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Perspective paper

Medicinal plants and antimicrobial activity

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Abstract

In the present paper, we analyze the past, present and future of medicinal plants, both as potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infection agents. In the past few decades, the search for new anti-infection agents has occupied many research groups in the field of ethnopharmacology. When we reviewed the number of articles published on the antimicrobial activity of medicinal plants in PubMed during the period between 1966 and 1994, we found 115; however, in the following decade between 1995 and 2004, this number more than doubled to 307. In the studies themselves one finds a wide range of criteria. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds such as alkaloids, flavonoids, sesquiterpene lactones, diterpenes, triterpenes or naphtoquinones, among others. Some of these compounds were isolated or obtained by bio-guided isolation after previously detecting antimicrobial activity on the part of the plant. A second block of studies focuses on the natural flora of a specific region or country; the third relevant group of papers is made up of specific studies of the activity of a plant or principle against a concrete pathological microorganism. Some general considerations must be established for the study of the antimicrobial activity of plant extracts, essential oils and the compounds isolated from them. Of utmost relevance is the definition of common parameters, such as plant material, techniques employed, growth medium and microorganisms tested.

Keywords: Antimicrobial activity; Medicinal plants; Antibacterial; Antifungal

1. Introduction

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. For example, the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (Heinrich et al., 2004). That being said, it has generally been the essential oils of these plants rather than their extracts that have had the greatest use in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin. In the case of *Melaleuca alternifolia*, for example, the use of the essential oil (tee tree oil) is a common therapeutic tool to treat acne and other infectious troubles of the skin (Vanaclocha and Cañigueral, 2003).

In the present paper, we analyze the past, present and future of medicinal plants, both as potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infection agents.

2. Past

In the past few decades, the search for new anti-infection agents has occupied many research groups in the field of ethnopharmacology. Recio et al. (1989a) reviewed the most

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relevant articles on this subject published between 1978 and 1988, compiling a list of 75 species in which the authors had established the activity of the extract along with both the spectrum of and the principles responsible for this activity. In general, the review showed that phenolics are the predominant active chemical in these plants, with Gram positive bacteria being the most sensible germs. The review also revealed, however, the major problem with this type of research, namely the lack of uniformity in the criteria selected to study the activity. This has in the past lead to relevant contradictions between the results obtained by different groups and even for the same authors studying the same sample with different methods (Pellecuer et al., 1976). To try to solve this important problem, Ríos et al. (1988) published a review of the experimental methods used for studying the activity of both plant extracts and essential oils to date. They proposed the use of diffusion methods for studying polar compounds of small or medium molecular size and determining the antimicrobial spectrum because this method allows researchers to test different compounds against one microorganism. The solid dilution method was recommended for studying polar and non-polar substances as well as all types of complex extracts. This method is especially good for determining the relative potency of extracts or essential oils and for establishing their antimicrobial spectrum as it facilitates the use of different strains against the extract on the same plate. Finally, the liquid dilution method is the best way to establish the real potency of a pure compound, but solubility is an obvious requisite.

The protocols proposed by Ríos et al. were widely accepted by many research groups as can be seen by the numerous times the paper has been cited. The proposed methods have been used principally in the study of plant extracts of medium or no polarity. The article has been most often cited in articles published in the *Journal of Ethnopharmacology* (23% of all the citations of the article), followed by those appearing in *Pharmaceutical Biology* (12%), but all told it has been cited in 42 journals, some of them extremely specific.

To examine the problem of a lack of unified criteria more in depth, we can look particularly at the study of the antimicrobial activity of essential oils. Janssen et al. (1987) reviewed the characteristics of these kinds of complex mixtures as well as the techniques used for studying them and concluded that the results are difficult to compare as the test methods differed so widely. They thus proposed that in future the strain number of the tested microorganism, the composition of the essential oil and the conditions under which it was obtained be included as an integral part of the report.

Recently, Kalemba and Kunicka (2003) reviewed the classical methods commonly used for the evaluation of the antibacterial and antifungal activities of essential oils, including the agar diffusion method (paper disc and well), the dilution method (agar and liquid broth) and the turbidimetric and impedimetric monitoring of microorganism growth in the presence of tested essential oils to draw conclusions about the factors that influence the in vitro antimicrobial activity of essential oils and their mechanisms of action. Moreover, they include an overview of the susceptibility of human and food-borne bacteria and fungi towards different essential oils and their constituents. The most relevant ones, which include the essential oils of thyme, origanum, mint, cinnamon, salvia and clove, have antimicrobial properties.

3. Present

When we reviewed the number of articles published on the antimicrobial activity of medicinal plants in PubMed during the period between 1966 and 1994, we found 115; however, in the following decade between 1995 and 2004, this number more than doubled to 307. Focusing the search specifically on the antimicrobial activity of essential oils, 187 references appeared in PubMed between 1971 and 2005; however, in a search processed by the ISI web of knowledge, the number of references for essential oils was much higher (323 between 1986 and 2005). These figures demonstrate the increased interest for this type of research among that portion of the scientific community dedicated to the investigation of the medicinal properties of plants. In the studies themselves one finds a wide range of criteria. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine (Ngwendson et al., 2003), essential oils (Alma et al., 2003) or isolated compounds such as alkaloids (Klausmeyer et al., 2004), flavonoids (Sohn et al., 2004), sesquiterpene lactones (Lin et al., 2003), diterpenes (El-Seedi et al., 2002), triterpenes (Katerere et al., 2003) or naphtoquinones (Machado et al., 2003), among others. Some of these compounds were isolated or obtained by bio-guided isolation after previously detecting antimicrobial activity on the part of the plant. These papers comprise about 65% of all the articles on microbial activity and medicinal plants published and compiled by PubMed. Although this type of research is the most common, set criteria to study the activity is often lacking: either the selection of microorganisms is not well established, the assayed doses are extremely high, the positive control is not clearly defined or the methods are inadequate. In fact, many of these papers view the antimicrobial activity as merely a complement of the study, without showing interest in the plant's therapeutic potential.

A second block of studies focuses on the natural flora of a specific region or country. Examples of such articles that have been published recently include studies of medicinal plants from Brazil (Duarte et al., 2005), Thailand (Wannissorn et al., 2005), Turkey (Uzun et al., 2004), Lebanon (Barbour et al., 2004), Argentina (Salvat et al., 2004), Colombia (López et al., 2001), India (Jeevan Ram et al., 2004), Malaysia (Wiart et al., 2004), Ghana (Konning et al., 2004), Peru (Rojas et al., 2003), Uganda (Olila et al., 2001), Cameroon (Nkuo-Akenji et al., 2001), Qatar (Mahasneh, 2002), the Ivory Coast (Atindehou et al., 2002) and the Democratic Republic of the Congo (Otshudi et al., 2000). Others prefer to study a wider region that includes different countries such as Asia (Almas, 2001) or Africa (Tshibangu et al., 2002), or a wide zone within one country such as Siberia (Kokoska et al., 2002). These papers represent 17% of the articles on microbial activity and medicinal plants compiled by PubMed. This criterion may be useful for understanding the activity of plants used in folk medicine in different parts of the world, but it is much more random than an ethnopharmacological criterion, which is more adequate here.

The third relevant group of papers is made up of specific studies of the activity of a plant or principle against a concrete pathological microorganism. These studies have in the past focused on activity against *Candida albicans* (Duarte et al., 2005), *Helicobacter pylori* (O'Gara et al., 2000), enterohaemorrhagic *Escherichia coli* (Voravuthikunchai et al., 2004), sexually transmitted diseases (Tshikalange et al., 2005) including *Neisseria gonorrhoeae* (Shokeen et al., 2005), bacteria resistant to known antibiotics such as *Staphylococcus aureus*, which is resistant to methicillin (Machado et al., 2003) or vancomycin-resistant enterococci (Fukai et al., 2004), as well as activity against multi-drug resistant bacteria such as *Salmonella typhi* (Rani and Khullar, 2004).

Finally, another criterion was the study of plants used for cosmetic or alimentary purposes, especially as a preservative, or the study of spices to justify their use as antimicrobial agents. While spices are thought to be antimicrobial agents against human pathogenic bacteria and yeasts, when Arora and Kaur (1999) tested different spices, only garlic and clove were found to exhibit antimicrobial activity. Indeed, some bacteria, which showed resistance to certain antibiotics were sensitive to extracts of both garlic and clove.

All the cited criteria seem sufficient to justify the studies, but we believe that the research should be focused on achieving definitive knowledge about the plant and its properties. For example, in one of our studies we screened 140 medicinal plants (two extracts of each) used in the Mediterranean region as anti-infection agents (Ríos et al., 1987; Recio et al., 1989b), and then selected one of them to study comprehensively, with the isolation and identification of the active principles as well as the subsequent determination of the spectra and potency of the isolated compounds. The selected species was *Helichrysum stoechas*, from which we isolated 10 principles. In the end, however, only four of them exhibited activity in a range of 3–25 μg/ml against Gram positive bacteria (Ríos et al., 1991).

4. Future

Over the next few years, the study of medicinal plants as antimicrobial agents should be focused in part on ascertaining specific information about the plant's antimicrobial activity, avoiding studies in which researchers use this criterion merely as a complement to a phytochemical study. The isolation of active compounds should be undertaken in light of the known activity of the plant and likewise follow a guided isolation of potential principles. Thus, when the activity of fractions and compounds is inferior to the total extract or fraction, rather than invalidating the results, this should confirm the known anti-infection properties of the plant. In the last 25 years, there was a predominant tendency to publish the activity of plants or natural products in isolation, but we think that the next 25 years should be spent in part on probing this activity more in depth. The fact that a plant extract exhibits activity is of interest, but it is only a preliminary piece of data and should be followed by the identification of the active compounds by means of a bio-guided assay. Finally, research should be kept up in order to uncover as much potentially interesting data as possible, including toxicity against animal or human cells, mechanisms of action, effects in vivo, positive and negative interactions with common antibiotics and so forth. In this vein, Shibata et al. (2005) studied the effect of ethyl gallate on β-lactam susceptibility in methicillin-resistant and methicillin-sensitive strains of *Staphylococcus aureus*. They demonstrated that it intensified the antibiotic effect and that the synergistic activity of the alkyl gallates is specific for β-lactam antibiotics since no significant changes were observed in the potency of the other classes of antibiotics tested. This study supports the possible use of these principles together with known antibiotics to increase their potency and avoid undesirable side effects. Another interesting study reports on the effect of 5′-methoxyhydnocarpin, a compound isolated from chaulmoogra oil, on the activity of berberine (Stermitz et al., 2000). While 5′-methoxyhydnocarpin exhibited no antimicrobial activity on its own, it greatly enhanced the action of berberine against *Staphylococcus aureus*. The authors observed that the level of accumulation of berberine in the cells increased sharply in the presence of 5′-methoxyhydnocarpin, allowing this natural product to effectively disable the bacterial resistance mechanism against berberine since otherwise this alkaloid is readily extruded by multi-drug resistance pumps of the human pathogen *Staphylococcus aureus*. In this case, this study supports the potential use of a weak antimicrobial natural product together with another compound to increase its activity. This type of finding could further boost the use of medicinal plants, extracts or natural products, either alone, combined or together with antibiotics.

5. Proposals and conclusions

Some general considerations must be established for the study of the antimicrobial activity of plant extracts, essential oils and the compounds isolated from them. Of utmost relevance is the definition of common parameters, such as plant material, techniques employed, growth medium and microorganisms tested.

Scientific criteria should be used in the selection of the plant material. Moreover, to avoid the use of random criteria, the selection of plants should be made from an ethnopharmacological perspective. All the species tested should be perfectly described and identified; this must include the specifics of collection including location, season, date and time of day. The use of commercial samples should be limited to cases of standardized extracts or defined phytomedicines.

The solvent and the extraction system may both modify the final results. The most appropriate method would be that in which the extract were the same as that used in folk medicine or phytotherapy, although in the lab the use of methanol or ethanol extract is much more common, sometimes making room for the essential oil. The results are notably affected. Thus, in the in vitro test carried out by Ross et al. (2001) with garlic, the activity of garlic powder against most bacteria was higher than for the plant or crude drug; however, these authors specify that garlic oil offers even greater therapeutic potential. For their part, Nostro et al. (2000) demonstrated that species such *Helichrysum italicum* or *Phytolacca dodecandra* showed moderate activity against *Escherichia coli* when the diethyl extract obtained after extraction of the aqueous suspension of the drug powder was used, but that there were no active extracts or fractions against this bacteria when a sequential extraction with petroleum ether, dichloromethane, dichloromethane–methanol (9:1) and methanol were used. In contrast, all the extracts were active against *Propionibacterium acnes*.

The pH of compounds in dilutions also modifies the results, as can sometimes be observed when phenolic or carboxylic compounds are present in the extract. Not only do ionisable compounds change the activity; indeed, it has been reported that the different effects of neutral essential oil depend on the pH. Thus, for example, anise oil had higher antifungal activity at pH 4.8 than at 6.8, while the oil of *Cedrus deudora* was most active at pH 9 (Janssen et al., 1987).

The methodology employed is another point, which needs to be considered in more depth. For non-polar extracts, the use of diffusion techniques seems to be inadequate, although many reports with these kinds of techniques have been published. Our own experience leads us to propose the use of solid dilution techniques for studying plant extracts or non-polar compounds. Only when a small amount of sample is available is the use of diffusion techniques possibly more appropriate.

The composition of the growth medium could also influence the activity of the tested extracts or compounds. Thus, in Ross et al. (2001) study on the effects of garlic powder and garlic oil, the antimicrobial activity of garlic oil was found to be greater in media lacking tryptone or cysteine, which led to the hypothesis that the effects may involve sulfhydryl reactivity.

As was previously noted by Janssen et al. (1987), the selected microorganisms should be adequately chosen and the strain number of the test organism should be mentioned. Tests may be run with collection strains, but further assays with isolated pathogens would be of interest in the case of active extracts or compounds. However, a common mistake in many papers is to claim positive activity for slight dilutions or excessively high concentrations. For example, experiments with quantities higher than 1 mg/ml for extracts or 0.1 mg/ml for isolated compounds should be avoided, whereas the presence of activity is very interesting in the case of concentrations below 100 μg/ml for extracts and 10 μg/ml for isolated compounds.

In summary, it is our firm belief that the study of medicinal plants as antimicrobial agents is necessary for gaining insight into medicinal flora and their real value, but the use of a standard method for investigation is essential. Likewise, the concentrations or dilutions used must be appropriate. Moreover, research in this area should be carried on until the agent responsible for the activity has been determined or, as the case may be, the most active fraction or extracts have been discovered. Finally, different kinds of studies on the mechanisms of action, interactions with antibiotics or other medicinal plants or compounds, and the pharmacokinetic profile of the extracts should be given high priority.

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