CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

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8.1 Screening of plants for bioactive agents

The screening of bioactive agents from plants is one of the most intensive areas of natural product research today, yet the field is far from exhausted. Sandberg and Bruhn (1979) reported that only around 10% of all plants had been investigated in detail for bioactive agents. For this reason alone it could be argued that further investigation on *Helichrysum* species is worthwhile.

In their computer analysis of data on worldwide research on plant derived drugs, Farnsworth and Bingle (1977) reported that 1650 compounds of novel structure were reported from plants in the year 1975 alone, while, 3077 compounds of known structure were also reported from other plants in the same year. In the same year, more than 400 patents were issued for substances isolated from higher plants. 325 compounds were reported for having one or more types of biological activity in systems having relevance to their potential use as drugs. Out of the 325 compounds, 93 were of novel structures reported for the first time and 232 had previously known structures.

Another reason for screening *Helichrysum* species for bioactive agents is that by isolating such agents it is possible to demonstrate that the reported medicinal activity of the plant is a reality. The fact that the antimicrobial activity of *H. caespititium* has been shown to be due to a particular chemical compound makes detailed pharmacological and other academic studies possible. It is not always possible, however, to isolate the bioactive agent in a plant and cases are known where attempts at such isolation have proved fruitless, even though an extract of the plant may be active, for example, a plant containing highly unstable compounds (Harborne,1992). Nevertheless, such attempts should continue as characterization of the active agent enables structure-related activity studies to be carried out, leading to the possible synthesis of a more potent drug with reduced toxicity. The

mode of action of the whole plant or plant part producing the biological effect can also be better investigated if the active principle is characterized. Often such studies lead to a better application of the drug, a better formulation into appropriate dosage forms, and may even lead to a better understanding of the disease itself.

The results of this study confirm that the co-occurrence of acidic phenolic hydroxyls and lipophilic residues is an important chemical feature for the expression of antifungal activity (Tomas-Barberan et al., 1990). The same requirements for a hydroxyl group and a degree of lipophilicity are also found in the simpler commercial phenolic fungicides such as p-pentyl phenol, dinitrophenol and pentachlorophenol (Laks and Pruner, 1989). These compounds exert their toxicity through the acidity of the hydroxyl group by uncoupling oxidative phosphorylation (Laks and Pruner, 1989). It is evident from Table 3.1 that over 96% of the species tested are worthy of closer investigation and isolation of their active compound.

Some drug registration bodies, like the Food and Drug Administration of the USA, the Dunlop Committee of the UK and the Republic of South Africa Patent Act of 1978, require the information on the structure(s) of the active agent(s) in a vegetable drug before it can be approved for general administration (Farnsworth, 1980). The pure compound is required to assess the possible lethal toxicity or side-effects (chronic and acute toxicity) of the drug. It was recently reported in the Los Angeles Times that medicines kill about 100 000 people each year:

> "More than 100,000 Americans are inadvertently killed every year by prescription drugs– one of the leading causes of death in the country. Some people die of drug reactions that are completely unexpected, the stuff of dramatic headlines and heavy lawsuits. But the majority of such deaths are preventable, the result of mistakes or confusion about dosage, dangerous drug interactions from mixing medications or known allergic reactions. Some patients, especially the elderly, die because their liver or

> kidneys are so weakened by other illnesses that they cannot effectively process new drugs....
>
> Over the past two years, the Food and Drug Administration has recalled five drugs and moved to re-evaluate several others, including the diabetes drug Rezulin, and the Parkinson's medication Tasmar, both of which have caused instances of liver failure."

Academically, the isolation of bioactive agents helps to provide chemotaxonomic evidence for the classification of genera or species, especially those whose classification on morphological grounds alone is unclear. When two plants are taxonomically identical but do not produce the same chemical constituents (either quantitatively or qualitatively) they are classified into chemical races. This difference can be due to genetic variation and is not merely because of a change in the environment or a difference in their geographical location (Soforowa, 1982; Harborne, 1992; Gillian *et al.*, 1998). For example, at least three chemical races of *Ocimum gratissimum* have been established from the major constituent of the volatile oil they produce: these are the thymol type, the eugenol type, and the citral type (Soforowa, 1982). The same plant may have been given different names by different taxonomists. For example: *Hunteria umbellata* Roxb = *Picralima nitida* Th. and Hel. Durr. and *Catharanthus roseus* G.Don = *Vinca rosea*. L.

The introduction of chemotaxonomy into plant systematics also complicates this problem further because of the repeated change of plant names or their families along with the change in name to the appropriate taxonomist. Phytochemists also often fail to confirm the identity of the plant they are investigating and the wrong plant names therefore sometimes get published along with their chemical findings. As a result, the chemical agent reported cannot be tallied in future with the biological activity claimed for the plant. This latter problem is generally disappearing, however, because it is now accepted practice to deposit a voucher specimen (with a voucher number) of any plant investigated in a recognized herbarium for future reference.

It is important to realize that the products of primary metabolism are usually innocuous, except for some toxic proteins, and were therefore of little interest to us investigating drug activity in *Helichrysum* species. Secondary metabolites such as alkaloids, acetophenones, phloroglucinols, flavonoids etc., are usually biologically active in animals and man. The isolation of bioactive agents from plants in general, can be grouped into two broadly fundamental procedures, namely:

(1) Biological screening, i.e. searching for a specific physiological effect.

(2) Phytochemical screening, i.e. randomly searching for bioactive compounds

8.2 Scope of research

Research in the field of Helichrysum species, was multi-disciplinary in approach and objectives were

set. Our objectives, among others were, to:

(a) Identify the agents in Helichrysum species which may possibly be used to produce useful drugs and,

(b) Quantify (mg/ml) the compounds and determine the MIC

The problems to be solved will depend on the overall objective to be achieved. It must be borne in mind, however, that in traditional medicine, medicinal plants are esteemed for their occult powers as well as their therapeutic effect.

Two major approaches were made in the investigation of the Helichrysum species studied:

(a) Performing a purely scientific exercise which may or may not result in the isolation of bioactive agents and,

(b) Recommending further investigations into the incorporation of useful and harmless Helichrysum species (pharmacological and therapeutical) into the modern health care system.

8.3 Acceptance of the hypotheses

The following hypotheses of this study may be accepted, namely, that:

(a) Crude extracts *Helichrysum* species exhibit significant antimicrobial activity and properties that support folkloric use in the control of bacterial and fungal related infections as broad spectrum agents. Secretions from leaf trichomes exhibit signicant antibacterial activity and properties that support folkloric use.

(b) Epicuticular extracts of *Helichrysum* species exhibit a relatively higher antimicrobial activity (minimum inhibition concentration (MIC)) compared to homogenized extracts. Antimicrobial compounds are probably sequestered in trichomes in *H. caespititium*. Shaken extracts proved to be more active than homogenized extracts.

(c) The previously studied *H. caespititium*, may in addition to the compound isolated (caespitin) by Dekker *et al*., (1983) contain novel constituents that can be discovered by bioassay directed fractionation methodology. This hypothesis can be accepted on the basis that a new phloroglucinol derivative, caespitate, was isolated in addition to the previously isolated caespitin.

(d) Mixtures of several closely related structures of the same class are produced by the plant and it is likely that synergism might occurs. A synergistic antibacterial bioassay demonstrated that the combination of caespitate and caespitin enhanced activity. The hypothesis can therefore, be accepted.

(e) Persistence on the use of *H. caespititium* among people of urban and rural communities in South Africa is good evidence of its non-toxicity and efficacy. The hypothesis can be accepted as caespitate proved to be non-toxic at biologically active concentrations.

8.4 REFERENCES

CORDELL, G.A. 1981. Introduction to the alkaloids: Biogenetic approach. John Wiley and Sons. New York.

FARNSWORTH, N.R. and BINGEL, A. S. 1977. Problems and prospects of discovering new drugs from higher plants by pharmaceutical screening. H. Wagner and P. Wolff, eds. In: New Natural products and Plant Drugs with pharmacological or Therapeutical Activity. Springer Verlag, Berlin. pp 1-22.

FARNSWORTH, N.R. 1980. Rational approaches applicable to the search for and discovery of new drugs from plants. *First Latin American and Caribbean Symposium on naturally occuring Pharmacological agents.* Havana Cuba. pp 23-28.

GILLIAN, A., COOPER, D. and MADHUMITA, B. 1998. Role of phenolic in plant evolution. *Phytochemistry* 49: 1169-1174.

HARBORNE, J. B. 1973. Phytochemical methods: A guide to Modern Techniques of Plant analysis. Charpman and Hall. London. pp. 279.

HARBORNE, J.B. 1992. Chemicals as defence agents. In: Introduction to ecological Biochemistry. Harborne, ed. Academic Press. Harcourt Brace and Co Publishers. New York. pp 131-158.

HILLIARD, O. .M. 1983. In: Flora of Southern Africa (Asteraceae). Vol. 33. Asteraceae. Lo.eistner, O.A. ed. Botanical Institute of South Africa. pp. 61- 310.

MITSCHER, LA, PARK, V.H., CLARK, D.. and BEAL, J.L. 1980. Antimicrobial agents from higher plants. *Journal of Natural Products* 43:259.

MITSCHER, LA and REGHAR RAO, G.S. 1984. In: Natural Products and Drug Development. Krogsgaard-Larsen, S. Brogger Christensen and H. Kofod, eds.. Munksgaard, Copenhagen. pp 193- 212.

SPECIAL CORRESPONDENT. PRETORIA NEWS. Tuesday, May 11, 1999.

SANDBERG, F. and BRUHN, J. G. 1979. Screening of plants for biologically active substances. In African Medicinal Plants E.A. Soforowa, ed. University of Ife Press. Lagos. pp 119.

SOFOROWA, E. A. 1982. Methods of obtaining information on medicinal plants. In: Medicinal plants and Traditional Medicine in Africa. Soforowa, E.A. ed. John Wiley and Sons. Chichester. New York. pp. 114-125.

TOMAS-BARBERAN, F.A., INIESTA-SANMARTIN, E. and TOMAS-LORENTE, F. and RUMBERO, A. 1990. Antimicrobial phenolic compounds from three Spanish Helichrysum species. *Phytochemistry* 29: 1093-1095.