

Datamining a medieval medical text reveals patterns in ingredient choice that reflect biological activity against the causative agents of specified infections

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Abstract (150 words)

The pharmacopeia used by physicians and lay people in medieval Europe has largely been dismissed as placebo or superstition. While we now recognise that some of the *materia medica* used by medieval physicians could have had useful biological properties, research in this area is limited by the labour-intensive process of searching and interpreting historical medical texts. Here, we demonstrate the potential power of turning medieval medical texts into contextualised electronic databases amenable to exploration by algorithm. We use established methodologies from network science to reveal statistically significant patterns in ingredient selection and usage in a key text, the fifteenth-century *Lylle of Medicynes*, focusing on remedies to treat symptoms of microbial infection. We discuss the potential that these patterns reflect rational medical decisions. In providing a worked example of data-driven textual analysis, we demonstrate the potential of this approach to encourage interdisciplinary collaboration and to shine a new light on the ethnopharmacology of historical medical texts.

1 Infection remedies from medieval Europe often use ingredients known to possess some
2 antimicrobial or immunomodulatory effects, at least in *in vitro* assays ^{1, 2}. Further, these texts
3 suggest complex preparations of multiple ingredients, and contingencies of treatments for the
4 same symptoms. A recent study found that an Anglo-Saxon remedy for eye infection used
5 ingredients that when combined produced a strong antibacterial cocktail ³. The existence in the
6 medieval pharmacopeia of groups of ingredients that are repeatedly combined to treat the
7 symptoms of infection, and that, individually, have useful biological activity, gives evidence to
8 the hypothesis that medieval medicine had some rational basis ^{4, 5}. It also suggests that these
9 remedies may contain combinations of natural materials that could yield clinically-useful
10 biologically active molecules.

11
12 Network science is a powerful tool to model complex systems and extract meaningful
13 information from data sets representing interactions between elements, such as the co-
14 occurrence of ingredients in recipes. Network analysis has been used to characterise and analyse
15 interactions between people or animals ^{6, 7, 8, 9}, computer networks ¹⁰, gene regulation networks
16 ¹¹ and combinations of ingredients in culinary recipes ¹². The network approach represents a
17 system as a graph, in which the nodes correspond to discrete elements (individual organisms,
18 computers, genes or ingredients), and the links to dyadic relations between them
19 (communication, coordinated behaviour or co-localisation) ^{13, 14, 15, 16}. The network paradigm
20 provides researchers with mathematical tools to define and study quantitative properties of real-
21 world systems, such as the typical distribution of links amongst nodes ^{17, 18}, the relation between
22 the number of links of the nodes sharing a link ^{19, 20}, the ability of nodes to synchronize their
23 dynamics ^{21, 22}, and the existence of communities ^{11, 23, 24}. In this context, communities (also
24 known as modules) are groups of nodes whose density of internal connections is higher than that
25 of the links between different modules ²⁵.

26
27 Community structures have been identified in numerous real-world systems, where the module
28 elements often share a common function. Some typical examples are closer friendship groups
29 within sport clubs ²⁶, ensembles of closely collaborating jazz players ²⁷, and sets of chemical

1 substrates that participate in the same metabolic reactions^{28, 29}. Detecting such modules is one
2 of the main techniques that are routinely employed to create organic descriptions of complex
3 systems; a large number of algorithms have been created to perform this task^{23, 24, 30, 31, 32}. Here,
4 we use community detection to analyse a set of medieval medical remedies and discover
5 communities of ingredients that are commonly combined to treat symptoms of microbial
6 infection.

7
8 Our work has two goals. First, we aimed to determine the tractability of turning medical texts
9 into contextualised electronic databases amenable to quantitative analyses of hidden patterns in
10 the use and preparation of historical *materia medica*. Network analyses appear eminently suited
11 to interrogating medical texts, and have successfully been used to analyse patterns of ingredient
12 co-occurrence in culinary recipes¹². We therefore wished to determine whether community
13 detection procedures could be used with a medieval medical text, given the difficulties of working
14 with medieval manuscripts (orthography, linguistic change and the need for contextualisation of
15 language based on a knowledge of the medical and material culture of the period). Our second
16 goal was to apply community detection algorithms to determine whether an exemplar text
17 contained detectable patterns of ingredient combination and/or assignation of specific
18 ingredients to specific symptoms of disease (in our case, microbial infection), and consider, based
19 on the ethnopharmacological literature, whether these patterns could reflect rational medical
20 decision-making on the part of the medieval physician.

21
22 The limited availability of medieval medical texts, as well as the constraints of researchers mining
23 these texts by hand, has thus far meant that ethnopharmacological research conducted on
24 medieval European medicine has concentrated on single specific ingredients (e.g. a body of
25 literature on *Plantago* spp.^{33, 34, 35}), or single recipes from select medieval texts^{3, 36}. The use of
26 digital technologies to turn these texts into databases amenable to quantitative datamining
27 requires a careful interdisciplinary approach, but it could provide an entirely new perspective on
28 medieval science and rationality. It could even suggest combinations of *materia medica* worthy
29 of further study to determine their potential for drug development.

1 We chose as our exemplar text the fifteenth-century Middle English manuscript known as the
2 *Lylye of Medicynes*. This is a Middle English translation of Bernard of Gordon's *Lilium medicinae*,
3 originally completed in 1305. The text is extant in one manuscript, Oxford, Bodleian Library MS
4 Ashmole 1505. Bernard of Gordon was a significant medieval medical doctor and lecturer in
5 Montpellier^{37,38}. His magnum opus is a lengthy treatise on disease etiology, medical philosophy
6 and history (citing Galen, Hippocrates, Ibn Sīnā, and many others), personal case studies, and
7 treatment procedures and recipes. The first owner of the *Lylye of Medicynes* is thought to have
8 been Robert Broke, apothecary to Henry VI; appropriately, the Middle English translation is
9 notable for its pharmaceutical content^{39, 40, 41}. There are 360 recipes in the *Lylye* specifically
10 notated with *Rx*, as well as nearly 6,000 ingredient names throughout the whole text. The recipes
11 in the *Lylye* are presented in a standard format: they begin with an indication of what type of
12 remedy it is, such as ointment, syrup, plaster, and usually the phase of the illness during which it
13 should be applied (beginning, middle, or end). This is followed by a recipe of ingredients, often
14 accompanied by quantity, and preparation instructions, such as to boil, powder, or infuse. In the
15 *Lylye* and other medieval medical texts, it is common practice to record recipes without defined
16 measurements, and to include long lists of ingredient substitutions instead of exact quantities,
17 even when hazardous materials are involved. Often, specific details are not given because it is
18 assumed that the reader is already familiar with the methodology. Furthermore, some diseases
19 or treatments carried so many variables that the selection of ingredients and quantities was left
20 to the judgement of the physician in charge of the patient.

21
22 The *Lylye* is a unique translation of a significant text by a notable medieval physician. Its
23 pharmaceutical content, association with a medieval apothecary and Tudor barber-surgeons,
24 large number of ingredients, and chapters on infectious disease are all factors which make this
25 text an attractive starting off point for an analysis of significant antimicrobial ingredient
26 combinations.

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1 Results

2

3 *Turning the Lylye of Medicynes into an electronic database*

4

5 As a starting point, the 360 recipes of the *Lylye* were entered into a spreadsheet (**Supplementary**
6 **Data**). These recipes contain 3,374 individual ingredient names for the treatment of 113 medical
7 conditions. Of those disease conditions, 30 (containing 86 recipes) may be classified as external
8 infections, mainly of the skin, mouth, or eyes. The *Lylye of Medicynes* uses language which clearly
9 indicates *infection*, as it might be translated in modern terms. For example, in describing skin
10 swellings, abscesses, lesions, wounds, carbuncles, erysipelas and fistula, symptoms from the text
11 include broken skin, purulence, itching, foul smell, heat, moisture, aching, pricking and burning
12 sensations, redness, yellowness, ulceration, and black crusts, which all are indicators of infection
13 (Book 1, fols 24r-28v).

14

15 In addition to the 360 recipes identified by the text with Rx, there are multiple other ingredients,
16 which are not prefaced by Rx, but clearly are set up in the format of a recipe. To the principal
17 data set, 36 additional recipes for the treatment of 19 medical conditions (specifically for external
18 infection) containing 174 ingredients were added for a total data set of 3,548 ingredients (747
19 unique names) and 124 unique disease names, of which 41 may be classified as potential external
20 infections. Ingredients which were excluded from the data set were those not in recipe format,
21 such as lists of ingredient substitutions and lists of purgative ingredients.

22

23 There are numerous challenges to constructing and processing a medieval data set using modern
24 analytical tools. These challenges include medieval spelling and language variation, multiple
25 synonyms for the same ingredient, translation of medieval ingredients and diseases into modern
26 equivalents (many of these terms have multiple possible interpretations or ambiguous
27 definitions), and the variation within the modern system of botanical binomial nomenclature.
28 Middle English spellings may be standardized and hand-checked using the Vard 2 software^{42, 43,}
29⁴⁴; however, this program was developed specifically for Early Modern English spelling variation

1 and was deemed not appropriate for our data set from the Middle English *Lylle of Medicynes*.
2 Spelling variants were normalized by hand with preference given to the English word (e.g. *oil*
3 instead of *oleum*). Additionally, the frequency of occurrence in the text of the *Lylle* and the
4 headword used in the *Middle English Dictionary (MED)*,⁴⁵ were taken into consideration when
5 normalizing the spelling. The Middle English characters of thorn, yogh, and long z were
6 modernized. The Middle English usage of *u/v* and *i/j* were also standardised to modern practices.
7
8 R. James Long⁴⁶ has stated that in translations of Latin botanical terms ‘as many as 15 synonyms
9 for the same plant have been counted and it is not unheard of that two or more words for the
10 same herb appear in the same recipe.’ This is an accurate description of ingredient terminology
11 in the *Lylle’s* recipes. To eliminate redundancy and to aid the processing of our data set,
12 synonyms were combined under one term where possible with preference given to the Middle
13 English term appearing most frequently in the *Lylle* or as the headword in the *MED*. For instance,
14 *fenel* was chosen as the headword for textual appearances of *fenel*, *fenell*, *feniculi*, *feniculum*,
15 *marathri*, *maratri*, *maratrum*. Due to variations in active ingredients in different parts of a plant,
16 those designations were included (e.g. root of fenel, seeds of fenel, juice of fenel). The imperative
17 of this study was to identify significant ingredient combinations. Therefore, preparation
18 instructions were not included with the ingredient names, except in cases where the preparation
19 changed the nature of the base ingredient [e.g. *calx* (lime); *calx vive* (calcium oxide); *calx extinct*
20 (calcium hydroxide)]. The Middle English ingredient names and disease names were not
21 translated into Modern English in the spreadsheet or for the analyses. Modern English
22 translations are provided for the ingredients referenced in this article. As many medieval medical
23 terms are ambiguous and definitions vary between modern dictionaries, caution was used in
24 translating these terms into Modern English and in identifying the scientific names of plants. The
25 definitions were cross-checked using the *MED*, *Oxford English Dictionary*⁴⁷, the *Dictionary of*
26 *Medical Vocabulary in English, 1375-1550*⁴⁸, Kew Medicinal Plants database⁴⁹, and the Plant List
27 database⁵⁰.
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1 *Network analysis of ingredients in the Lylve of Medicynes*

2

3 To build a network from the recipes, we created a node for each individual ingredient
4 encountered. Every time two ingredients were found together in the same recipe, a link was
5 placed between them. In cases where pairs of ingredients were found in more than one recipe,
6 the corresponding links were “strengthened” by assigning them a weight proportional to the
7 number of times the pair occurred across recipes. As an example, Fig. 1 shows the network
8 created from one recipe for *fistula in lacrimali* (lacrimal fistula) and one for *pascionibus oris*
9 (diseases of the mouth). The ingredients for the former (shown in yellow) are *galle* (bile salts),
10 *hony* (honey), *pomegarnettes* (pomegranates), *ruta* (a plant of the genus *Ruta*, especially
11 common rue, *Ruta graveolens*), and sumac (sumac, *Rhus coriaria*); those for the latter (shown in
12 blue) are *galle*, *hony*, *olibanum* (frankincense), *pomegarnettes*, *sumac*, and *vinegre* (vinegar).
13 Note that four ingredients, namely *hony*, *galle*, *pomegarnettes*, and *sumac*, are found in both
14 recipes. Thus, the corresponding nodes are colored both yellow and blue. Since, in this example,
15 six pairs of ingredients are found in both recipes, the links that represent them are thicker. When
16 this procedure was carried out on all the recipes, we obtained a network with 354 nodes and
17 3073 weighted links.

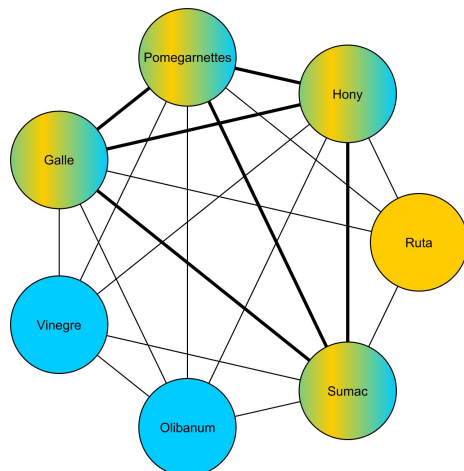
18

19 **Figure 1** Example of an ingredient network. The nodes in yellow are ingredients of a
20 recipe for *fistula in lacrimali*; those in blue are for *pascionibus oris*. Ingredients found
21 in both recipes are colored both yellow and blue. Thicker links join pairs of ingredients
22 that appear in both recipes.

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3 To normalize the link weights, we used a linear transformation that did not affect the network
4 properties^{51, 52}. More specifically, if nodes i and j were connected, their link weight $W_{i,j}$
5 transformed as

6

$$7 \quad W_{i,j} \rightarrow \frac{(1-2\varepsilon)W_{i,j} + (2\varepsilon-1)W_{min}}{W_{max}-W_{min}} + \varepsilon \quad (1)$$

8

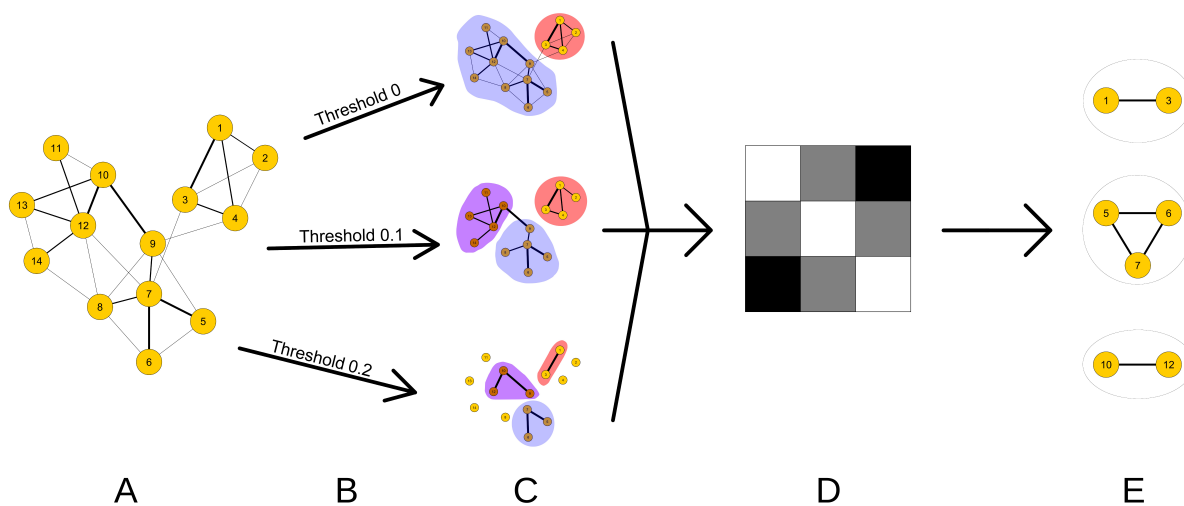
9 where W_{min} and W_{max} are the minimum and maximum original weights, and ε is a small parameter
10 used to avoid cutting links whose original weight is W_{min} . In our case, we used the common choice
11 $\varepsilon = 0.01$. In principle, one could perform the analyses of ingredient communities on the full
12 weighted network. However, the results could be affected by noise in the data, such as could be
13 originated by pairs of ingredients whose links were too weak to be significant, but strong enough
14 to spuriously alter the local structure of the network. To avoid this type of problem, we
15 introduced a variable threshold t . Starting from the full weighted network, for every given value
16 of t we created a thresholded, unweighted network by removing all the links whose weight was
17 $\leq t$ and considering all other links as having equal unitary weight. By studying how the
18 communities change with the value of the threshold, we were able to find groups of ingredients
19 that always or most often belonged to the same community, regardless of the assignment of the
20 other nodes around them. This allowed us to identify combinations of ingredients that were
21 statistically relevant and thus warranted further investigation.

1 To illustrate the procedure, consider the fictitious network in Fig. 2. For threshold 0, we identify
2 two communities (red and blue), while for thresholds 0.1 and 0.2 we find three communities (red,
3 blue, and violet). Note that, in going from threshold 0 to 0.1, the blue community splits into two
4 sub-communities (blue and violet). This is an example of hierarchical structure: some
5 communities are themselves composed of other, smaller communities. Comparing the results for
6 the three thresholds, we find the combinations of nodes 1 and 3, nodes 5, 6 and 7, and nodes 10
7 and 12 to be important. Note that, in the original network, nodes 5 and 6 are connected with the
8 weakest (thinnest) link. Nonetheless, this procedure identifies their co-occurrence, together with
9 ingredient 7, as potentially relevant.

10

11 **Figure 2** Fictitious example of the process for identifying relevant combinations of
12 ingredients via thresholding and community detection. (A) The starting network.
13 (B, C) Apply thresholding procedure and choose partitioned network with maximal
14 modularity ($q_{\{c\}}$, equation 2). (D) Draw heatmap to visualise strengths of associations
15 between ingredient pairs. Darker pixels indicate stronger association. For example, in
16 this example network ingredients 1 and 3 are more strongly connected by their recipe
17 co-occurrences than are ingredients 1 and 2 or 2 and 3. (E) Identify most strongly
18 associated group of ingredients and manually search database for recipes including
19 these combinations.

20



21

1 We chose 6 thresholds: 0, 0.01, 0.15, 0.29, 0.43 and 0.571. Our choice was based on the fact that
2 the number of edges that are cut increases with t , causing more nodes to become isolated, and
3 the network to fragment (see Fig. 3). Thus, we chose the values at which such changes in the
4 thresholded network happened, stopping at 0.571 because for that threshold the number of non-
5 isolated nodes becomes only 10.

6

7 To carry out the community detection on our data, we used the algorithm developed by Treviño
8 *et al.*³². This is a spectral method that uses a synergy of refining steps, including local and global
9 tuning, as well as agglomeration, to estimate the network partition that maximizes modularity, a
10 widely used objective function. For a network with N nodes and m links, its partition into a set of
11 communities has modularity

12

13

$$14 \quad q_{\{c\}} = \frac{1}{2m} \sum (A_{i,j} - \frac{k_i k_j}{2m}) \delta_{c_i c_j} \quad (2)$$

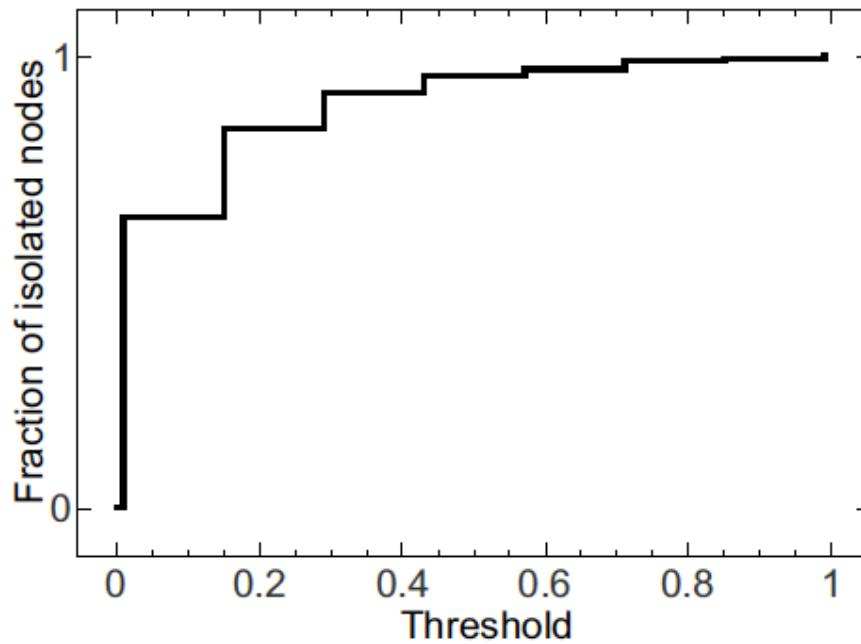
15

16 In the expression above, A is the adjacency matrix of the network, whose elements $A_{i,j}$ are 1 if
17 nodes i and j are linked, and 0 otherwise; k_i is the number of links involving node i , c_i is the
18 community to which node i belongs, δ is Kronecker's symbol; and the sum is over all possible
19 pairs of nodes. Thus, this definition promotes partitions in which the number of links within
20 communities is higher than its expectation value for a randomized version of the network.

21

22 **Figure 3** Fragmentation of the network with threshold. As the threshold is increased,
23 more nodes lose all their links and become isolated.

24



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3 *Communities of ingredients in the Lylve of Medicynes*

4

5 For each value of link threshold, we ran our community detection algorithm 10,000 times, and
6 selected the partition with the highest modularity. Having obtained the best community
7 structures, we counted the number of times every possible pair of ingredients was assigned to
8 the same community, producing a matrix of co-occurrences. To visualize this matrix, we sorted
9 the ingredient list using the Cuthill-McKee algorithm⁵³, and represented the result as a greyscale
10 heat map, in which the saturation of each pixel of coordinates (i, j) is proportional to the number
11 of community co-occurrences of ingredients i and j (Fig. 4).

12

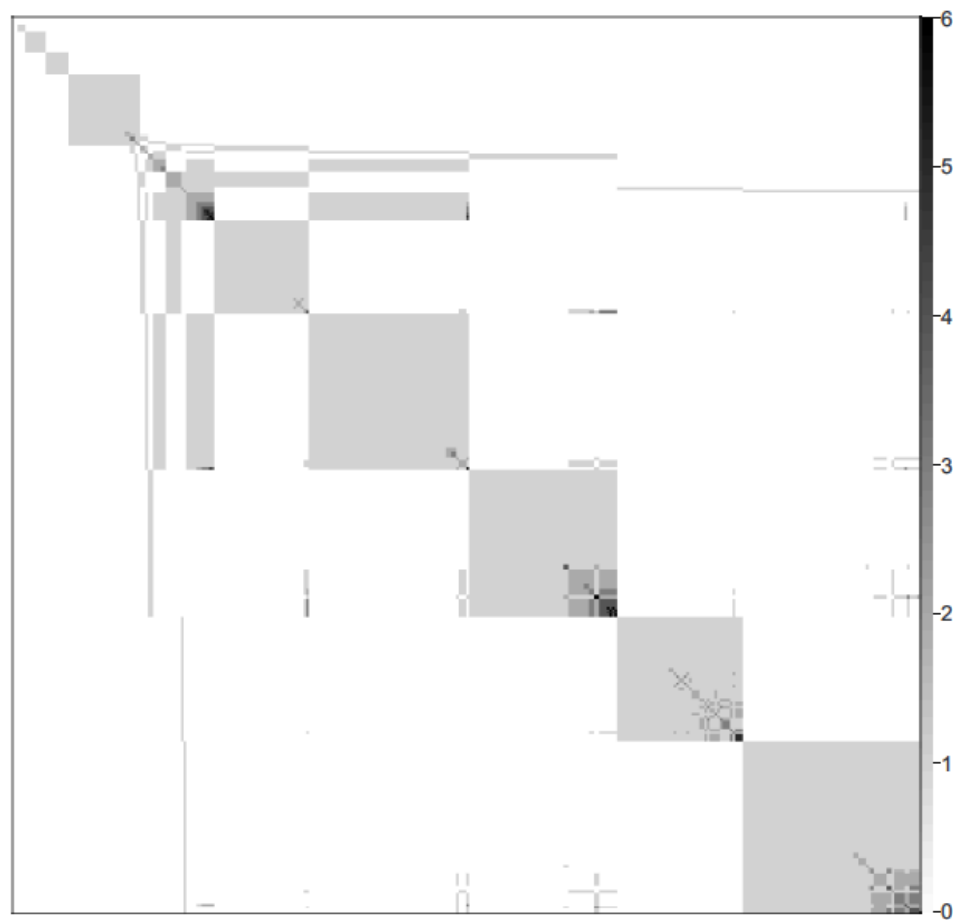
13 The results clearly show the existence of a hierarchical structure within the recipes. Each larger
14 community is composed of other, smaller sub-communities, all with a common kernel of
15 ingredients. This allows us to identify three core combinations and four core individual
16 ingredients. The combinations are *aloen* (aloes; *Aloe vera*)+*sarcocolla nutrite* (gum which exudes
17 from one of several Persian trees, including *Penaea mucronata* and *P. sarcocolla*, *Astragalus*

1 *fasiculifolius* and *A. sarcocolla*; *nutrite* means infused, typically in breast milk); *ceruse lote* (white
2 lead, washed or purified)+*eris ust* (copper calcined), and *olibanum*+*sumac*. The single ingredients
3 are *balaustia* (blossoms of pomegranate), *galle*, *hony*, and *vinegre*. Of these, we excluded *ceruse*
4 and *eris* from our consideration. Although certain metals, such as copper, have shown
5 antimicrobial properties, medicinal plants and plant-derived products were the main focus of this
6 enquiry. Thus, we focus on the remaining ingredients.

7

8 **Figure 4** Community co-occurrence of ingredients. Every pixel in the figure
9 corresponds to a pair of ingredients. The saturation of the color is proportional to the
10 number of times the pair of ingredients is found in the same community.

11



12

13

1 *Choice of two exemplar remedies*

2

3 From the data set, 62 recipes were manually selected as candidates for further investigation
4 based on the results of network analyses. These 62 recipes were placed into seven groups in
5 order of potential relevance (number of ingredients or pairs of ingredients that occur in the
6 recipe; **Supplementary Data**). Remedies containing several alchemical metals, opiates, or
7 lengthy, complex lists of ingredients (e.g. one recipe contains 32 ingredients) were excluded. The
8 two most interesting remedies were selected based on the exclusion criteria, the antimicrobial
9 significance of the ingredients as individuals and as combinations, and the description contained
10 in the text of the condition they were intended to treat. These two recipes were compared with
11 an early printed edition of the Latin *Lilium medicinae* (Lyon 1559) to see if there was any
12 significant variation in the recipes between texts.

13

14 The first recipe occurs in a chapter for the treatment of eyes, including many infectious
15 conditions. This particular recipe is for the treatment of *fistula in lacrimali*, which may be a
16 lacrimal fistula, by modern definition; it is described by the text as ‘a fester in the corner of the
17 eye’ (fol. 99r). This recipe is described as an ointment to ‘regendre flesh’ (i.e., to cause new flesh
18 to grow/to repair/to heal). This recipe states to make an ointment of *aloen*, *olibanum*, *sarcocolla*
19 [*nutrite*], *sumac*, and *rind of pomegarnettes*. The inclusion of *nutrite* was informed by the Latin
20 text and by the frequent occurrence of *sarcocolla nutrite* throughout the Middle English *Lylle*. It
21 is possible that it was omitted in this recipe by scribal error or an errant (or variant) exemplar.

22

23 The second recipe occurs in the chapter *De pascionibus oris* for the treatment of ‘pustules, ulcers,
24 *apostemata* (swelling/inflammation), cancer, fistula, *herpestiomenus* (gangrene), and
25 *carbunculus* (carbuncle; suppurating boil)’ (fol. 126r). This recipe is a *gargarisme* (a medicine for
26 washing the affected area of the mouth or throat) to be used if the condition is deemed to be
27 caused by the phlegmatic humour. The mouthwash is to be made of *sumac*, *galle*, *psidia* (the rind
28 of pomegranate or the bark of the tree), *balaustia*, *mastic* (resin exuded from the mastic tree,

1 *Pistacia lentiscus*), *olibanum*, *hony*, and *vinegre*. We suspect it likely that these two recipes are
2 especially good candidates for further research.

3

4 *Structured review of evidence for efficacy of selected ingredients.*

5

6 To perform a thorough literature search for evidence of antimicrobial or immunomodulatory
7 effects of the selected ingredients, we (a) searched the Cochrane Database of Systematic Reviews
8 (www.cochranereviews.com) for reviews containing each ingredient's name, and (b) conducted
9 a wider literature search using Scopus.com (*TITLE-ABS-KEY (ingredient_name) AND TITLE-ABS-*
10 *KEY (antibacterial OR antifungal OR antibiotic OR antimicrobial)*). For plant species, common
11 names and Latin binomials were searched; further terms were added as necessary to specifically
12 search for active compounds within ingredients (e.g. lactoferrin for breast milk). Searches were
13 conducted during the week of 25-29th October, 2017. In empirical literature on the antimicrobial
14 effects of ingredients of component compounds, we assessed the rigour and relevance of the
15 results. This included not only a judgement on whether sufficient quantitative data were
16 presented, but also an assessment of the relevance of experiments to an *in vivo* context, i.e., we
17 judged experiments testing the effects of substances on bacteria growing as biofilm⁵⁴ as more
18 relevant than those using disk diffusion assays on agar plates or inhibition of planktonic growth,
19 and those using clinical isolates of bacteria as more relevant than those focusing purely on
20 domesticated lab strains of the same bacteria.

21

22 We found poor evidence for useful antimicrobial or healing effects of aloe, frankincense, mastic,
23 and sarcocolla. Aloe extracts are used in a wide variety of medicinal and cosmetic products
24 marketed for skin healing, and numerous patents cover the use of acetylated mannans from *Aloe*
25 *vera* due to their supposed immunomodulatory effects^{55, 56}. However, two recent Cochrane
26 reviews found that published studies on the wound healing and antiseptic properties of this plant
27 were generally of low quality and that the results published of human trials returned variable
28 results, spanning help and harm to the patients^{57, 58}. The available literature on the biological
29 composition and activity of mastic was even less clear. This resin has a history of use as a chewing

1 gum to promote oral health, and does appear to contain compounds with the ability to inhibit
2 bacterial growth in disk diffusion assays *in vitro* ⁵⁹. However, we found that the few publications
3 available which specifically addressed the effect of mastic gum on oral bacteria did not present
4 clear quantitative data on the effects of mastic on bacterial cell viability ^{60, 61, 62, 63}. Frankincense
5 may be obtained from a number of trees in the genus *Boswellia*, most usually *B. sacra*. It may
6 have some immunomodulatory and thus healing effects via its effects on the NF-κB and TNFα
7 pathways, but little research has been conducted on its chemistry; this is further complicated by
8 the fact that different species have different chemical compositions and crude extracts can have
9 different effects on inflammatory pathways than purified constituent molecules ^{64, 65}. Finally,
10 while medicinal use of sarcocolla is mentioned in Latin, Greek, and Arabic historical sources, we
11 were unable to find any modern research into its biological properties.

12
13 Slightly better evidence was found suggesting antibacterial potential of vinegar, sumac, bile, and
14 pomegranate rind. Vinegar is generally acknowledged to act as a mild disinfectant, and pure
15 acetic acid has been shown to exhibit various bactericidal effects at similar or lower
16 concentrations to those typically found in vinegar. This includes the ability to kill cells of key
17 opportunistic pathogens living as monospecies biofilms (clinical isolates of the Gram-negative
18 species *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella*
19 *pneumoniae*, *Enterobacter cloacae* and *Proteus mirabilis*, plus the Gram-positive *Staphylococcus*
20 *aureus* ^{66,67}. As a weak acid, it can readily cross cell membranes and collapse the cross-membrane
21 proton gradient necessary for ATP synthesis; once inside the cell, acetic acid alters the
22 cytoplasmic pH and this can cause DNA damage and protein unfolding ⁶⁷. A clinical trial into the
23 effectiveness of acetic acid for treating burn wound infections is underway in the UK ⁶⁸. Vinegar
24 has also been shown to kill the fungus *Candida albicans*, the causal agent of oral thrush, *in vitro*
25 ⁶⁹. Like vinegar, an aqueous extract of sumac is acidic ⁷⁰; this property in itself could provide some
26 antibacterial effect, but even after increasing the pH to neutrality, aqueous extracts of dried
27 sumac fruit show bactericidal effects against Gram-positive and Gram-negative bacteria grown
28 as planktonic cultures, and this has been partly attributed to tannins ^{70,71}. The bactericidal effects
29 of bile are long established: bile salts disrupt bacterial membranes and this quality contributes to

1 host-mediated control of bacterial overgrowth in the gut ^{72, 73}. Interestingly, different fractions
2 of aqueous or alcohol extracts of pomegranate peel have different spectra of antibacterial
3 activity as measured in disk diffusion assays, and different authors present conflicting data on
4 the inhibitory effects of aqueous extract fractions ^{74, 75, 76, 77, 78, 79}. But we note that antibacterial
5 effects have been observed, even when using dried rather than fresh peel, and that pomegranate
6 peel extracts have also been reported to exhibit antiviral ⁸⁰ and antifungal ^{75, 76, 79} effects. We
7 were unable to find any relevant data on the *in vivo* effects of these four ingredients with regard
8 to treating soft-tissue wounds or infections.

9
10 The most interesting ingredients are undoubtedly honey and breast milk. Here we must add the
11 caveat that honey's sweetness must explain a large part of its ubiquity in pre-modern medicine
12 – a mouthwash containing gall, vinegar, and sumac would certainly be unpalatably bitter in the
13 absence of a sweetening agent! However, the antimicrobial and wound-healing properties of
14 honey have been relatively extensively studied, and honey preparations and dressings are used
15 in clinical settings (e.g. the UK NHS, ⁸¹). Honey's antimicrobial, immunostimulatory and healing
16 effects are attributed to its osmotic properties, an enzyme that produces hydrogen peroxide, bee
17 defensin, phenolics and flavonoids: while the most potent medical-grade honey (manuka honey)
18 also contains highly active compounds from the plant from which it is made, a range of European
19 honeys have been shown to have measurable antimicrobial effects *in vitro* ^{82, 83, 84, 85}. Four
20 Cochrane reviews find moderate to good evidence that honey reduces healing times for burns
21 and surgical wounds ^{58, 86}, weak evidence for honey helping to prevent mouth ulcers in cancer
22 patients ⁸⁷ and suggest that honey may be better than no treatment or placebo in relieving
23 childhood cough ⁸⁸.

24
25 Breast milk is similarly recognised as antimicrobial. It contains lysozyme, an enzyme which
26 destroys the structural peptidoglycan layer surrounding bacterial cells, and which is recognised
27 as a key component of innate immunity ⁸⁹. It also contains the protein lactoferrin, which
28 sequesters vital iron away from pathogenic bacteria. In acid conditions, lactoferrin is cleaved to
29 produce a peptide called lactoferricin, which has a direct bactericidal effect by binding cell walls

1 and triggering membrane damage^{89, 90, 91}. Lactoferrin can kill a range of microbes, including
2 antibiotic-resistant *K. pneumoniae* and *Candida* spp.; it can also enhance the sensitivity of these
3 microbes to modern antibiotics^{90, 91}. Clinical research into the antimicrobial potential of breast
4 milk or its component proteins is, however, lacking⁹². It is interesting that donkey milk is
5 suggested as a substitute (*Lylve of Medicynes*, fol. 16v) as the proteome of donkey milk is very
6 similar to that of human milk, and it contains appreciable amounts of lactoferrin and lysozyme^{93,}
7⁹⁴. Raw breast milk also contains a range of commensal bacteria, and if milk was not prepared in
8 such a way that these were killed, it is possible that the milk microbiome could contribute to its
9 anti-infection properties. One study found that colostrum could help alleviate infant
10 conjunctivitis because commensal bacteria in the milk outcompeted pathogenic bacteria when
11 applied to the eye⁹⁵.

12
13 There are several reasons why mixing these ingredients could represent a rational medical
14 decision. First, combining ingredients in a cocktail could increase efficacy against a particular
15 target microbial species by attacking several cellular targets at the same time, or allowing for
16 chemical activation of particular component molecules. For instance, acid pH promotes
17 proteolysis of lactoferrin into peptides called lactoferricins, which have greater antimicrobial
18 activity than the original protein⁹⁶. When milk is directly consumed this happens in the gut and
19 inside neutrophils, but we note that the sumac in *fistula in lacrimali* would decrease pH of the
20 recipe and potentially process lactoferrin into lactoferricins in the ointment itself. A further
21 possibility is that using multiple ingredients allows for contingency. We note that the available
22 evidence suggests for instance, that Gram-positive bacteria seem to show more sensitivity to
23 sumac than Gram-negative bacteria, whereas the opposite is true for vinegar^{67, 69}. Another type
24 of contingency which could be covered by combining multiple antimicrobial ingredients would
25 be protection against potential variation in the composition and quality of particular ingredients,
26 e.g. which *Boswellia* species was the available frankincense sourced from, and did this vary⁶⁴?
27 Finally, we must also consider the physical properties of each ingredient. Mastic, for instance, is
28 not well evidenced as an antimicrobial, but could act as a thickening agent to work other, active,
29 ingredients into an ointment suitable for topical application to an infection site.

1 **Discussion**

2

3 Turning a historical medical text into a database suitable for network analysis was achieved
4 through detailed interdisciplinary consideration of the text and its structure. The outcome of this
5 study was dependent on the collaborative efforts and exchange of knowledge between multiple
6 disciplines: medieval studies to translate and contextualise recipes, ingredients, and symptoms;
7 maths and computer science to organise and analyse the resulting database; and
8 microbiology/immunology to determine if “blind” statistically-driven results make sense from an
9 applied perspective.

10

11 As described, ‘normalizing’ or ‘cleaning’ a medieval data set for analysis with modern
12 computational tools is not a standardized methodology. The process of normalization presented
13 here is likened to a series of editorial decisions, such as preference for a Middle English spelling
14 over a Latin spelling or inclusion of plant parts in the terminology for a base ingredient, but
15 exclusion of preparation instructions. The normalized data set was accomplished by open
16 interdisciplinary dialogue using expertise in medieval language and scientific analytical
17 processing. In documenting our approach to this data set, we hope to add to other ongoing
18 conversations about structuring medieval data in a modern digital space.

19

20 This work demonstrates the possibility to use algorithms from complex networks to explore a
21 medieval medical data set for underlying patterns in ingredient combinations related to the
22 treatment of infectious disease. The preliminary results from this pilot study on a single medieval
23 medical text study speak to larger questions regarding medieval medical rationality in
24 constructing treatment recipes, and the application of historical medical knowledge to the
25 current search for novel antimicrobial drugs. Application of this methodology to an expanded
26 data set of ingredients drawn from multiple representative medical and surgical sources may
27 provide a foundation to begin to build evidenced answers to such questions.

28

29

1 **Data Availability Statement**

2 Our final electronic database of remedies and ingredients in the *Lylve of Medicynes* is provided
3 as **Supplementary Data**. All codes and algorithms used in network analysis are available via
4 C.I.D.G.'s website (https://warwick.ac.uk/fac/cross_fac/complexity/people/staff/delgenio/)
5

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13

14 **Author contributions**

15 E.C. conceived the project, created the medieval data set from the *Lylve of Medicynes*, and
16 provided interpretation of Middle English and medieval Latin terminology. C.I.D.G. conducted
17 the network analyses. F.H. conducted the literature review and interpreted the suitability of
18 the results for antimicrobial activity. E.C., C.I.D.G., and F.H. interpreted the data and wrote the
19 manuscript. All authors discussed the results and commented on the manuscript.
20

21 **Competing interests**

22 The authors declare that they have no competing interests.
23

24 **Materials and Correspondence**

25 General correspondence to Erin Connelly. Specific queries on network analysis to Charo I. del
26 Genio

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