

Taste shaped the use of botanical drugs

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Abstract

The perception of taste (here defined as a combination of taste, odour and chemesthesis) enables animals to find high-value foods and avoid toxins. Humans have learned to use unpalatable and toxic substances as medicines, yet the importance of taste perception in this process is poorly understood. Here we generate tasting-panel data for botanical drugs and apply phylogenetic generalised linear mixed models to test whether taste intensity, complexity and particular tastes can predict ancient Graeco-Roman drug use. We found taste to be strongly predictive of therapeutic use: botanical drugs with high therapeutic versatility have simple yet intense tastes, and 21 of 22 tastes predicted at least one therapeutic use. In addition to the common notion of bitter tasting medicines, we also found starchy, musky, sweet, cooling, and soapy tasting drugs associated with versatility. In ancient Greece and Rome, illness was thought to arise from imbalance in bodily fluids or humours, yet our study suggests that uses of drugs were based on observed physiological effects that are often consistent with modern understanding of taste receptor pharmacology and medicine.

eLife assessment

This **valuable** study uses phylogenetic linear mixed models in a Bayesian framework to explore the relationships between taste qualities and the therapeutic use of botanicals from the ancient Graeco-Roman vademecum. The evidence supporting the authors' conclusions is, however, **incomplete**, and the paper would benefit from a more exhaustive methodological description. The work is nevertheless of broad relevance to ethnobotanists, pharmacologists, and scientists working on drug discovery, particularly those interested in natural products.

Introduction

'A bitter pill to swallow' is just one of many common idioms referring to the taste of medicine, which from ancient to early modern times has relied mostly on botanical drugs (Touwaide, 2022). In ancient Greece and Rome, the expected effects of botanical drugs were explained by their taste (Jones, 1959 ; Einarson and Link, 1990 ; Jouanna and Allies, 2012 ). According to the prevailing medical theory, disease resulted from a disequilibrium between bodily fluids or humours including phlegm, blood, yellow bile and black bile; drugs affected the flow and balance of these

humours (Jones, 1959 [↗](#); King, 2013 [↗](#)). Theophrastus (300 BCE) attributed sweet the capacity to smoothen, astringent with the power to desiccate and solidify, pungent with the capacity to cut or to separate out the heat, salty with desiccating and irritating properties, and the bitter with the capacity to melt and irritate (Einarson and Link, 1990 [↗](#)). Other systems of medicine, such as Ayurveda and Traditional Chinese Medicine continue to classify *materia medica* according to taste qualities (Dragos and Gilca, 2018 [↗](#); Shou-zhong, 2018), and indigenous societies use taste cues to select botanical drugs (Casagrande, 2000 [↗](#); Leonti et al., 2002 [↗](#); Shepard, 2004 [↗](#)).

Botanical drugs are chemically complex and often used for a range of different health problems (Wagner et al., 2007 [↗](#)). Chemical complexity determines pharmacological complexity, since different chemicals interact with different targets (Gertsch, 2011 [↗](#)), and influence taste complexity and intensity (Spence and Wang, 2018 [↗](#); Breslin and Beauchamp, 1997 [↗](#); Green et al., 2010 [↗](#)). Today, some medicinal tastes and uses, such as bitter botanical drugs for stomach problems or astringent ones for diarrhoea are still recognised in Western herbal medicine (Wagner et al., 2007 [↗](#)). These observations establish a role for taste qualities in medicine. Yet, drug taste is largely irrelevant to modern medicine, aside from the pharmaceutical industry looking for ways to mask bad tastes.

Whether ancient systems of traditional medicine could be relevant to modern science is questionable. The theory of humours was the principal underlying concept of Western medicine from ancient Graeco-Roman times until the Industrial Age, but as an entirely discredited theory it might be expected that associated practices also lack scientific basis. However, we propose that the taste perception of botanical drugs links humoral theory to modern medicine. We hypothesize that underlying physiological effects mediated by taste receptor pharmacology co-explain the diverse therapeutic uses of botanical drugs in ancient times. If tastes, as determined by a modern-day tasting panel, do predict uses as recorded in ancient texts, this would be an unprecedented insight into how tastes were perceived and guided human behaviour in pre-scientific times. To test this hypothesis, we assessed the relationships between tastes, intensity of tastes and complexity of tastes with therapeutic uses of botanical drugs described in Pedanios Dioscorides' pharmacognostic compendium, *De Materia Medica* (*DMM*; Matthioli, 1967 [↗](#)-1970; Staub et al., 2016 [↗](#)), the foremost pharmaceutical source of antiquity. *DMM* compiles knowledge on the sourcing, preparation and therapeutic uses of botanical drugs traded and used in the Eastern Mediterranean region of the Roman Empire during the 1st century CE (Riddle, 1985 [↗](#)). We collected 700 botanical drugs described in *DMM*, (Supplementary Table 1) and used descriptors to represent their taste by a trained tasting panel scoring each botanical drug for presence and intensity of each taste (**Fig. 1** [↗](#), Extended Data Table 1, Supplementary Table 2). We classified the therapeutic uses associated with each botanical drug into categories according to affected organs, therapeutic functions, diseases, and symptoms described in *DMM* (**Fig. 1** [↗](#), Extended Data Table 2, Supplementary Table 1). By applying phylogenetic generalised linear mixed models (PGLMMs) to our data and a plant phylogeny (Zanne et al., 2014 [↗](#); see Methods) we tested whether taste, taste intensity and taste complexity predict recorded therapeutic uses whilst simultaneously accounting for the confounding effects of the shared uses and tastes of drugs from closely related species.

Results

Diversity of tastes and therapeutic uses

We used 22 taste descriptors to represent taste perceptions of 700 botanical drugs and identified 46 categories to represent therapeutic uses as described in *DMM* (**Fig. 1** [↗](#), Extended Data Table 2, Supplementary Table 1). A group of eleven panellists conducted 3973 taste trials (see Methods) and reported 10,463 individual perceptions of taste. The most frequently reported tastes were bitter (1556 reports; 39% of all assessed samples), herby/leafy (1146 reports; 29%), aromatic (795 reports;

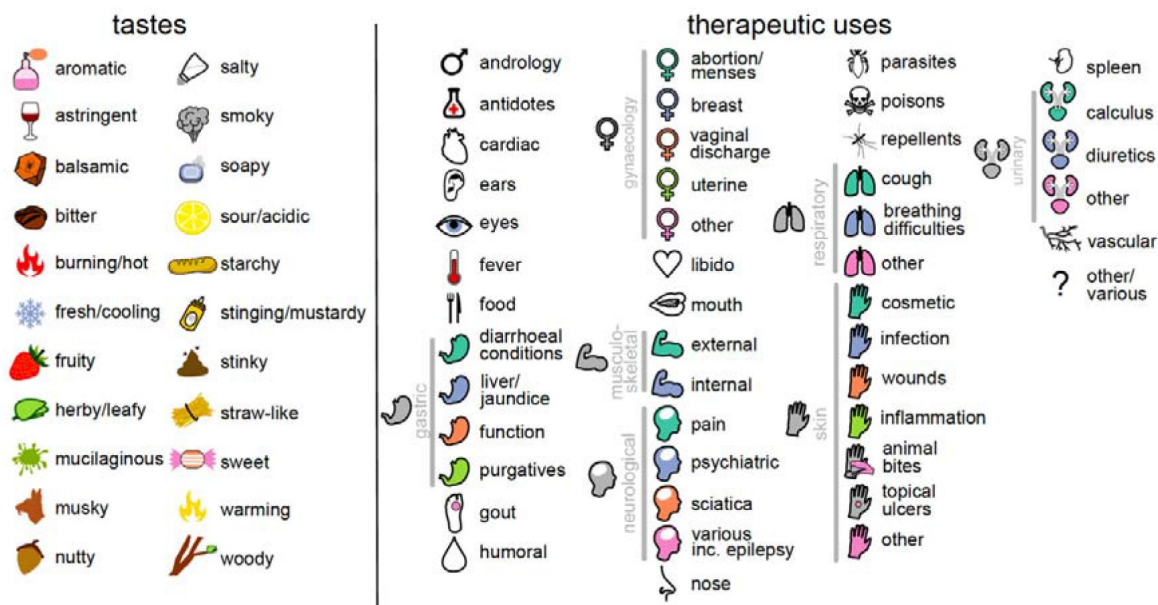
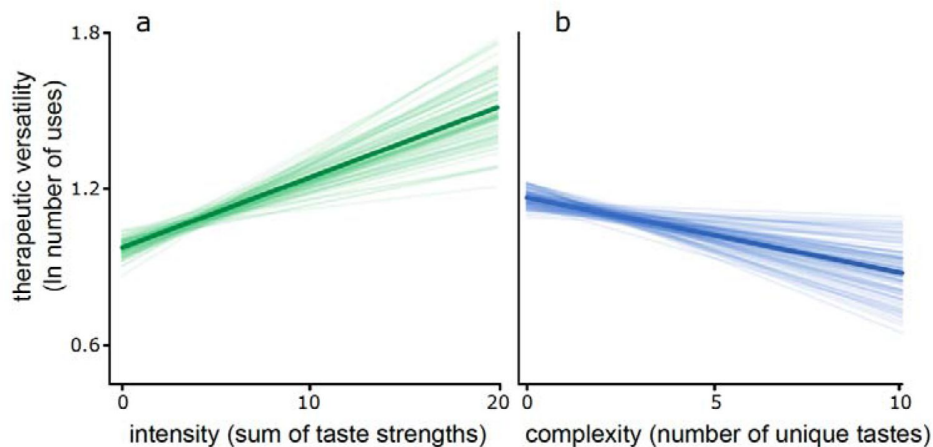


Fig. 1.

The 22 taste categories and 46 therapeutic uses studied here. Each taste and use is represented by an icon that is used throughout the manuscript.



Figs. 2a,b.

Strong-tasting drugs with relatively few perceived unique tastes predict therapeutic versatility a) Botanical drugs with more tastes are significantly positively associated with the number of therapeutic uses, $p_x = 0.008$. b) Botanical drugs with strong tastes are significantly negatively associated with the number of therapeutic uses, $p_x < 0.001$. For both relationships, we plot the mean regression line as estimated from the parameter estimates of our phylogenetic Bayesian regression analyses (dark line) along with 100 random samples of the posterior distribution (faded lines).

20%) and stinging (730 reports; 18%), while the least was mucilaginous (128 reports; 3.2%). Weak tastes were most frequently reported (5244 reports; 50%); 3899 (37%) of reports were of medium strength and 1320 (13%) were perceived as strong. Balsamic (69 reports; 24%), burning/hot (41 reports; 22%) and fresh/cooling (49 reports; 21%) were the tastes most often perceived as strong, while smoky (87 reports; 61%), sour (224 reports; 60%) and starchy (190 reports; 59%) the tastes most often perceived as weak. Panellists perceived between zero (e.g. *Anemone coronaria*, root) and ten (*Cinnamomum verum*, bark) individual tastes per drug. The mean number of therapeutic uses per botanical drug was 6.3 with the most frequent category of use being ‘gynaecology – abortion and menses’ (272 use records) and the least frequent ‘cardiac problems’ (seven use records).

Simple and intense tastes are associated with therapeutic versatility

We show that taste strongly predicts how many specific therapeutic purposes botanical drugs are used for (henceforth referred to as ‘therapeutic versatility’, Figs. 2a,b). Taste complexity (number of taste categories a botanical drug is scored for, see Methods) is negatively associated with therapeutic versatility ($p_x = 0.008$), whereas the relationship between taste intensity (the sum of all taste scores across all taste categories, see Methods) and therapeutic versatility is positive ($p_x < 0.001$). Importantly, the multiple regression framework we use allows for each trait to covary whilst simultaneously predicting their underlying relationship with therapeutic versatility.

Strong tastes predict specific therapeutic use and versatility

All models we analyse have strong phylogenetic signal (modal $h^2 > 0.9$, see Methods) which may have led to misleading impressions about medicinal tastes in the past in analyses which did not account for such statistical non-independence. Indeed, while we do find a significant association between bitterness and therapeutic versatility ($p_x = 0.011$), the most versatile drugs were not only the bitter-tasting ones; contrary to popular concepts of bad-tasting medicine (Mennella et al., 2013). We also find that starchy ($p_x = 0.013$), musky ($p_x = 0.002$), sweet ($p_x < 0.001$), cooling ($p_x = 0.022$), and soapy ($p_x = 0.001$) tastes were positively associated with therapeutic versatility (Fig. 3) while sour and woody tastes were negatively associated with versatility ($p_x < 0.001$ and 0.028, respectively) (Fig. 3).

We find that taste intensity and complexity were significantly associated with seventeen uses (Fig. 4). For seven individual uses, we find that more intense and less complex tastes increased the probability of a drug being used i.e., there was a preference for stronger and simpler tastes. Nine uses had a significant positive association with taste intensity and were predicted by stronger taste alone. A single use (various urinary problems) was uniquely significantly associated with increased taste complexity. We found that no therapeutic use was predicted by weaker flavours (Fig. 4).

Associations between specific tastes and specific uses

All therapeutic uses (with the exception of ‘gynaecology – other’) are significantly associated with at least one specific taste, whether positively (99 instances) or negatively (50 instances) and often with high magnitude of effect (Fig. 5, Supplementary Table 3). Thus, the presence or absence of several specific tastes significantly confer the ‘taste of medicine’ (less than 5% of their estimated parameter distribution crosses zero, $p_x < 0.05$) with high magnitude of effect (Fig. 5).

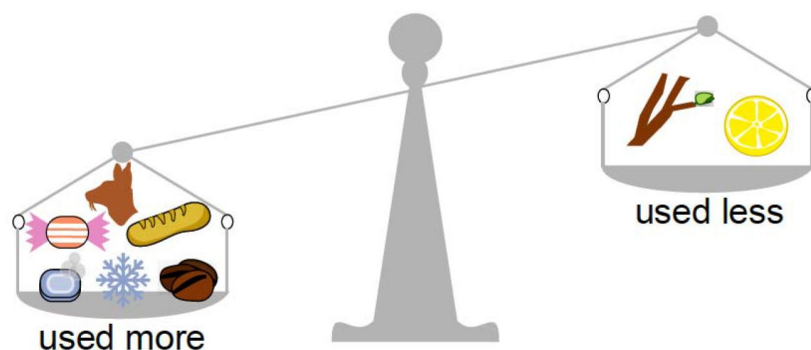


Fig. 3.

Diversity of use predicted by taste. The balance is a pictorial representation of how the strength of specific tastes contributes to significant associations with fewer or more uses.

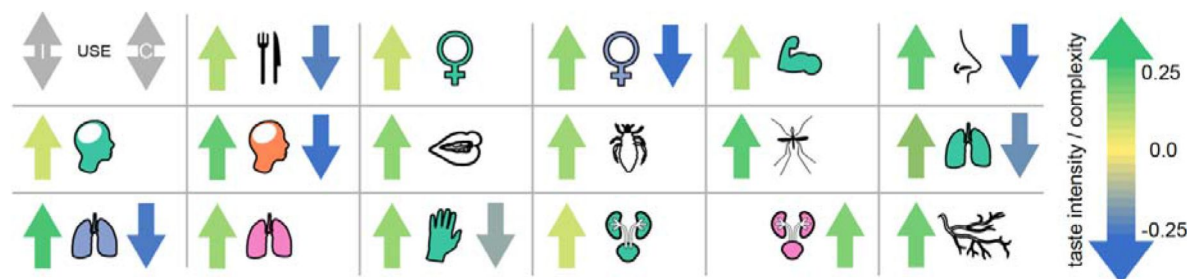


Fig. 4.

The relationship between individual therapeutic uses and taste intensity/complexity. Uses predicted by intensity and complexity of taste. An arrow is shown indicating the direction of any significant association ($p_x < 0.05$) with either taste intensity (left) or taste complexity (right). An arrow points upward if a drug is more likely to be used for a given purpose with increasing taste intensity or complexity. Arrows are shaded by the strength of the relationship (magnitude of estimated parameter, see scale).

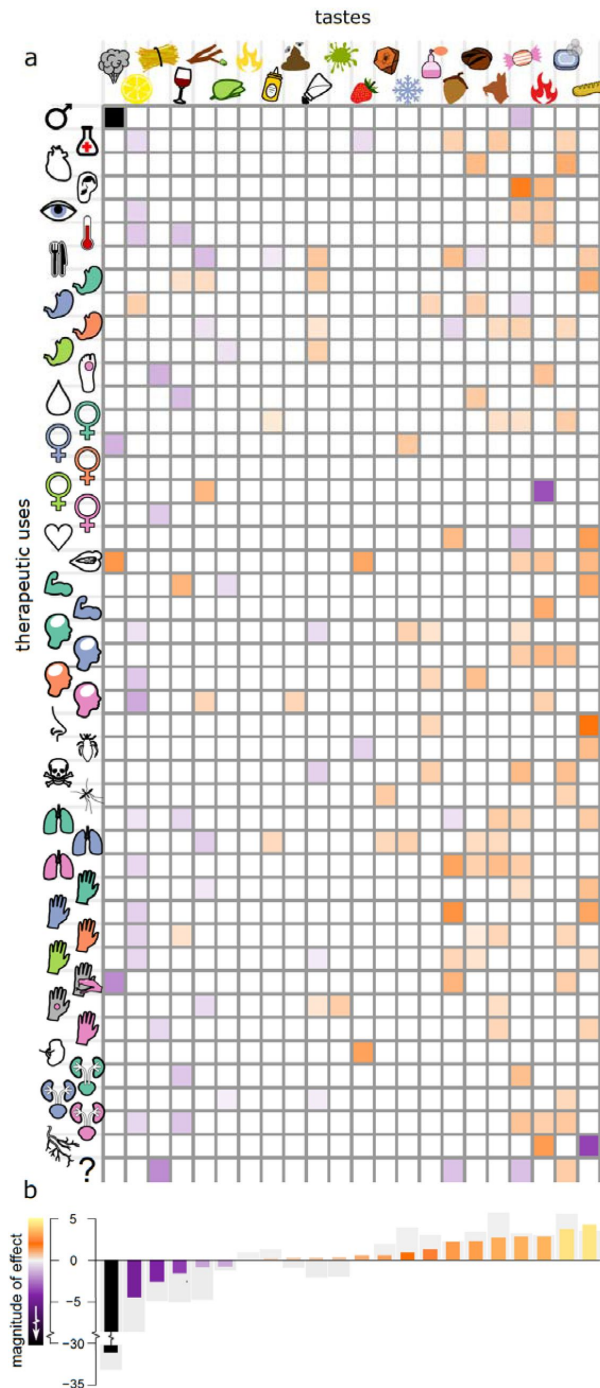


Fig. 5.

The magnitude of effect for 22 different specific tastes across 46 different therapeutic uses. a) A heat map showing the strength of the association between specific tastes (columns) and therapeutic uses (rows). Where we find a significant association between the strength of taste and whether a drug is used for a particular therapeutic purpose in our phylogenetic binary (probit) response model, the corresponding cell is coloured according to its estimated parameter value; see colour scale in panel (b). All other variables are zeroed and coloured white for visualization. b) An overall 'magnitude of effect' is calculated for each taste by summing the significant betas ($p_x < 0.05$, i.e. those displayed in the heat-map above) across all different uses and plotted as bars coloured by their magnitude; see colour scale alongside axis. The sum of all betas regardless of significance are shown as grey bars. Note that the large parameter estimate for "smoky" tastes is owing to the fact that no botanical drug with any smoky taste (scored by any participant) is ever used for androgynous therapeutic purposes and thus is poorly estimated in that model (see supporting information).

Unexpectedly, botanical drugs eliciting fewer but intense taste perceptions were more versatile (Fig. 2). Not only are taste intensity and complexity positively associated, but also taste complexity is popularly interpreted with a higher complexity of ingredients (Spence, and Wang, 2018). However, simple tastes can be associated with complex chemistry when intense tastes mask weaker tastes, or when tastants are blended (Breslin and Beauchamp, 1997; Green et al., 2010). For example, starchy or sweet tastes can be sensed when bitter and astringent antifeedant compounds are present below a certain threshold while salts enhance overall flavour by suppressing the perception of bitter tastants (Breslin and Beauchamp, 1997; Johns, 1990). On the other hand, combinations of different tastants or olfactory stimuli do not necessarily result in increased perceived complexity (Spence and Wang, 2018; Weiss et al., 2012). The detected relationships between intense taste and specific uses (Fig. 4) are consistent with a perceived link between strong tastes and strong effects that is probably innate (Ganchrow et al., 1983; Glendinning, 1994). Strong effects are needed in the case of unwanted pregnancy (abortifacients and emmenagogues), infestation by parasites and repelling harmful animals. Interventions in the case of psychiatric disorders also included drugs of very potent effect, such as the root of mandrake (*Mandragora officinarum*), or opium (*Papaver somniferum*). Both drugs are pharmacologically complex, but high concentrations of very strong-tasting alkaloids contribute to the perceived low taste complexity. Moreover, several botanical drugs are strong tasting because they are rich in essential oils, which have rubefacient and anti-inflammatory properties when applied topically (e.g. calamint (*Clinopodium nepeta*) or marjoram (*Origanum majorana*) leaves used to treat musculo-skeletal conditions).

Low therapeutic versatility of sour tasting drugs (12 negative associations; Fig. 3) may best be understood by considering the uses that these tastes were negatively associated with in our PGLMMs (Fig. 5). For example, the significant association between sour tastes and treatment of coughs ($p_x = 0.038$) and other respiratory problems ($p_x = 0.042$; Fig. 5, Supplementary Tables 3,4) might be related to coughing triggered by acid signalling in the airways (Kollarik et al., 2007). Moreover, acidic solutions and tissue acidosis are associated with inflammation and nociception while acid reflux is a common cause of chronic cough probably triggered by an oesophageal-bronchial reflex (Irwin, 2006). The negative associations of sour but also salty drugs with pain management ($p_x = 0.021$ and 0.022 , respectively; Fig. 5, Supplementary Tables 3,4), are probably related to the avoidance of nociception known by personal experience to result from the contact between acidic or salty solutions and skin or mucous membrane lesions.

The therapeutic versatility of drugs perceived as sweet (13 positive associations) and starchy (12 positive associations) (Figs. 3 and 5) is influenced by the many staples and edible fruits that are also used for medicine (e.g. protein-rich Fabaceae seeds were often perceived as having starchy tastes). The daily use of food items, generally with a low toxicity, has allowed for a more detailed comprehension of their postprandial effects, which arguably has led to a diversification of uses. Sweet sensations are known to induce analgesia (Kakeda et al., 2010) reflected here by a positive association of sweet tasting drugs with the treatment of pain ($p_x = 0.032$). Both starchy ($p_x = 0.001$) and salty tastes ($p_x = 0.002$) are associated with the treatment of diarrhoea and dysentery. Today, mixtures of glucose, starch and electrolytes are used to treat secretolytic and inflammatory diarrhoea caused by bacterial enterotoxins (Thiagarajah et al., 2015; Binder et al., 2014). These oral rehydration solutions exploit sodium/glucose co-transport by sodium/glucose co-transporter 1 in the small intestine and sodium absorption through sodium-hydrogen antiporter 2 stimulated by short chain fatty acids synthesized from starch by bacteria in the colon (Thiagarajah et al., 2015; Binder et al., 2014). Starchy tasting drugs are also positively associated with the treatment of topical ulcers ($p_x = 0.001$), skin infections ($p_x = 0.025$), other skin conditions ($p_x = 0.035$) and the topical treatment of venomous and non-venomous animal bites ($p_x = 0.001$) where plasters with lenitive properties might bring benefit. Moreover, fresh Fabaceae seeds contain

protease inhibitors (Birk, 1996; Wink and Waterman, 1999) such as trypsin and chymotrypsin inhibitors, which have the potential to inhibit bacterial proteases and control bacterial virulence during cutaneous infections (Frees et al., 2013; Culp and Wright, 2017).

Bitter was the most frequently reported taste sensation and showed nine significant associations with therapeutic use – including the negative association with foods (Fig. 5). Though many bitter compounds are toxic, not all bitter plant metabolites are (Glendinning, 1994; e.g., iridoids, flavonoids, bitter sugars). In part, this may be the outcome of an arms race between plant defence and herbivorous mammals' bitter taste receptor sensitivities, resulting in the synthesis of metabolites capable of confounding herbivore's perception of potential nutrients and mimicking tastes of toxins. Anti-inflammatory pharmacology of several bitter tasting compounds explains the positive associations with treatments of sciatica ($p_x = <0.001$). Bitter tasting drugs to treat sciatica derive from several disparate lineages containing a range of different anti-inflammatory compound classes such as iridoids (Viljoen et al., 2012; *Ajuga*, *Centaureum*), sesquiterpene lactones (Coricello, 2020; *Artemisia*, *Inula*), triterpene saponines (Gepdiremen et al., 2006; Wang et al., 2014; *Citrullus*, *Ecballium*, *Leontice*), and coumarins (Kirsch et al., 2016; *Opopanax*, *Ruta*) (Fig. 5, Supplementary Tables 1,2).

Generally, overarching explanations for versatility based on taste perception (Figs. 3,5) are not feasible. However, we hypothesize that many of the associations we found (Fig. 5, Supplementary Tables 3,4) are grounded in observed physiological effects mediated by taste receptor pharmacology (Chandrashekar et al., 2006; Palmer and Servant, 2006). Cumulative evidence suggests that together with transient receptor potential channels (TRPs), extraoral taste receptors and ectopic olfactory receptors form a chemosensory network involved in homeostasis, disease response and off-target drug effects (Foster et al., 2014; An and Liggett, 2018; Kang and Koo, 2012; Hauser et al., 2017; Clark et al., 2012). Bitter receptors, for instance, are located also in human airways where their activation mediates bronchodilation and the movement of motile cilia which leads to improved respiration and evacuation of mucus (Shah et al., 2009; Deshpande et al., 2010). Indeed, bitter showed positive associations with the treatment of breathing difficulties ($p_x = 0.027$) and other respiratory problems ($p_x = 0.004$). We find that drugs used to treat pain, including pain associated with breastfeeding (breast inflammation and lactation), are significantly more likely to have fresh and cooling tastes ($p_x = 0.01$ and 0.037 , respectively). This is likely owing to interactions with TRP channels (McKemy et al., 2002; Behrendt et al., 2004; Proudfoot et al., 2006; Memon et al., 2019). Specifically, the TRPM8 channel mediates cold stimuli and is known to be activated by cooling essential oil constituents producing analgesic effects (McKemy et al., 2002; Behrendt et al., 2004; Proudfoot et al., 2006). Cooling menthol and eucalyptol have been shown to act as counterirritants inhibiting respiratory irritation caused by smoke constituents (Willis et al., 2011) explaining the positive association between drugs used to treat breathing difficulties and fresh and cooling tastes ($p_x = 0.04$). The strong positive association of burning and hot drugs with oral applications for treating musculoskeletal problems ($p_x = 0.004$) might be related to analgesic properties mediated by desensitizing effects upon TRP channel interaction (Marwaha et al., 2016; Aroke et al., 2020). Burning and hot drugs are also positively associated with vascular problems ($p_x = 0.042$) including varices and haemorrhoids and can be explained by vasorelaxant effects transmitted by TRP channels on the endothelial cells (Montell, 2005; Pires and Earley, 2017). TRPV channels are also involved in kidney and urinary bladder functioning (Montell, 2005) and TRPV1-knockout mice urinate more frequently without voiding their bladder (Birder et al., 2002). Interactions with TRPV1 and other TRPV receptors might therefore explain the use of hot botanical drugs to treat other urinary problems including urinary retention, incontinence, kidney and bladder problems ($p_x = 0.047$).

The positive association of bitterness with liver problems and jaundice ($p_x = 0.002$) as well as with humoral management ($p_x = 0.004$) is explained by uses intended to purge and resolve bitter bile and seems to include what we call cholagogues and choleretics today, such as the roots of the

great yellow gentian (*Gentiana lutea*) or the herb of the common vervain (*Verbena officinalis*). Astringency and woody tastes showed positive associations with the treatment of diarrhoea and dysentery ($p_x = 0.029$ and $p_x = 0.019$) where there is a need for controlling the flow of fluids and for drying them up. Similarly woody tastes, often correlating with astringency (Scalbert et al., 1989) were also positively associated with the staunching of vaginal discharge ($p_x < 0.001$). Curiously, astringency showed positive associations with external applications for musculoskeletal conditions ($p_x = 0.001$) where drying internal bleeds and solidifying tissues and bones are needed. Negative associations of astringency emerged with conditions where the flux of humours, perspiration and the evacuation of liquids and mucus are treatment strategies, such as in fever ($p_x = 0.045$), humoral management ($p_x = 0.02$), urinary calculus ($p_x = 0.02$), other urinary problems (retention, incontinence, kidney and bladder problems, $p_x < 0.001$) and cough ($p_x = 0.002$). More obviously, the intake of salt aggravates fluid retention and oedema, and this is likely the reason why remedies for dropsy and oedema (diuretics) are negatively associated with salty tastes ($p_x = 0.023$). The concepts of humoral medicine may have derived from the observation that the appearance and consistency of bodily fluids during disease change and that they can be modified. Tastes of botanical drugs and their physiological effects probably explain the persistence of humoral medicine until the 19th century.

Looking forwards, our study highlights the potential of taste receptor pharmacology in the development of herbal medicines. Botanical drugs with taste profiles not aligned with those of their use group might represent drugs working beyond taste receptor interactions. Besides the associations discussed, other therapeutically relevant taste receptor functions might be involved in the associations currently lacking general explanations. Our approach demonstrates the power of phylogenetic methods applied to human behaviour and assessment of plant properties. We show that taste perception connects ancient knowledge with modern science, even though aetiologies were fundamentally different in Graeco-Roman times. Our study offers a blueprint for macroscale re-evaluation of pre-scientific knowledge and points towards a central role for taste in medicine.

Methods

Botanical fieldwork

A total of 700 botanical drugs (ca. 70% of the botanical drugs described in *DMM*) associated with 407 species were collected from the wild, cultivated in home gardens or purchased from commercial sources between 2014 and 2016. Plant collection included at least one voucher per species and one or more bulk samples of the therapeutic plant part (botanical drug). Latin binomials follow plantsoftheworldonline.org. Botanical vouchers were deposited at the herbarium of the University of Geneva (G) and the herbarium of the National and Kapodistrian University of Athens (ATHU). Bulk samples for sensory analysis were dried at 40–60° C and stored in plastic containers. Collection permits were obtained from the Greek Ministry of Environment and Energy (6Q8K4653Π8-AK7).

Data extraction from *DMM*

Information about the therapeutic uses (use record) of botanical drugs was extracted from Matthioli's 1568 edition of Dioscorides' *De Materia Medica* (Matthioli, 1967–1970; Staub et al., 2016). Therapeutic uses were classified into 46 categories defined by organ, therapeutic function, disease and symptom (Fig. 1, Extended Data Table 2, Supplementary Table 1). Each of the 700 botanical drugs was then assigned a binary variable defining whether it was used (1) or not (0) for each of the 46 therapeutic categories based on their descriptions in *DMM*. When the description in *DMM* permitted identification (Taxon ID, Supplementary Table 1) only to genus level (due to ambiguities or the possibility that different closely related taxa were subsumed) we chose one species as the representative for the whole genus (Taxon panel, Supplementary Table 1).

Unidentified taxa or taxa identified only to the family level were not considered in this analysis. The category ‘food’ includes only those drugs where edibility was specifically mentioned. As a measure of therapeutic versatility, we summed up the total number of therapeutic categories each botanical drug was used for.

Tasting panel

The chemosensory properties of each of the 700 botanical drugs were assessed experimentally by trained human subjects using the sensory analysis technique Conventional Profiling (ISO 11035) performed in accordance with the Declaration of Helsinki ([World Medical Association of Helsinki, 2013](#)) and the ethics guidelines of the Institute of Food Science & Technology (Institute of Food Science and Technology, 2020). The study design was approved by the Ethics Committee of the University Hospital of Cagliari (NP/2016/4522). Informed consent was obtained from all research participants. We used 22 taste descriptors to represent taste, flavour and chemosensory perceptions (**Fig. 1**, Extended Data Table 1, Supplementary Table 2). The evaluation took place at the Hospital of *San Giovanni di Dio* (Cagliari, Italy) during a period of five months in 2016. The panel consisted of eleven healthy Caucasian volunteers (six males and five females; age 30.7 ± 7.4). Working language was Italian. Based on a random subset of 40 (out of 700) samples, four initial training sessions were held to establish a consensus regarding taste descriptors. Hedonic (e.g. “good” or “bad”), self-referential (e.g. “minty” for mint) and infrequent descriptors were excluded to reduce subjectivity and to increase discriminatory power of the analysis. Synonymous and closely related descriptors (e.g., “astringent” and “tannic”) were pooled to reduce attribute redundancy. The qualities ‘astringent’ and ‘slimy’, which are considered tactile sensations ([Green, 1993](#)) were also included among the taste descriptors.

Samples (0.1 to 2 g) were randomly labelled with three-digit codes, randomly assigned to individual panellists, and presented in identical 125 ml plastic containers at room temperature. Panellists were instructed to spit, rinse their mouth with drinking water and to take a break before tasting the next sample. For each sensory trial a separate evaluation sheet was used. Quality intensities were evaluated on a 4-point ordinal scale: absent (0), weak (1), medium (2) and strong (3). Overall, 3973 individual sensory trials were conducted, with an average of 361 ± 153 trials per panellist and 5.7 ± 1.3 trials per botanical drug. From the ordinal data, two additional metrics were calculated to represent taste *complexity* (the total number of different taste categories a botanical drug was assigned to as non-zero by each panellist) and taste *intensity* (the sum of all taste scores assigned across all taste categories by each panellist).

Phylogenetic tree

A species-level phylogeny was constructed based on the supertree of land plants ([Zanne et al., 2014](#)). Missing species were attached to the most recent common ancestor of the respective genus or, if absent, the family. Branch lengths of added taxa were set to retain ultrametricity. From this modified tree a phylogeny comprising only analysed species was pruned out. Tree manipulations were made in R (R Core Team, 2016) using the packages APE and PHYTOOLS ([Paradis et al., 2004](#); [Revell, 2012](#)).

Statistical procedure

In order, to determine whether tastes and therapeutic uses were linked, we used two sets of phylogenetic linear mixed models implemented in a Bayesian framework within the R package MCMCglmm ([Hadfield, 2010](#)). The first set of models considered therapeutic versatility (number of categories of use) modelled as a zero-truncated poisson distributed response variable. We ran two models: (i) considering all 22 individual tastes as ordinal-scale independent variables and (ii) considering taste intensity and taste complexity as independent variables. Together, these models tell us whether any particular tastes as well as intense or complex tastes predict how often a drug is used for any therapeutic purpose. We then ran a second set of probit models which considered

each individual therapeutic use as a binary response. That is, we ran 46 models examining the relationship between individual tastes and therapeutic use (one for each use, where all tastes are included as independent variables) and an additional 46 to test the relationship between each therapeutic use and taste intensity and complexity.

The significance of all parameters is assessed using the proportion of the posterior distribution of parameter estimates that cross zero (p_x). If a parameter has a substantial effect on a model, we expect the distribution of parameter estimates to be shifted away from zero (i.e., $p_x < 0.5$). All models were run for a total of 500,000 iterations after burn-in, sampling every 5,000.

Phylogenetic signal was calculated using heritability (h^2) which has an identical interpretation to Pagel's lambda. All models showed high heritability values ($h^2 > 0.9$) demonstrating the importance of species' shared ancestry (h^2 ranges between 0 and 1, and values close to 1 indicate very strong phylogenetic signal). In all models we included plant part, panellist ID, and the phylogenetic matrix as random effects. We also included the number of days between sample collection and when each sample was tasted, in order, to account for any degradation of samples over time that may have affected taste. For all fixed factors in all models, we used largely uninformative priors (implementing a normal distribution with a mean of 0 and a variance of $1e10$). Residual variance was estimated using an inverse gamma prior ($V = 1$, $\nu = 0.002$). We used parameter-expanded priors (roughly uniform standard deviations) for all random effects including phylogenetic variance (Hadfield and Nakagawa, 2010 [\[link\]](#)).

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Author Contributions

All authors conceived the study. PS extracted the data from *DMM* and collected the botanical drugs. PS and LC conducted the tasting panel. JB conducted the statistical analysis. ML, JH and JB wrote the paper. JB produced the figures.

Competing Interest Statement

The authors declare no competing interest.

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Reviewer #1 (Public Review):

Summary:

The authors explored correlations between taste features of botanical drugs used in ancient times and therapeutic uses, finding some potentially interesting associations between intensity and complexity of flavors and therapeutic potential, plus some more specific associations described in the discussion sections. I believe the results could be of potential benefit to the drug discovery community, especially for those scientists working in the field of natural products.

Strengths:

Owing to its eclectic and somehow heterodox nature, I believe the article might be of interest to a general audience. In fact, I have enjoyed reading it and my curiosity was raised by the extensive discussion.

The idea of revisiting a classical vademecum with new scientific perspectives is quite stimulating.

The authors have undertaken a significant amount of work, collecting 700 botanical drugs and exploring their taste and association with known uses via eleven trained panelists.

Weaknesses:

I have some methodological concerns. Was subjective bias within the panel of participants explored or minimized in any manner? Were the panelists exposed to the drugs blindly and on several occasions to assess the robustness of their perceptions? Judging from the total number of taste assessments recorded and from Supplementary Material, it seems that not every panelist tasted every drug. Why? It may be a good idea to explore the similarity in the assessments of the same botanical drug by different volunteers. If a given descriptor was reported by a single volunteer, was it used anyway for the statistical analysis or filtered out?

The idea of "versatility" is repeatedly used in the manuscript, but the authors do not clearly define what they call "versatile".

The introduction should be expanded. There are plenty of studies and articles out there exploring the evolution of bitter taste receptors, and associating it with a hypothetical evolutionary advantage since bitter plants are more likely to be poisonous. Since plant secondary metabolites are one of the most important sources of therapeutic drugs and one of their main functions is to protect plants from environmental dangers (e.g., animals), this evolutionary interplay should be at least briefly discussed in the introductory section. Since the authors visit some classical authors, Paracelsus' famous quote "All things are poison and nothing is without poison. Solely the dose determines that a thing is not a poison" may be relevant here. Also note that some authors have explored the relationship between taste receptors and pharmacological targets (e.g., *Bioorg Med Chem Lett.* 2012 Jun 15;22(12):4072-4).

Reviewer #2 (Public Review):

Summary:

This is an unusual, but interesting approach to link the "taste" of plants and plant extracts to their therapeutic use in ancient Graeco-Roman culture. The authors used a panel of 11 trained tasters to test ~700 different medicinal plants and describe them in terms of 22 "taste" descriptors. They correlated these descriptors with the plant's medical use as reported in the *De Materia Medica* (DMM 1st Century, CE). Correcting for some of the plants' evolutionary phylogenetic relationships, the authors found that taste descriptors along with intensity measures were correlated with the "versatility" and/or specific therapeutic use of the medicine. For example, simple but intense tastes were correlated with the versatility of a medicine. Specific intense tastes were linked to versatility while others were not; intense bitter, starchy, musky, sweet, cooling, and soapy were associated with versatility, but sour and woody were negatively associated. Also, some specific tastes could be associated with specific uses - both positive and negative associations. Some of these findings make sense immediately, but others are somewhat surprising, and the authors propose some links between taste and medicinal use (both historical and modern use) in the discussion. The authors state that this study allows for a re-evaluation of pre-scientific knowledge, pointing toward a central role of taste in medicine.

Strengths:

The real strength of this study is the novelty of this approach - using modern-day tasters to evaluate ancient medicinal plants to understand the potential relationships between taste and therapeutic use, lending some support to the idea that the "taste" of a medicine is linked to its effectiveness as a treatment.

Weaknesses:

While I find this study very interesting and potentially insightful into the development and classification of certain botanical drugs for specific medicinal use, I would encourage the authors to revise the manuscript and the accompanying figures significantly to improve the reader's understanding of the methods, analyses, and findings. A more thorough discussion of the limitations of this particular study and this general type of approach would also be very important to include.

The metric of versatility seems somewhat arbitrary. It is not well explained why versatility is important and/or its relationship with taste complexity or intensity. Similarly, the rationale for examining the relationships between individual therapeutic uses and taste intensity/complexity is not well explained, and given that a similar high intensity/low complexity relationship is common for most of the therapeutic uses, it restates the same concepts that were covered by the initial versatility comparison. There are multiple issues with the figures - the use of icons is in many cases counterproductive and other representations are not clear or cause confusion (especially Figure 3). The phylogenetic information about the botanicals is missing. Also missing is any reference/discussion about how that analysis was able to disambiguate the confounding effects of shared uses and tastes of drugs from closely related species.