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Review

Antimicrobial natural product research: A review from a South African perspective for the years 2009–2016

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ABSTRACT

Ethnopharmacological relevance: This review provides information on the antimicrobial research which has taken place on South African natural products for the last eight years (2009–2016). This important field is the backbone of all studies involving the use of medicinal plants against infectious diseases and hence can form the mainstay for future studies.

Materials and methods: All publications within the years 2009–2016 were considered. Exclusion criteria were studies not involving South African medicinal natural products and those publications where full articles could not be accessed. An overview of the most common experimental methods used and new advances in terms of antimicrobial investigations are provided. Disease categories selected for further investigation were skin and wounds, respiratory, gastrointestinal, sexually transmitted and ophthalmic infections amongst others. Alternate natural products and combinations studies were also included.

Results: The minimum inhibitory concentration (MIC) was the most commonly used experimental method to determine antimicrobial activity. *Staphylococcus aureus* was the most commonly tested skin pathogen and *Klebsiella pneumoniae* was the most common pathogen implicated in respiratory disorders. Only 20% of gastrointestinal studies included commonly implicated pathogens such as *Shigella flexneri* and *Campylobacter* species.

Conclusion: Multidisciplinary studies have emerged as a strong support for antimicrobial investigations and show the importance of including toxicity when studying antimicrobial efficacy. Alternate approaches (for example biofilms and quorum sensing) at examining antimicrobial effects are encouraged. Studies on resistant strains require more insight and future recommendations should look at consistent dosing and investigations on compound interactions amongst others.

1. Introduction

The use of natural products for medicinal properties is an ever-growing market and currently billions of people in developing regions of the globe are utilising medicinal plants and other natural products as a means to treat infectious diseases. South Africa is no exception to this, and due to the diversity of Southern African plant species and the rich cultural heritage of traditional healing practises, there have been numerous well cited publications regarding the anti-infective properties of South African natural products. A review was undertaken on South African medicinal plants used for anti-infective properties (van Vuuren, 2008), and certain recommendations for future research were proposed. After eight years it is certainly time to take stock and re-evaluate the antimicrobial research undertaken on South African natural products. Are we as researchers adding new knowledge or just filling in the gaps? Have we taken cognisance of past errors and provided valuable insight into newer trends of research? This review aims to investigate what antimicrobial research has been undertaken on South African natural products over the last eight years (2009–2016). Inclusion criteria included any study that would highlight antimicrobial effects of South African plants and/or other natural products. Exclusion criteria were assays pertaining to parasites, veterinary and general studies pertaining to plants distributed throughout Africa. Furthermore, any highlights, changes, advances, shortfalls

Abbreviations: AQ, Aqueous extract; AIDS, Acquired immune deficiency syndrome; ATCC, American type culture collection; DCM, 1:1 mixture of dichloromethane: methanol; EO, essential oils; HIV, Human Immunodeficiency Virus; INT, *p*-iodonitrotetrazolium chloride; MIC, minimum inhibitory concentration; INT, *p*-Iodonitrotetrazolium salt; MRSA, Methicillin-resistant *Staphylococcus aureus*; RA, Rheumatoid Arthritis; ΣFIC, sum of the fractional inhibitory concentration; STI, sexually transmitted infections; WHO, The World Health Organization

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and / or new trends that have occurred since the last review (van Vuuren, 2008), are discussed. This review encompasses additional topics such as the ethnobotany, combination studies, specific disease categories, studies that combine complimentary biological activities such as anti-inflammatory, cytotoxicity, anti-oxidant and acetylcholinesterase activity as well as a small section dedicated to the farming sector.

2. Antimicrobial method development

The aim of antimicrobial studies on natural products should be to yield results that are comparable with other studies in order to ensure reliability (Othman et al., 2011). Lack of standardization with respect to *in vitro* research methods may lead to a generation of inconsistency which ultimately leads to a lack of comparable evaluation. Van Vuuren (2008), highlighted this aspect where the need for standardization amongst researchers with regard to methodology and techniques needs to be encouraged and adopted. Based on the recommendations made in the 2008 review to avoid the disc diffusion method as a means to determine antimicrobial activity of medicinal plants, recent literature has shown that it is no longer a commonly used antimicrobial method and when used, it usually is accompanied by the minimum inhibitory concentration (MIC) assay. The most frequently used method for antimicrobial screening according to the 2008 review, was the MIC assay. In more recent years it appears that the MIC method has remained the method of choice (Fig. 1). This has been further confirmed by comparative studies where dilution methods are preferred (Othman et al., 2011). Other assays include the use of bio-autography (Mahlo et al., 2010) antiquorum sensing activity (Aliyu et al., 2016), biofilm (Nyila et al., 2012) and time-kill effects (Samie et al., 2010) etc.

By no means is the MIC method perfect and without flaw. One needs to take cognisance when performing doubling dilutions that absolute values are not entirely accurate as one dilution difference may yield quite a variation. For this reason reporting standard deviations may be pointless, unless confirming reproducibility of experimental repetitions. Furthermore, subtle changes by differing research methods can lead to major variances. For example, when performing the ever popular MIC microdilution assays using the colour indicator *p*-Iodonitrotetrazolium salt (INT) (Eloff, 1998), some researchers add indicator and wait two hours before reading. Other studies report a waiting period of three to six hours. This can lead to variability in the recording of results. Realistically, every pathogen grows and responds differently to the addition of INT. If the Clinical and Laboratory Standards Institute (CLSI) for standard laboratory guidelines (2012) (CLSI, 2012) for antimicrobial susceptibility testing is used, the recommendation of a control is always added. This can and should be incorporated in all assays. The idea of a control is that when the pathogen (without test substance) is exposed to the INT, it will darken red indicating viable growth. Once this is visible, the remainder of the test assay results can be read. Each pathogen will respond differently and results will be read at the correct response time for the particular pathogen been tested. Past experience using this approach has shown quite a varied response time with Gram-positive pathogens responding quicker than the Gram-negative counterparts and of course the yeasts taking the slowest (24 h) to respond.

One aspect of natural product antimicrobial analysis that has been lacking is the reporting of the minimum bactericidal or fungicidal concentration (MBC or MFC). This is a simple addition to the MIC assay but yields information that demonstrates the killing effect rather than just the inhibitory effects. This information is becoming more important for the future as the impact of cidal over inhibitory activity reduces the possibility of antimicrobial resistance. Suleman et al. (2015), is one such study that has demonstrated the killing effect of South African propolis, one of the most potent natural products studied to date, and MBCs have been reported for all samples. Another study to report cidal activity is that by Okem et al. (2012).

An important aspect that needs to be considered when reporting antimicrobial activity of natural products is the way in which noteworthy activity is measured and compared. In conventional antibiotic studies breakpoint MICs are used whereby susceptible and resistant patterns are reported for pathogens against known conventional antibiotics. Such criteria does not exist for natural products due to the complexity and chemical variety that exists between plant species. Van Vuuren (2008) suggested some noteworthy values as a yard stick with which to compare notable activity when investigating natural products. Other researchers over the years have contributed, and an overview of the more recent (2009–2016) factors of what is considered noteworthy can be observed in Table 1. When comparing the criteria for good activity from the past (pre 2009), with that of more recent publications, it is quite clear that what was considered antimicrobially active in the past can no longer be assumed to be worthy of publication. Far stricter criteria are currently applicable. It is only with defining activity for isolated compounds that no new criteria have been established. One also needs to take cognisance that each researcher may be using their own results as a measurable comparator, and as such, the criteria may alter accordingly. When examining the broadened criteria stated by the different researchers, a more up to date set of criteria should be adopted and hence the general trends as observed in more recent (2009–2016) years (Table 1) should be considered. In reviewing the antimicrobial data, noteworthy activity will only be considered for activities against medicinal plant extracts of ≤ 160 μg/ml, against essential oils (≤ 1000 μg/ml) and compounds (≤16 μg/ml) keeping in line with the most recent criteria published.

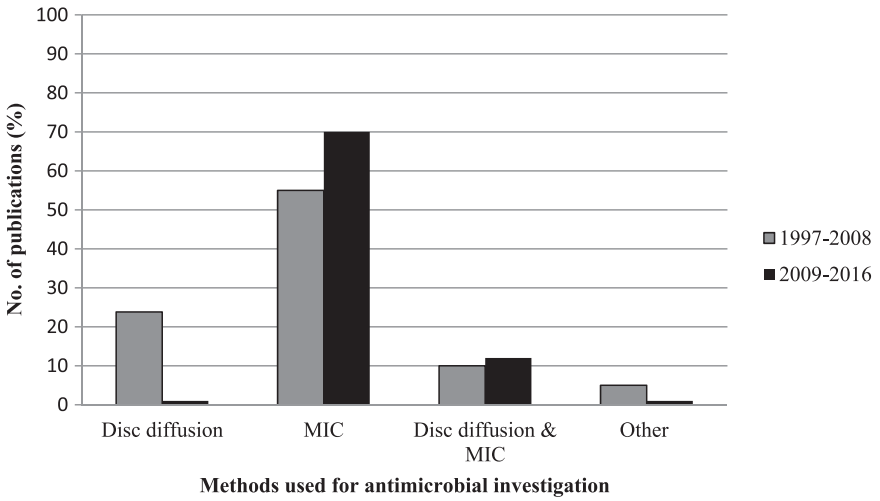

Fig. 1. Comparison of methods used to assess antimicrobial activity of South African natural products where the period 1997–2008 has been adapted from van Vuuren (2008).