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ETHNOGRAPHY AND BIOASSAY: COMBINED METHODS FOR A PRELIMINARY SCREEN OF HOME REMEDIES FOR POTENTIAL PHARMACOLOGICAL ACTIVITY

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Summary

This paper introduces a combined set of anthropological and biological research techniques that allow a single researcher to conduct a field-based screen of ethnopharmaceutical resources, even under difficult field conditions. The results of one such screen, presented here, indicate that the most commonly used remedies in an ethnomedical system are also those most likely to contain active constituents. Several pragmatic and theoretical considerations deriving from these results are discussed.

Introduction

This article presents a combined set of ethnographic and biological research techniques that can be used to screen home remedies as potential pharmacological resources. The data in the article were gathered as part of a test of the hypothesis that the most commonly used home remedies in any folk medical system have the highest probability of containing pharmacologically active ingredients.

An earlier analysis of Mexican American ethnopharmacology (Trotter, 1981a) established a method for determining a list of the most common home remedies in use in a particular region. It also produced a listing of the most common home treated illnesses in the lower Rio Grande Valley of Texas. Further analysis of the data (Trotter, 1981b) demonstrated that both the types and frequency of home treated illnesses, and the remedies used to treat them, varied in relationship to the sex and age of the informant providing the data. It was, therefore, decided to use the basic remedy/ailment data derived for these articles to test the hypothesis mentioned above.

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Only the 25 most common remedies* were selected for testing for pharmacological activity.

The problem of conducting this test of hypothesis resolved into one of testing for potential biological/pharmacological activities in the remedies in a way that was also sensitive to the ethnographic data that has been collected. The most obvious solution was to conduct a general bioassay of extracts of the remedies. However, in choosing a particular bioassay system, several additional critical parameters had to be met. First, the bioassay had to be sensitive to a wide range of activities, rather than narrowly directed at a particular set of compounds or reactions. Second, the system had to be capable of being done in-house, extremely inexpensively, without the benefit of extensive equipment or sophisticated facilities, since the research was not externally funded†. And, finally, the process chosen had to replicate, to the extent possible, the conditions of preparation of the remedies identified in the original ethnographic research.

Fortunately, such a technique has just been developed (Meyer et al., 1982), using brine shrimp (*Artemisa salina* Leach) as the test organism. As stated by Meyer, "The organism is now suggested as a convenient probe for pharmacologic activities in plants which may be manifested as toxicity towards the newly hatched nauplii." The fact that many active plant principles are toxic to the young shrimp when they are subjected to elevated doses of the material makes them extremely useful for both general screening and for fractionation studies. The technique does not require asepsis, nor a difficult-to-maintain colony of animals, nor is it expensive‡.

Methodology

As suggested in the title, this article proposes a combination of ethnographic and biological procedures united into a single screening process. The ethnographic techniques are used to isolate the most promising set of

*The list in Table 1 is modified slightly from those reported in Trotter (1981a,b). Marijuana (*Cannabis sativa* L.) was not analyzed, since the author does not as yet have a Federal registration number for research on controlled substances. *Nopal* (prickly pear cactus; *Opuntia* sp.) was eliminated because it is normally not taken internally, nor used for antisepsis on wounds, according to the existing ethnographic reports. The list was filled out by adding the next two most common remedies.

†The authors would like to express their appreciation to Dr. Sammy Sides, Professor and Chair, Department of Biology, Pan American University. Dr. Sides made available the laboratory space, glass wear, pipettes, scales, and other equipment necessary to complete the bioassay. Without this help, the project would have been made extremely difficult.

‡The only purchases necessary for the completion of the project were the brine shrimp eggs, the remedies, the Ocean 50 and distilled water to make the artificial sea water, the three plastic pans, and the glue for the partition. The costs averaged less than $2.00 U.S. per remedy tested, excluding personnel costs, which, in this case, were donated to the project.

remedies (and the ailments they treat) in a particular ethnomedical system. The bioassay procedures are then used to screen the most promising remedies for the presence of pharmacologic activity.

The methodology for collecting and analyzing the ethnographic data has been previously described in detail (Trotter 1981a,b). Briefly, a sample of cases of the home treatment of illness is collected through the use of a standardized data collection form. The form, derived from extensive qualitative data collection of home remedies, records the remedy used, the illness treated, the method of preparation and administration of the remedy, a case example of its use, and demographic data on the informant providing the information. This information is then coded and processed for computer analysis, producing a list similar to the list of remedies in Table 1. Since each remedy is linked with a particular ailment, the combined screening procedure not only indicates the presence or absence of pharmacologic activity, it also provides a target ailment for that activity.

The bioassay procedure used to test for pharmacologic activity was based on that proposed by Meyer et al. (1982)*. Samples of the remedies being tested were either purchased locally at a *yerberia* (herb store), or were, in a few cases, picked from living plants growing in the area. The choice of whether the sample was prepackaged or fresh depended upon the most common selection process for the same remedy found in the ethnographic data. Thus, the process of acquisition of the remedies approximated the ethnographic condition as closely as possible. The remedies processed in their fresh form included *savila* (Aloe vera; *Aloe vera* L.), *hojas de naranjo* (orange leaves; *Citrus aurantium* L.), *ajo* (garlic; *Allium sativum* L.), *hojas de mesquite* (mesquite leaves; *Prosopis glandulosa* Torr.), and *cebolla* (onion; *Allium cepa* L.).

With one exception, the remedies were prepared by placing 28 g of the crude remedy in 500 ml of distilled water, bringing the water to a boil, and boiling for 5 min. The exception to this procedure was *Savila* (*Aloe vera* L.). The *Savila* was prepared by cutting and peeling a leaf, then steeping it in distilled water at room temperature (approximately 21°C) for 24 h. The aqueous extract thus formed closely approximates the ethnopharmacological use of *Savila* steeped in water to treat ulcers. Boiling the plant produced a solution with a rose-pink tint, which indicated that some significant change in the biochemistry of the plant may have taken place. Therefore, the boiled

*In Meyer's, and earlier experiments using brine shrimp, a drop of dry yeast suspension was added to each vial to feed the shrimp. However, control experiments comparing mortality between vials with and without yeast suspension (5 replicates of 10 shrimp for each condition) indicated there was no mortality at 24 h and 36 h in the vials with no yeast, compared with an 8% mortality in the vials with yeast. Therefore, the yeast suspension was eliminated from the protocol. Controls were also run adding 1 ml and 0.5 ml of distilled water, respectively, to 5 replicates of 10 shrimp for each condition, then adding artificial sea water to make 5 ml total volume. No deaths were recorded within 24 h, indicating that the change in salinity caused by the addition of the herbal teas was not responsible for the deaths recorded in the experiment.

solution was not tested. The teas, or aqueous extracts, were separated from the crude plant materials used to make them and tested at various concentrations with the brine shrimp.

A 100-ml aliquot of each of the solutions tested was reserved and frozen to test the weight of extract per unit volume of water for each of the remedies. However, the equipment necessary to reduce the extracts in vacuo is not yet available. Thus, it has not yet been possible to determine the LC_{50} values for the teas. This is the most significant departure of this project from the protocol set up by Meyers et al. However, since equal amounts of each remedy were prepared in equal amounts of solvent, the technique still produces a method for a relative comparison of the medicinal teas themselves. This modification appears acceptable, since it allows for a preliminary screening process that can be carried out under even the most difficult field research conditions; using equipment limited to scales, a shallow pan, rock salt (see Tarpley 1958), something to measure the water, a vial of brine shrimp eggs, a magnifying glass, and small glass containers for the brine shrimp replicates. However, since the LC_{50} levels would allow the activities of the remedies to be more universally compared with other known compounds, it is far more desirable to use an aliquot of each aqueous solution to determine the amount of extract per unit of water, if that is possible.

The brine shrimp were hatched from encysted eggs (Living World, Metaframe, Inc., Elmwood Park, NJ 07407, U.S.A.) in shallow rectangular plastic dishes ($20 \times 26 \times 8$ cm). A plastic divider, with 30–35 3-mm holes bored in it, was glued with epoxy resin approximately 7.5 cm from one end of each tray. The tray was filled with artificial sea water made with Oceans 50 Instant Sea (Jungle, Inc., Sanford, FL 32771) and distilled water. Approximately 50 mg of eggs were sprinkled on top of the water in the larger compartment, and the compartment darkened by wrapping several layers of thick construction paper around it. The narrow end of the tray was left uncovered. After 48 h, the general illumination in the room caused the phototropic nauplii to swim into the smaller compartment, leaving their shells and the unhatched eggs behind. Ten brine shrimp were then transferred to 7 ml vials, using a 20-cm disposable pipette (Scientific Products). The shrimp can be easily counted in the narrow section of the pipette, when it is held against an illuminated colored background.

Five replicates of ten shrimp each were exposed to each of four concentrations of the aqueous solution prepared from the remedy. The five replicates of solution A contained 1 ml of undiluted tea combined with 4 ml of artificial sea water. Solution B consisted of 0.5 ml of tea in 4.5 ml of artificial sea water, in each replicate. The base stock for Solution C was created by diluting 1 ml of the tea with 9 ml of artificial sea water. Each replicate for Solution C contained 0.5 ml of Solution C base stock and 4.5 ml of sea water. Solution D was made by diluting 1 ml of Solution C with 9 ml of artificial sea water, then adding 0.5 ml of D to 4.5 ml of artificial sea

water in each of the five replicates. There were virtually no deaths recorded in the replicates for Solution D for any of the remedies tested, thus only the percent of deaths for Solutions A, B, and C are reported. The vials were maintained under general room fluorescent illumination. Survivors were counted at the end of 24 h. Two methods of counting were compared; one, using a 3× magnifying glass, and the second by pipetting the shrimp out of the vial. Pipetting appeared to be the most consistently accurate method across researchers but also the more time-consuming of the two methods. One or two control vials of ten shrimp each in 5 ml of artificial sea water were set up for each set of four solutions tested. Where control deaths occurred, the data for that set of solutions were corrected using Abbott's formula (1925). The results of the exposure of the shrimp to the remedies is reported in Table 1.

Conclusions

The results presented in Table 1 support the hypothesis that the more commonly used remedies within an ethnopharmacological system are the ones most likely to contain pharmacologically active ingredients. In fact, there is a strong correlation between the rankings produced by ordering the remedies according to the percent of deaths caused by various solutions compared with the ranking produced by ordering the remedies according to the frequency with which they occur in the sample of cases (i.e. Trotter 1981a,b) that produced the list of remedies being tested. (Rank correlation coefficient $=0.66$; 23df; $P > 0.005$)*. This confirms that not only are the more common remedies the ones most likely to contain pharmacologic activity, but also that the relative strength of the pharmacologic activity appears to vary in relation to the frequency of use of the remedy in an ethnographic community. Both of these conclusions need further testing in other ethnopharmacological systems, but, even as preliminary findings, they have some major practical implications.

This new combined method of screening home remedies may be of particular significance for those situations where ethnopharmacological research has been neglected or poorly supported, to date. While it provides valuable information for more sophisticated pharmacological research systems, the method was designed to be simple enough to be carried out by a single researcher working in a field setting where ideal laboratory conditions do not exist. It meets this criterion. Yet, it also produces results of sufficient scientific accuracy and validity to make obsolete the past method of simply recording the illness, the treatment, and preserving botanical specimens for later identification.

In addition, this proposed screening process can be used to verify or

*Procedures for calculating the rank correlation coefficient (r') and t are according to Edwards (1958).

TABLE 1

REMEDIES RANKED BY PERCENT DEATHS

Remedy[a]	% Death Solution A	% Death Solution B	% Death Solution C	Rank by frequency in survey sample
1. *Ruda*	100	100	18	3
2. *Oregano*	100	100	8	9
3. *Yerba Buena*	100	84	2	5
4. *Savila*	100	64	0	2
5. *Manzanilla*	100	62	0	1
6. *Anise*	99	68	4	4
7. *Canela*	96	8	10	12
8. *Ajo*	60	14	8	10
9. *Albacar*	46	0	2	8
10. *Pelos de Elote*	33	3	3	11
11. *Cebolla*	22	2	4	25
12. *Estafiate*	18	2	0	6
13. *Hojas Se*	16	2	2	24
14. *Borraja*	6	2	2	14
15. *Flor de Azajar*	6	2	0	23
16. *Cenizo*	5	3	0	15
17. *Nogal*	5	2	4	19
18. *Rosa de Castillo*	4	0	14	16
19. *Hojas de Naranjo*	4	2	0	7
20. *Zacate de Limon*	2	0	0	22
21. *Romero*	1	2	1	13
22. *Golondrina*	0	2	2	21
23. *Comino*	0	0	2	18
24 *Hojas de Mesquite*	0	0	2	20
25. *Salvia*	0	0	0	17

[a]The binomial designations for the 25 remedios caseros, and the English translation for each are as follows: *Manzanilla* (camomile) *Matricaria chamomilla* L.; *savila* (aloe vera) *Aloe barbadensis* Mill.; *ruda* (rue) *Ruta graveolens* L.; *yerba aniz* (anise) *Pimpinella anisum* L.; *yerba buena* (mint) *Mentha spicata* L.; *estafiate* (wormwood) *Artemisia mexicana Willd.*; *hojas de naranjo* (orange leaves) *Citrus aurantium* L.; *albacar* (sweet basil) *Ocimum basilicum* L.; *oregano* (oregano) *Monarda menthaefolia* Graham; *ajo* (garlic) *Allium sativum* L.; *pelos de elote* (corn silks) *Zea mays* L.; *canela* (cinnamon) *Pulchea orodata Cass*; *romero* (rosemary) *Rosmarinus officinalis* L.; *borraja* (borrage) *Borago officinalis* L.; *cenizo* (purple sage) *Leucophyllum texanum* Benth.; *nopal* (*prickly pear cactus*) *Opuntia* sp.; *Rosa de Castillo* (rose) *Rosa centifolia*; *salvia* (sage) *Salvia leucantha* Cav.; *hojas de mesquite* (mesquite leaves) *Prosopis glandulosa* Torr.; *nogal* (pecan) *Carya illonoenis* Koch; *comino* (cumin) *Arracacia atropurpurea* Benth. et Hook; *golondrina* (swallowwort) *Euphorbia prostrata* Ait.; *sacate de limon* (lemon grass) unidentified local plant; *el azajar* (orange blossoms) *Citrus aurantium* L.; *hojas se* (American tar bush) *Fluorensia cenura* DC.; *cebolla* (onion) *Allium cepa* L.

disprove existing folk knowledge and rapidly provide a source of alternate pharmaceutical resources for physicians and other health officials in areas where economic conditions and/or the cultural environment make the use of manufactured pharmaceuticals more difficult. For example, using the

findings of this study, it would be possible for a physician to recommend *te de manzanilla* (camomile tea) for colic or other childhood stomach complaints, instead of more expensive over-the-counter or prescription medicines. For the appropriate patient, this could provide an economically and culturally feasible alternative, while the physician can be assured that this type of alternative was derived from scientific screening of the herb, rather than folk wisdom, which many physicians find suspect.

These findings may also have an important impact on the theoretical under-pinnings for the whole of ethnopharmacology. While scientific testing was not carried out by the discoverers of the various folk remedies, there was an obviously successful empirical system in operation. This suggests a second hypothesis: that, within the overall variation the treatments available for a specific illness, the most commonly occurring remedies should also be those that are the most pharmacologically active. This hypothesis will shortly be tested using the existing sample of cases and the brine shrimp bioassay techniques.

Finally, the findings of this research project also suggest that the function of magical remedies within folk medical systems needs to be re-assessed. Our ethnographic data indicates that many of the remedies in Table 1 are not only used as medicinal teas, they are also significant elements in the magical curing rituals performed by *curanderos* (folk healers) working in the area. It is therefore possible that an as yet undiscovered link exists between the pharmacologic (not hallucenogenic) activities of plants and their selection as constituents of magical rites. This question will also be explored in future research efforts.

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