Antimicrobial effects of natural products

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Natural products, such as plants and their components, have been used to treat and cure diseases all around the globe since ancient times, long before the discovery of current pharmaceuticals. Natural products have long been used for anti-inflammatory, analgesic, and antipyretic remedies as well as alternatives for hormone replacement therapy. In this paper, an extensive review of the role of natural products' antimicrobial effects will be performed using in vitro and in vivo studies.

Keywords: Antimicrobial agents; Herbal medicine; Plant roots

1. Introduction

Since ancient times, natural products—including plants and their components—have been utilized to treat and cure diseases worldwide, long before the discovery of current modern drugs [1]. Natural products have been used for centuries as anti-inflammatory, analgesic, and antipyretic solutions and as alternatives for hormone replacement therapy [2-3]. Today's high occurrence of resistance to all major classes of known antibiotics represents a new challenge, and new classes of antibacterial compounds are urgently needed to respond to this unmet clinical need [4].

Many naturally occurring compounds have been studied and found to be effective in their potential role as antimicrobial agents against pathogenic microorganisms [5]. In this paper, an extensive review of the antimicrobial effects of natural products will be performed through in vitro and in vivo studies.

2. Extraction methods

The extraction of natural products may be performed by various methods, including water and ethanol [6-7]. "Essential oils also called volatile or ethereal oils are aromatic oily liquids obtained from different plant parts and volatile oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties" [7]. The dry roots of the natural plants is boiled in distilled water under reflux for several hours. The resulting extract is centrifuged and the supernatant is concentrated under reduced pressure using a rotary evaporator. The concentrates can then be freeze-dried using lyophilizer [8-9]. The pulverized natural plants can be extracted with oragnic solvents including absolute ethanol, 70 % ethanol, absolute methanol, 70 % methanol, water, and boiling water for several hours, and finally the extraction can be dried under a vacuum rotary evaporator.

Antimicrobial activities of water and ethanol extracts of anise (*Pimpinella anisum* L.) seed (PAS) were investigated and the water extract of PAS exhibited greater antioxidant capacity than that of ethanol [6].

Essential oils and methanol extracts obtained from aerial parts of Thymus vulgaris and Pimpinella anisum seeds were evaluated for their single and combined antibacterial activities against nine Gram-positive and Gram-negative pathogenic bacteria and combinations of essential oils and methanol extracts showed an additive action against most tested pathogens [7].

3. Methods for antimicrobial testing

There are various techniques to test antimicrobial activity, including the agar diffusion method, agar dilution method (direct contact assay and vapor phase assay), turbidimetry, bioimpedimetry, bioautography, the microatmospheric method, and the time-kill assay [10].

Screening strategies include whole-cell assays, drug-resistant bacteria assay, whole-cell reporter assay, wild-type resistance pair assay, target upregulation assay, reduced expression of target, antisense-all target screening assay and cell-free enzyme assay [11].

4. Antimicrobials of plant origin

The antimicrobial activity of ethanol extracts from several plants, all of them currently used in the Peruvian traditional medicine for the treatment of several infectious and inflammatory disorders, was tested by means of the agar-well diffusion assay against four bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas*

aeruginosa) and four fungi (*Candida albicans*, *Trichophyton mentagrophytes*, *Microsporum gypseum* and *Sporothrix schenckii*) and 69% of the extracts showed some degree of antimicrobial activity against at least one microorganism [12].

Wood and bark extracts of eastern North American hardwood tree species which were used traditionally as medicine by First Nation's people were screened for antimicrobial activities and the extracts were more active against gram positive bacteria than gram negative bacteria and against filamentous fungi than yeast-like fungi [13].

5. Usage

Naturally occruing antimicrobial compunds can be applied as food preservatives to protect food quality and extend the shelf life of foods and beverages [5]. The antimicrobial activity of prepared mouthwashes was found to be effective against various strains of bacteria [1]. It also suggests that the prepared herbal mouthwashes may provide an alternative to those containing chemical entities, with enhanced antimicrobial properties and better patient compliance.

6. Mechanisms of antimicrobial activity

Various mechanisms have been proposed for the antimicrobial activities of natural antimicrobials. Iron deficiency may play a great role in the inhibition of microbial growth [14]. Considering the large number of different groups of chemical compounds present in the extracts of natural products, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell [15].The hydrophobicity of the components may enable themselves to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering more permeable [15]. Saponins are naturally occurring glycosides in many plants and saponins interact with steriols and fatty acids on microbial membranes [5]. Eugenol may exert antimicrobial activity by acting on the cell wall [16].

Indolo[2,3-α]carbazole-based inhibitors from natural products may also serve as antibacterial agents [17]. Some plant-derived products do, however, have the potentially valuable property of modifying complex bacterial phenotypes that could benefit the new paradigms of antivirulence and resistance modification [18].

7. Factors affecting antimicrobial activity

The activity of natural antimicrobials depends on a variety of factors that regulate the structure and functional properties of antimicrobial agents [5]. For instance, temperature, pH, time and water activity could all affect the potency of antimicrobials [10]. The hydrophobicity of essential oils enables them to partition in the lipids of the cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents and physical conditions that improve the action of essential oils are low pH, low temperature and low oxygen levels [19]. Carvacrol and thymol may exhibit inhibitory effects on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms by disintegrating the outer membrane of the bacteria [20].

8. Combination effects

Many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target and the data from previous study showed that the combination effects of these plants had antibacterial enhancement (additive effects) against most pathogenic bacteria especially *Pseudomonas aeruginosa* which resisted all single actions of essential oils, methanol extracts and the standard drug [7]. Synergism has been observed between carvacrol and its precursor p-cymene and between cinnamaldehyde and eugenol [19]. Mixing carvacrol and thymol at proper amounts may exert the additive effect by oregano essential oil [15].

9. Future aspects

Natural antimicrobials are gaining interest for their use as alternatives to physical- and chemical-based antimicrobial treatments [5]. While natural products discovery programs have been gradually abandoned by big pharma, smaller biotechnology companies and other research organizations are taking the lead in the discovery of novel antibacterials [4]. This is in part due to the development of new technologies such as combinatorial chemistry, metagenomics and high-throughput screening; however, the new drug discovery approaches did not fulfilled the initial expectations and this has lead to a renewed interest in natural products [21].

Even though only some of the extracts may yield pure substances appropriate for the commercial pharmaceutical stream, some relatively unrefined materials may be considered for development as phytomedicines which can be registered in traditional medicines [13].Thanks to modern advances in selective organic synthesis, ribosome

crystallography, chemical biology tools for target elucidation, and novel methods for uncovering new natural products, this area would continue to provide new medicines towards unmet patient needs [22]. Thus, further research is warranted regarding which compounds are responsible for their biological activity and bioassay-guided isolation and identification of the active principles should be further evaluated.

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