Traditional pharmaceutical recipes from a metabolomics perspective: Interviews with traditional healers in Mali and comparison with modern herbal medicines

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ABSTRACT

Scientific data on traditional pharmaceutical recipes are largely missing in the literature, despite the relevance of this information within the process of herbal medicine preparation; the chemistry of the finished product in fact, can be dramatically affected by the recipes adopted. A fieldwork on the topic of traditional pharmaceutical recipes was performed in Mali; traditional and modern practices to prepare herbal medicines were compared. The nascent scientific discipline named metabolomics identify a new paradigm in natural product chemistry. Under the umbrella of metabolomics, the traditional practices recorded during the field survey in Mali were interpreted from a biochemical perspective and presented in this paper.

Keywords: Medicinal plants, herbal medicines, traditional healer, ethnopharmacy, ethnochemistry, inter-kingdom communication.

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INTRODUCTION

Traditional medicine represents the major healing system in Mali and it is strongly based on the use of natural remedies prepared from medicinal plants (Coppo et al., 1992; Diallo et al., 2001; Diallo et al., 2006; Bah et al., 2007; Wilcox et al., 2008). Traditional healers in Mali are pharmacists and medical doctors at the same time. Most of the traditional healer’s heritage has been orally transmitted, strictly meaning that there are no local written records of the traditional heritage on herbal medicines. However, such knowledge is usually transmitted to the new generations by the direct involvement of the younger monitored by the elderly members in different practices related to the preparation of natural medicines. The traditional medical system has been passed on through generation during centuries but it is nowadays at risk of vanishing (Balique, 1998). Major causes for that are the modernizations of the social structure and the establishment of new monotheistic religions (that is, Muslim and Christianity) which strongly affect the local context and the relationships with the animal and plant kingdom. There is therefore an urgent need to preserve such knowledge.

The present work consists of a field survey based on participant observation and interviews with key informants performed in Bandiagara province, North of Mali. Bandiagara is a city of about 15000 inhabitants in the region of Mopti (14°21′N 3°37′W) within the Dogon plateau. This work area, a savannah plateau characterized by the presence of a cliff at the borderline with the Sahara desert, is mainly populated by an ethnic group named Dogon; the cultural heritage of this ethnic group is well documented especially in the anthropology literature (van Beek et al., 1991). The Dogon traditional medicine is well known in Mali and the Dogon traditional healers are important point of reference for all Mali population.

In 1968 the traditional medical system in Mali was legalised by the national government and, successively, the Division of Traditional Medicine was created with the aim to develop research centers of traditional medicine for each region of the country, each one specialised in one or more specific medical treatment. Additional
objectives of the Division were the valorisation and conservation of the intangible cultural heritage related with traditional medicine as well as the know-how regarding the medicinal use of local botanical resources. Successively the Department of Traditional Medicine, which still represent the institutional reference for the activity in this research field, was created within the University of Bamako.

One of the authors (L.P.) was constantly involved from 1977 to present in field research activities related with the topic of traditional medicine in different parts of Mali. Starting from 1986, for six years, L.P. took part in the planning and performing of an international collaboration project between Italy and Mali which allow the creation of the Regional Centre of Traditional Medicine (fifth region) in Bandiagara. Major objectives of this centre were to involve traditional healers and midwives in primary health care teams, and to harmonize the traditional system with the conventional one in particular in the psychiatric field. The first step was to perform the regional census of the local traditional healers recording their practices and knowledge related with traditional medicine. One major result of this six year project was the botanical identification of the main local medicinal plants used by the healers and their finished remedy (Coppo and Keita, 1990; Losi and Pisani, 1992; Keita and Coppo, 1993). At the end of this project, the Federation of the Traditional Therapists was funded in Bandiagara (FATTB), following the national strategies to organize a network of traditional healers all over the country. At the time of the work presented in this paper, the FATTB, which was the local reference institution for the present work, counted 32 local associations for a total of around 700 members of which around 130 were female.

Herbal medicines can be scientifically defined as complex mixture of natural products obtained from the plant kingdom (Phillips, 2007). In order to obtain such products, two major sequential steps are necessary: identification of the plant species and processing of the collected material. Ethnobotany is a well established scientific discipline concerning the investigation of the human traditional knowledge related with plants including their medicinal uses (Heinrich, 2003; Gurib-Fakim, 2006). However, most of the ethnobotanical reports on medicinal plants are focused on the botanical identification of the plant species used as sources of medicaments and related therapeutic indication, while very little has been investigated regarding the manufacturing of the remedies; this missing data is here defined as "traditional pharmaceutical recipes".

The term “Pharmaceutical Anthropology” concerns the study of the human behaviour with respect to (mostly modern) pharmaceuticals available to the public (Nichter and Vuckovic, 1994; Oldani, 2004). "Ethnopharmacy" is a recently defined discipline aimed at the interdisciplinary study of the perception and use of pharmaceuticals (especially traditional medicines, but not only), within a given human society (Pieroni et al., 2002; Pieroni et al., 2004). Both disciplines intend to cover very broad research fields in which the peculiar study of the traditional pharmaceutical recipe (definable "ethnopharmaceutics” as mentioned in Pieroni 2004 (Pieroni et al. 2004)) can be included but risk to be marginalized. Few authors (Agelet and Valles, 2003; Bonet and Valles, 2003; Ghorbani, 2005; Rigat et al., 2007) use the terms “Pharmaceutical Ethnobotany” in order to give emphasis to the traditional pharmaceutical recipe. These works describe the manufacturing procedures of the herbal medicines based on indirect observation; this means that the informant is asked to recall the recipe but the preparation itself is not directly observed by the interviewer. In this case, indirect observation could be very risky. Traditional pharmaceutical recipes can be a complex practice, extremely difficult to be described orally, and performing direct observation should be an advantage.

Metabolomics identify a new paradigm in natural product chemistry (Sumner et al., 2003). Metabolomics deals with the qualitative/quantitative identification of all the metabolites present in a crude mixture. If herbal medicines are complex mixtures of natural products, they have to be studied using the metabolomics tools now on (Dunn et al., 2005; Allwood et al., 2008). Plants are living organisms and their metabolome change constantly. On the other hand, herbal medicine is a relatively static mixture of the plant metabolites that is obtained following specific pharmaceutical procedures. Such procedures are adopted by both traditional and conventional pharmaceutical systems. The focus of this paper, which is part of a more general work on the topic of pharmaceutical recipes and herbal medicine (Politi, 2013), concerns the discussion on similarities and differences between traditional and modern herbal medicinal products.

METHODOLOGY

A fieldwork was performed among the Dogon plateau in Mali. Collection of the field data was achieved by the means of direct participant observation and semi-structured interview with key informants represented by the traditional healers. The collected field data were about the practices of medicinal plant collection, the manufacturing of herbal remedy, and the administration of the finished medicines.

Before performing the field work, the aim of the research project was presented and discussed in a meeting organized in Bandiagara in December 2007 with the representatives of the 32 associations of FATTB. General objective of the study were unveiled, explained and discussed, and an agreement about the scope of the investigation was reached. The oral consent to perform the planned field activity was obtained and the availability of the single traditional healers for the participation at the interviews was recorded. Consensus agreement was also requested before each interview.

Interviews were performed in repeated sections along a period of 3 months between 2007 and 2008 with the president of FATTB and with members of four local associations, including in particular the association of Bandiagara and the villages of Ama, Bodio and Ondugu. A total of 7 traditional healers (5 males and 2 females)
were directly interviewed. Moreover, two focus groups were organised involving a total of around 20 healers each time. Participant observation at the practice of collection of the plant material and preparation of the herbal remedies was achieved with 4 healers (3 males and 1 female) and related collaborators. Audio material and written records were collected and kept by the authors. The overall research fieldwork was supported by the non-lucrative association of volunteers named ORISS (Interdisciplinary Organization of Development and Health), which is actively working in Mali on the topic of traditional medicine, health and education since 1990 (within ORISS, L.P. is a founding member and member of the steering committee while M.P. is a member). Seed camp was based in Bandiagara at the local headquarter of ORISS.

RESULTS

Modern pharmaceutical recipes

Industrially prepared plant extracts can be distinguished in three classes as described in a monograph of the European Pharmacopoeia (2003) (Heinrich et al., 2004): Standardized extracts (extracts standardized to active constituents). Quantified extracts (extracts standardized to constituents that contribute to the activity). Other extracts (extracts standardized to lead compounds of unknown pharmacological relevance, which serve as quality markers). In all these cases, the quali/quantitative aspect of an herbal medicine is measured by referring to single or few molecules or classes of molecules. Herbal medicines, however, are complex mixture of natural products, although their quality is still officially assessed by measuring only few single chemical entities of the whole phyto-complex.

A list (M) summarizing the main steps adopted in the industrialised countries to prepare modern herbal medicines was prepared on the basis of the scientific literature (Heinrich et al., 2004) and following the general indications of the Italian and European pharmacopoeias. List M counts 9 steps which are:

1M) Taxonomic identification of the plant; 2M) Collection period (age of the plant, season, and eventually hour of the day); 3M) Cultivation condition or geographical origin if the plant is gathered from the wild; 4M) Selection of plant organs; 5M) Drying process; 6M) Manufacturing (e.g. powdering); 7M) Extraction (normally with organic solvents); 8M) Formulation; 9M) Storage condition.

List M served as reference for the comparison of traditional versus modern ways to prepare the herbal medicines.

Traditional pharmaceutical recipes

Semi-structured interviews with individual local healers were organized in order to collect information on the traditional way to prepare herbal medicine. Each session, lasting around 1 to 2 h, aimed at the identification of all the steps considered relevant for the process. The corresponding list (T) summarizing the necessary steps for the traditional herbal medicines preparation was also created by recalling previous experience of the author L.P. with the Dogon traditional healers on this research topic. Finally, list A was presented in the organized focus groups for further discussion and validation or confutation of each point of the list. List T counts 19 steps:

1T) Preliminary divination (to identify the exact plant and the specific treatment); 2T) Request of permission to the invisible owners of the plant; 3T) Day of the week useful for the collection (depending from the healer’s community of origin); 4T) Collection period (age of the plant, season, and hour of the day); 5T) Collection site (taking care also the physical and spiritual owners of the place); 6T) Keep silence before and after collection; 7T) Follow specific routes before and after collection; 8T) Ceremonial habits to be dressed or rather nudity during the plant collection; 9T) Request of permission to the plant before collection; 10T) Selection of plant organs (taking care also of their spatial orientation); 11T) Collection way (with or without specific utensils); 12T) Treatment of the wound caused to the plant (normally by applying some ground collected around the plant mixed with water); 13T) Compensation for the plant (sometimes leaving different objects near the plant including coins, food or particular seashells); 14T) Closing divination ceremony; 15T) Carrying of the plant material with specific utensils; 16T) Drying process (including specific site and conditions); 17T) Manufacturing (e.g. powdering); 18T) Formulation; 19T) Storage condition.

Observed traditional pharmaceutical practices

In our fieldwork, we observed three major ways to prepare aqueous extracts which cannot be described neither as infusion nor as decoction: 1) branches and leaves are boiled in 7 to 9 L of water in a argil jar and left boiling for around 20 min, then the water is cooled down and administered internally (drinking a cup) and externally (bath: the healer keep in his/her mouth and then spray out the aqueous extract directly on the patient, starting from the head) usually in the morning and sometimes also at night; the following days (3 for men and 4 for women) new water is added to the jar and the procedure is repeated. Then the treatment is normally concluded, but if it is necessary to go further, new plant material is added to the jar after removing the old one. 2) In a jar full of water (15 to 20 L) different organs of plants including branches, leaves, bark, roots as well as other materials including pieces of iron and stones are added and macerated in a sacred place sometimes for various days; water is added to restore the original level. 3) In a common metal pot, bark or roots are left 3 to 5 min in
tepider water and then orally administered; other times the powders made from these plant organs are directly added to a cup of water and drank as is.

The most general and common way observed concerning the preparation and storage of traditional herbal medicines consists of drying the plant material (in the shadow or under the sun depending on the plant) and successively powdering it with a wood or rock mortar and pestle. The powdering step helps to prepare complex formula, where two or more plants are mixed together to give the actual remedy. However, each single remedy is prepared following a specific pharmaceutical recipe. Depending on the recipe, the actual chemical content of the final product can differ strongly from the original metabolic profile of the plant itself. One of the most relevant steps observed which can trigger significant chemical modification regards the carbonisation of the dried plant material. Carbonisation consists of burning the plant material for few minutes and then adding water to stop the fire. For example, we observed the preparation of a traditional remedy which was obtained by using three different dried organs of a same plant (bark, small branch and fruit). These organs from the same plant were carbonised and successively powdered together. The fruit of a second plant species was added and powdered with the carbonised material (the final product was a black powder material that the healer administers in different ways including external bath, inhalation and infusion).

Other observed steps that could potentially affect the final chemical content of the traditional remedies include the powdering of different fresh plants species together rather than drying and powdering them separately and mixing at the end (this latter method represent the most common one observed to prepare powdered complex formula). In another case we observed the drying of powdered fresh aromatic plants in a specific room where flying insects were present (such insects were attracted from the aroma of the powdered material; this can potentially affect the chemical composition of the material, although from the healers point of view the vicinity of the insects to the drying material has exclusively a symbolic and spiritual grounds).

Finally, short prayers, chants and specific recommendations performed on the finished product (powdered plant) by the healers were also observed.

**DISCUSSION**

**Herbal medicines and pharmaceutical recipes: State of the art**

Discussion on the “theory of emergence” is affecting many area of scientific research including systems biology (Zhang, 2007) and bio-pharmaceutics (Testa and Bojarski, 2000). According to this theory, emergent structures are more than the sum of their parts because the emergent order will not arise if the various parts are simply coexisting; the interaction of these parts is essential. A question arises from the fact that herbal medicines are complex mixture of natural products. The so called mixture effects (Jones et al., 2012), which include synergisms for instance, can be seen as a typical emergent property of herbal medicines?

One of the most common ways to administer a traditional herbal medicine all over the world is through the preparation of aqueous extracts. This kind of preparation is defined ‘infusion’ or ‘decocion’ depending if the warm water is added to the plant (infusion) or if the plant is boiled in water for a period of time (decocion). However, in most of the phytochemical laboratory analysis, it is a common practice to extract the plant material using organic solvents such as chloroform or methanol. If a medicinal plant is consumed as infusion or decocion, the herbal extracts obtained with organic solvents cannot be used to justify the claimed medicinal property of the plant; in fact, the phyto-complexes obtained using organic solvents rather than water are largely different and the potential mixture effects can be lost (Politi et al., 2007). Moreover, traditional pharmaceutical recipes are many times ‘composed formulæ’ where different medicinal plants are mixed in a due dosage and sequence, and the potential mixture effects between metabolites from different plants is lost if the phyto/pharmacological investigation is applied on single medicinal plant species.

In this context, metabolomics assumes an important role dealing with the measurement of even slightly variation between single components in complex mixtures, with the aim to identify which variations are relevant for a due problem (Dunn et al., 2005). Such an approach can rapidly unveil potential mixture effects, therefore highlighting the emergent properties of the herbal extracts.

**Modern versus traditional herbal medicines**

Comparison of lists M and T highlights the higher complexity of the traditional preparation with respect to the modern one. Moreover, most of the steps listed in industrial procedure are included in the list for the traditional recipe, although some considerations are necessary. For example, point 1M (Taxonomic identification) is included in point 1T. However, from the traditional perspective the identification of the plant species sometimes is not enough; within the same botanical species, the collection of a specific individual plant was claimed to be essential. The “Preliminary divination” step allows the healer to identify exactly which plant has to be collected. This can occur through the direct ‘vision’ of the plant including its shape or its immediate environment, for instance. Although current
science is unable to explain how such vision can occur, from the metabolomics perspective it is plausible that the metabolic profiles of plants belonging to the same species can be different. This is because, as mentioned earlier, plants are living organisms and their metabolome change constantly depending, for example, on the environment condition. The ability of the traditional healer would be, therefore, to choose the best plant to be used as source of the remedy. To find out a possible parallelism with a modern context, the “choice of the best plant” is achieved essentially through the selection of the best cultivars of a due plant species.

Another example concerns points 10T and 4M, both about the selection of the plant organs. Apart from the identification of the specific organs such as leaves or roots for instance, care about their spatial orientation is taken in the traditional context. Again, metabolomics is the recommended approach to eventually unveil chemical differences between very similar mixtures of compounds, which would be the case of extracts obtained from the same organs attached in different position of a single plant as we were told by the Malian healers.

The above mentioned examples are in same way scientifically understandable. However, the traditional way to prepare the herbal medicines takes into consideration a large series of others pathways apparently much more difficult to understand. Such steps like 6T, 7T, 8T, 9T, 11T, 12T, and 13T are completely neglected within the industrialised approach to prepare modern herbal medicines mostly due to the lack of scientific evidences about possible influences of these steps on the chemistry of the finished product. These pathways involve a particular relationship between the healers and the medicinal plant which has to be collected.

On one hand, a great deal emerging from our interviews with traditional healers regards the meticulous procedure concerning, firstly, the self-preparation/self-purification before the collection of the plant; secondly, the demand for authorization to the plant’s owners for having the permission to go to collect the plant and thirdly, the negotiation with the plant itself before its collection. Communication between the healers and the plant including its invisible owners is central not only for the Malian healers here interviewed but it is a well known phenomenon also described in many other traditional cultures (Schultes and Raffauf, 1992) which surely merit further investigation as recently suggested by Zick and collaborators (Zick et al., 2009).

On the other hand, ecosystems of sender/receiving organisms communicating through chemicals are described in the scientific literature, and intra and inter-kingdom communication that works also through organic volatiles has been demonstrated (Weber et al., 2007). The sender produces some chemicals able to trigger intracellular reactions in the receiving organism. In other words, it is possible to state that “the sender needs to set up its metabolome in order to affect the metabolome of the receiving being”.

Such observation encourages a discussion about the human-plant communication phenomena observed also in our field study where, in order to obtain the optimal remedy, the healers need to follow specific rituals including negotiation with the plant itself. In terms of inter-kingdom communication, is this meaning that “the sender (healer) needs to set up its metabolome in order to affect the metabolome of the receiving being (plant)”? The plant therefore, will be collected when its metabolome is at a due state; is this meaning that only by following this procedure the right herbal medicine in terms of phytocomplex can be prepared? With these questions we would like to open the discussion about the potential biochemical consequences on the finished herbal medicines, of the rituals related with the collection of medicinal plants in traditional contexts.

Finally, the step of performing short prayers, chants and specific recommendations on the powdered plant material recall a recent investigation which indicate the different pharmacological efficacy of chocolate on human mood due to the “mind-matter interaction phenomenon” (Radin et al., 2007). This practice can be therefore also considered a relevant step of the traditional pharmaceutical recipes and merit further attention.

Conclusion

Traditional knowledge is every time more at risk of vanishing, therefore also how to prepare traditional herbal medicines. Compared to the ethnobotanical data, ethnompharmaceutical information is almost completely missing in the scientific literature. It seems to us that the deep knowledge held by traditional healers all around the world concerning the use of medicinal plants has been only partially investigated. The current approach in this research field is still about the search of new lead chemical entities from the traditional herbal medicines. However, the force of these medicaments could be kept in the mixture of compounds which hold the potential emergent property of the medicine. The study of the traditional pharmaceutical recipes could therefore represent a crucial point for future investigation on medicinal plants in the search of new drugs.

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