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Antibacterial screening, synergy studies and phenolic content of seven South African medicinal plants against drug-sensitive and -resistant microbial strains

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ABSTRACT

Extracts from *Bolusanthus speciosus*, *Cucumis myriocarpus*, *Ekebergia capensis*, *Protea caffra*, *Prunus africana*, *Searsia lancea* and *Solanum panduriforme* were screened for antigonococcal activity using microdilution and agar disk-diffusion techniques. In addition, combinations of the different plant extracts, as well as plant extracts with four antibiotics (ampicillin, cefotaxime, chloramphenicol and penicillin) were also evaluated for antibacterial synergistic interactions against multidrug-resistant (MDR) Gram-negative bacterial strains (*Escherichia coli* and *Klebsiella pneumoniae*) using the checkerboard titration method and the time-kill assay. Phytochemical analysis for phenolics were also conducted using aqueous (50%) methanol plant extracts. *B. speciosus* methanol and dichloromethane bark extracts, as well as dichloromethane leaf extracts of *P. africana* and *S. lancea* demonstrated moderate antigonococcal properties when screened using the microdilution assay, with minimum inhibition concentration values ranging from 313 to 625 μg/ml. The checkerboard assay detected antibacterial synergistic interactions in combinations of chloramphenicol with each of *B. specious* methanol leaf extracts, *P. africana* methanol and dichloromethane leaf extracts against MDR *E. coli* (fractional inhibitory concentration index ≤0.5). However, the time-kill assay did not detect any significant synergistic interactions in any of these three aforementioned combinations. Total phenolic content in the medicinal plant extracts investigated ranged from 2.38 to 62.73 mg GAE/g dry matter. Variations in the quantity of flavonoids and condensed tannins between these plant extracts were also observed. Overall, the current findings indicated that both drug-sensitive and -resistant bacterial strains could potentially be managed using efficacious medicinal plant extracts, used either in mono- or combination therapies.

1. Introduction

The misuse and widespread use of antibiotics in medicine have caused several pathogenic bacterial strains to mutate and acquire additional survival strategies (Poole, 2001). These bacterial mutations have often instigated the evolution of several kinds of antibiotic resistant bacterial strains which are now a menace to public health globally. Particularly, multidrug-resistant (MDR) bacterial strains remain a serious cause of concern since they cause treatment failures and impose huge economic burdens especially in developing countries (De Angelis et al., 2014; Ballani and Babby, 2016). This predicament is exacerbated by the precipitous rate at which antibiotic resistant bacterial strains are evolving which far surpasses the rate at which new antibiotics are being developed (Coates et al., 2002). Unlike the 'Golden era' of antibiotic discovery (mid 1940s–early 1970s) during which several classes of antibiotics were discovered, only two new classes of antibiotics were developed in the last 40 years (Singh and Barrett, 2006; Mundy et al., 2016). Furthermore, there is also an increasing concern that newly developed antibiotics might have shorter life spans because most MDR mutants often resist the effects of the antibiotics they have been previously exposed to as well as other chemically similar or unrelated antibacterial agents (Coates et al., 2002).

Most MDR bacterial infections can successfully be managed using combination therapies. As a result, modern therapeutic regimes usually consist of combinations of antibiotics with other antibiotics, non-bactericidal chemicals or crude plant extracts (Williamson, 2001; Andries et al., 2005). Combination therapies are often favoured in cases where the combined antibacterial effects of two antibacterial agents used in a given combination supersedes the sum effects of either agent used alone (Khan et al., 2009). Apart from curtailing the growth and development of antimicrobial resistant mutants, synergistic therapies are known to potentiate the efficacy of antibiotics, increase the antibacterial spectrum and the bioavailability of antibacterial agents into bacterial cells (Zhao et al., 2002; Tripodi et al., 2007; Chukwujekwu and van Staden, 2016).

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A few years ago, Gandhi et al. (2006) discovered *Mycobacterium tuberculosis* strains resistant to all known antibiotics in KwaZulu-Natal, South Africa, which perhaps served as early warning signs of an imminent 'untreatable bacterial infections' era. The need to accelerate the rate at which novel therapeutic drugs are being developed cannot therefore, be overemphasised. Thus, researchers including ethnobotanists should strive to, among other things, discover more clinically relevant highly efficacious antibacterial compounds and novel antibacterial resistance modifying compounds from natural sources especially plants. Given that some medicinal plants such as *Berberis fremontii* have been reported to produce both bactericidal compounds and antibacterial resistance inhibitors (Stermitz et al., 2000), understanding the intricate nature of combination therapies involving medicinal plant extracts will inevitably help achieve these two important research goals.

South Africa, a country of diverse agro-ecological regions, is home to approximately 10% of the world's vascular plant species (Germishuizen and Meyer, 2003). The rich floristic diversity found in South Africa provides an abundantly diverse renewable source of active ingredients for potential use in a wide range of pharmaceutical, cosmeceutical and nutraceutical products (Mander et al., 2007). It is however, intriguing that even though over 70% of the South African population use traditional phytomedicines (Scott et al., 2004), some commonly used medicinal plants from this country have not yet been tested pharmacologically. The interspecies synergistic interactions of medicinal plant extracts, as well as the interactions of most medicinal plant extracts with antibiotics remain largely unknown. Although literature is inundated with examples of phytocompounds with excellent antibacterial properties (Afolayan and Meyer, 1997; Drewes et al., 2006; Eldeen et al., 2006), little is known about the susceptibility of MDR bacterial strains to South African medicinal plant extracts (Van Vuuren and Muhlarhi, 2017). The present study was undertaken to screen extracts from seven South African traditional medicinal plants for antigonococcal activities as well as to evaluate the antibacterial synergistic interactions of different plant extract combinations and of plant extracts with antibiotic combinations against drug-sensitive and MDR Gram-negative bacterial strains. In addition, the phenolic content in the seven plants was evaluated to provide an indication of phytochemicals possibly responsible for the antibacterial activity.

2. Materials and methods

2.1. Plant collection

The selected plant species are used in folk medicine to manage a wide range of bacterial infections (Table 1). The plant materials were collected between July and September 2015. *Bolusanthus speciosus* (Bolos) Harms, *Ekebergia capensis* Sparrm, *Prunus africana* (Hook) Kalkman and *Searsia lancea* (L.f.) Barkley were collected from the University of KwaZulu-Natal (UKZN) Pietermaritzburg (PMB) Botanical garden, while *Protea caffra* Meisn was collected from the Walter Sisulu South African National Biodiversity Institute (SANBI) (27°50′40.7″E 26°05′13.8″S). Seeds of *Cucurmis myriocarpus* (Naud) E. Mey and *Solanum panduriforme* E. Mey were obtained from Bradfield, Bulawayo, Zimbabwe (20°10′40″S 28°35′06″ E) and grown in the UKZN PMB Botanical garden. All the plant species were positively identified by the curator, after which voucher specimens were prepared and deposited in the Bews Herbarium at UKZN (Table 1). Once collected, the plant materials were oven dried for 3–5 days at 50 °C, ground and stored in the dark at room temperature.

2.2. Source of chemicals

Organic solvents (dichloromethane, methanol and petroleum ether) were purchased from Radchem Laboratory Supplies (Pty) Ltd. (Alberton, South Africa). The antibiotics, catechin, dimethyl sulfoxide (DMSO), Folin and Ciocalteu reagents as well as gallic acid were supplied by Sigma-Aldrich Co. (Steinheim, Germany). The carbon dioxide generator, soluble haemoglobin, Mueller and Hinton growth media (broth and agar) and vitox were bought from Oxoid Ltd (Hampshire, United Kingdom). Other chemicals utilised in this study included sodium carbonate and sodium nitrate (BDH Chemicals Ltd, England, United Kingdom); ferric ammonium sulphate (Hopkins and Williams

Table 1
Ethnomedicinal applications of seven South African traditional medicinal plants.

Plant species Family	Part used	Voucher number	Use in traditional medicine	Reference
Bolusanthus speciosus (Bolos) Harms Fabaceae	Bark	NU0042512	Abdominal pains, emetic and tuberculosis	Palgrave (1977)
	Bark, leaf and stem		Blood cleansing, kidney ailments and sexually transmitted infections	Magobo (1990)
	Root		Emetic and abdominal pains	Amusan et al. (2002) Venter and Venter (2002)
Cucumis myriocarpus (Naud) E. Mey Curcubitaceae	Fruit	NU0042511	Gonorrhoea and syphilis	Semenya et al. (2013)
Ekebergia capensis Sparrm Maliaceae	Bark	NU0042516	Malaria, tonic, chronic coughs, headaches, dysentery, boils, dermatological diseases, laxative, emetic, sexually-transmitted diseases	Hutchings et al. (1996) Grierson and Afolayan (1999) Koch et al. (2005)
Prunus africana (Hook) Kalkman Rosaceae	Stem bark	NU0042513	Allergies, diarrhoea, gastrointestinal ailments, kidney and prostate gland diseases	Pujol (1990)
	Leaf		Abdominal pains, laxative, skin diseases, prostatitis	Gangoue-Pieboji et al. (2009)
	Not specified		Anti-inflammatory, aphrodisiac, appetite stimulants, chest pains, heart burn, fevers, kidney ailments, madness, malaria, urinary tract infections, wound dressing	Neuwinger (2000) Ndenecho (2009)
Protea caffra Meisn Proteaceae	Bark	NU0048533	Bleeding stomach ulcers and diarrhoea	Coetzee and LittleJohn (2000)
	Root		Healing broken bones	Zukulu (2012)
	Seed		Chlamydia	Semenya et al. (2013)
Searsia lancea Barley Anacardiaceae	Wood	NU0042514	Skin tanning	Watt and Breyer-Brandwijk (1962)
	Leaf		Colds, fever, papules and pustules	Hutchings et al. (1996)
	Root		Diarrhoea and gall sickness	Van der Merwe et al. (2001)
Solanum panduriforme E. Mey Solanaceae	Fruit	NU0042515	Gonorrhoea and wounds	Bruschi et al. (2011) Erasmus et al. (2012)
	Root		Diarrhoea, ingestion, pelvic pains, stomach aches, snake bites, tooth aches and ulcers	Bruschi et al. (2011) Hutchings et al. (1996)