 9x seemed more interesting; second, there was a restricted number of places for slides in our evaporation chambers. The patterns that are to be directly compared in the statistical analysis must be dried in the same chamber compartment (the upper or the lower part of the chamber) to avoid systematic differences; also, the systematic control experiments were set up to evaluate the experimental system's stability in the restricted setting of one chamber compartment. Correspondingly, modified research questions would require a different experimental layout.

## Conclusion

The lack of basic research studies investigating homeopathic complex remedies makes this discipline virtually a new research field, though homeopathic complex remedies already have a long tradition in clinical medicine. The present study represents a first step into basic research that is dedicated to homeopathic complex remedies. We were able to observe, based on an example of a bi-component combination of *Luffa* 4x – *Mercurius bijodatus* 9x, that patterns from evaporated droplets of the complex are clearly different from patterns of its single components. In other words, the complex does not correspond to a simple addition of the components in this phenomenological assay. Thus, some interactions between the components seem to take place. The exact nature of this underlying interaction is currently unknown and needs to be elucidated in further investigations.

### Highlights

- Homeopathic complex remedies, used to treat several common diseases, represent an almost unexplored area in homeopathy basic research.
- Self-assembled patterns from evaporating droplets can be used for a phenomenological comparison of homeopathic complexes compared with their single compounds.
- A two-component preparation of *Luffa* 4x and *Mercurius bijodatus* 9x was analyzed.
- The addition of *Mercurius bijodatus* 9x increased the homogeneity of the *Luffa* 4x pattern compared with the corresponding control sample (*Luffa* 4x – solvent)
- In comparison to the pattern of pure solvent, the addition of *Mercurius bijodatus* 9x decreased the pattern's homogeneity.
- Our results indicate that in the two-component preparation, *Luffa* 4x – *Mercurius bijodatus* 9x, some interactions between the compounds might take place.

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### Conflict of Interest

None declared.

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