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South African medicinal plants screened against *Pseudomonas aeruginosa*

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Background: *Pseudomonas aeruginosa* is amongst the three high-ranking pathogens on the World Health Organization's global priority list of antibiotic-resistant bacteria. The list highlights research priorities in drug discovery and development.

Aim: This study aimed toprovide a detailed account of efforts by researchers to find anti-*P. aeruginosa* compounds from South African medicinal plant species during the period 2000–2020.

Method: Various online research and journal databases were used to obtain information relating to South African medicinal plants and *P. aeruginosa*.

Results: During the study period (2000–2020), only 31 studies reported on the antibacterial properties of South African medicinal plants against the pathogen. Given that *P. aeruginosa* is a serious cause of morbidity and mortality worldwide, it was interesting to note that none of the published reports were dedicated solely to the pathogen. Furthermore, only one study included the antibiotic-resistant mutants of the pathogen as a test organism. Over 150 plant species belonging to 78 families were screened against the bacterium. *Barringtonia racemosa*, *Croton megalobotrys*, *Erythrina caffra*, *Leucosidea sericea*, *Maesa lanceolata*, *Morella serrata* and *Trichilia emetica* exhibited potent anti-*P. aeruginosa* properties (minimum inhibitory concentration [MIC] < 0.1 mg/mL). Plumbagin, a compound isolated from the leaves of *Aristea ecklonii* demonstrated promising activities (MIC = 0.008 mg/mL) against the bacterium. Essential oils extracted from some plants demonstrated noteworthy antibacterial synergistic effects (fractional inhibitory concentration index [FICI] < 0.5) when used in pairwise combinations with conventional antibiotics.

Conclusion: Overall, empirical evidence presented in the scantly available literature suggests that novel anti-*P. aeruginosa* agents could be developed from South African herbal extracts.

Keywords: Antibacterial; Drug-resistance; Medicinal plants; Phytochemistry; *Pseudomonas aeruginosa*; South Africa.

Introduction

South Africa (SA) is renowned for its extensive floral biodiversity which is perhaps more spectacularly displayed in the Western Cape Province, where over 9000 plant species are distributed within an area of approximately 90 000 km^2 (Manning & Goldblatt 2012). What makes the South African flora unique is that about half of the higher plant species (≥ 30 000) found in the country are endemic (Goldblati 1978). Owing to the country's vast cultural diversity, a considerable amount of indigenous plants are exploited by local herbalists. For instance, over 25% of plant species in the KwaZulu-Natal province are used as herbs (Hutchings et al. 1996). Given the plant species richness in the country, coupled with the fact that each plant species can potentially produce over 500 different secondary metabolites (Anil 2010; Miller 2011; Sibanda & Okoh 2007), there are reasonable prospects of discovering several novel drug scaffolds within the South African flora. The South African floral diversity has attracted the attention of researchers worldwide, as evidenced by a significant increase in the number of publications, citations and patents on the country's medicinal plant species over the past few decades (Van Wyk 2008).

For decades, medicinal plants have contributed immensely towards the development of therapeutic drugs. Approximately 25% of the drugs approved by the Food and Drug Administration (FDA, United States of America) and the European Medical Agency (EMA) in recent years were developed from efficacious medicinal plant extracts (Patridge et al. 2016). Artemisinin, aspirin, camptothecin, quinine and taxol are but a few examples of plant-based therapeutic drugs (Tshibangu et al. 2002). The scourge of drug-resistant pathogenic infections has necessitated an urgent need to develop new and effective antibacterial agents. Based on the urgency of the need

for novel antibiotics, the World Health Organization (WHO) described three categories of pathogens namely critical, high and medium priority. Carbapenem-resistant *Pseudomonas aeruginosa* is amongst the three bacterial strains classified as a critical 'research' priority (WHO 2017).

Pseudomonas Aeruginosa is an opportunistic pathogen that commonly infects individuals who are immune compromised, particularly those infected with the human immunodeficiency virus (HIV) and/or those suffering from cancer (Sandhu & Samra 2013). It is a common etiological agent of hospital-acquired pneumonia, urinary tract infections and bacteremia (Horcajada et al. 2013). The antibiotic-resistant mechanisms commonly employed by the pathogen include the extrusion and enzymatic inactivation of antibiotics (Wolter & Lister 2013). Given that the bacterium is Gram-negative, its semi-impermeable outer membrane also greatly restricts antibiotics from reaching their intracellular target sites.

Being a well-recognised intrinsic drug-resistant pathogen, it is conceivable that *P. aeruginosa* has captivated the attention of several researchers around the world. The current review provides a detailed account of efforts by researchers to find anti-*P. aeruginosa* compounds from SA medicinal plant species during the period 2000–2020.

Methodology

Online research and journal databases (Google Scholar, Science Direct, PubMed, Scopus and Springer Link) were used to obtain reports related to South African medicinal plants, phytochemical analysis, isolated compounds, antibacterial synergy and *P. aeruginosa*.

Antibacterial screening of crude plant extracts

Despite being a highly virulent and drug-resistant pathogen, *P. aeruginosa* has somehow, not received much attention from SA ethnobotanists as evidenced by the scantly available information in the accessed literature (Figure 1). In over two decades, only 31 ethnobotanical studies relating to the pathogen were published, averaging a meagre 1.6 publications per annum. Interestingly, no such publications were made within the first 3 years (2018–2020) after the pathogen was classified as a critical research priority by the World Health Organization (Figure 1) (WHO 2017). It was also quite interesting to note that none of the studies conducted were dedicated solely to *P. aeruginosa*. Apparently, little efforts were made to screen SA medicinal plants against antibiotic-resistant strains of the pathogen. Of the 31 reports published, only a study by Soyingbe et al. (2013) included the drug-resistant strain of the bacterium as a test organism.

A total of 152 plant species belonging to 78 families were screened against the bacterium (Tables 1, 2, 3, 4). The most represented families were Fabaceae (16.7%), Euphorbiaceae (12.8%) and Anacardiaceae (9%). The most investigated species were *Cussonia spicata*, *Ricinus communis*, *Sclerocarya birrea* and *Zizipus mucronata*. It was encouraging to note that

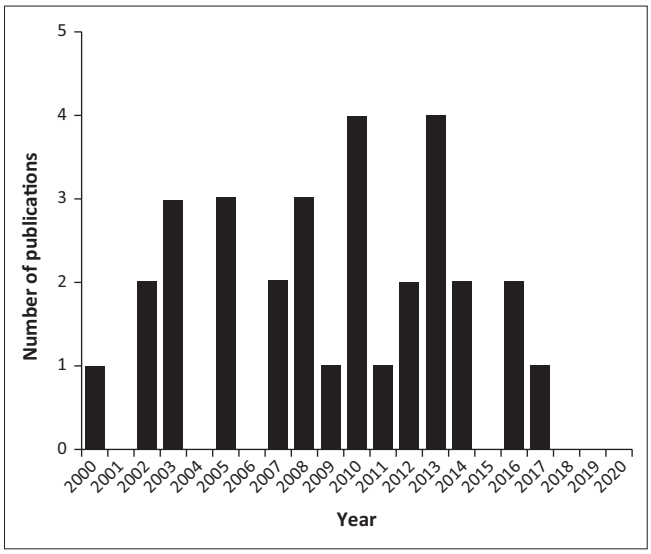

FIGURE 1: Number of scientific reports on South African medicinal plants screened against *Pseudomonas aeruginosa* published during the period 2000–2020.

almost half (45%) of all plant species evaluated demonstrated noteworthy antibacterial activities (minimum inhibitory concentration [MIC] < 1 mg/mL) against the pathogen. *Barringtonia racemosa*, *Croton megalobotrys*, *Erythrina caffra*, *Leucosidea sericea*, *Maesa lanceolata*, *Morella serrata* and *Trichilia emetica* yielded potent antibacterial activities (MIC range: 0.02–0.09 mg/mL, Table 1) and as such, warrants further investigations. Using the disc diffusion assay, Mongalo, Opoku and Zobolo (2012) also demonstrated that the acetone root extracts of *Waltheria indica* possess significant growth inhibitory properties against the pathogen. As the aforementioned plants belong to eight different families (Euphorbiaceae, Fabaceae, Lecythidaceae, Maesaceae, Meliaceae, Myricaceae, Rosaceae and Sterculiaceae), the principal bactericidal compounds in them are likely both structurally and functionally diverse. This presents encouraging prospects of finding an assortment of clinically relevant anti-pseudomonas compounds within them.

In addition to crude plant extracts, some researchers investigated the antibacterial properties of semi-purified extracts or isolated phyto compounds. Magama et al. (2003), for instance, screened semi-purified leaf extracts of *Euclea crispa* against the bacterium and reported weak-moderate antibacterial activities. However, the compounds isolated from the leaves exhibited poor antibacterial activities against *P. aeruginosa* in a separate study by Pretorius, Magama and Zietsman (2003).

Whilst some researchers screened medicinal plants from a variety of families, others focused on specific medicinal plant species including *Antidesma madagascariense*, *Erythrina caffra*, *E. crispa*, *Lavandula angustifolia*, *Morella serrata*, *S. schinus*, *Tulbaghia violacea* and many others (Gundidza et al. 2008; Pretorius et al. 2003; Seebaluck-Sandoram et al. 2017; Soyingbe et al. 2013). In some studies, only medicinal plants used by people within a specific geographical location were evaluated. The study areas covered were predominantly in the Eastern Cape, Limpopo,

TABLE 1: Antibacterial activities of South African medicinal plants screened against Pseudomonas aeruginosa during the period 2000–2020.

Family	Botanical names	Plant part	Bioassay used	Activity	Reference
Amaryllidaceae	*Crinum viridis*	LV	MIC	4	Kelmanson, Jäger and Van Staden (2000)
Asteraceae	*Vernonia cororata*	LV	MIC	4	
Dioscoreaceae	*Dioscorea sylvatica*	TB, BK	MIC	NA	
Melianthaceae	*Melianthus comosus*	LV	MIC	NA	
Caesalpinioideae	*Schotia latifolia*	BK	MIC	5	Masika and Afolayan (2002)
Combretaceae	*Combretum caffrum*	BK	MIC	5	
Salicaceae	*Salix capensis*	BK	MIC	1–5	
Parmeliaceae	*Usnea barbata*		MIC	5	Madamombe and Afolayan (2003)
Ebenaceae	*Eucleacrispa subspcrispa*	LV	Disk diffusion	X–XX	Magama et al. (2003)
Ebenaceae	*Eucleacrispa*	LV	Disk diffusion	X	Pretorius, Magama and Zietsman (2003)
Fabaceae	*Peltophorumafricanum*	LV, ST-BK, RT-BK	MIC	**0.16–0.63**	Chikoto et al. (2005)
Annonaceae	*Annona senegalensis*	LV	MIC Disk diffusion	> 12 X	Samie et al. (2005)
Euphorbiaceae	*Bridelia micrantha*	BK, SD	MIC Disk diffusion	6–12 X	
Euphorbiaceae	*Androstachysjohnsonii*	BK, LV, RT	MIC Disk diffusion	**0.62** X	
Fabaceae	*Mucuna coriacea*	RT	MIC Disk diffusion	> 12 X	
Fabaceae	*Peltophorum africanum*	BK, RT	MIC Disk diffusion	1.5–3 X	
Malvaceae	*Sida alba*	LV	MIC Disk diffusion	3–12 X	
Menispermaceae	*Cissampelos torulosa*	LV	MIC Disk diffusion	> 12 X	
Myrtaceae	*Syzygium cordatum*	BK, LV	MIC Disk diffusion	**0.31–0.35** X	
Olacaceae	*Ximenia caffra*	LV, RT	MIC Disk diffusion	1.5–6 X	
Papilionoideae	*Zornia milmeana*	WP	MIC Disk diffusion	3–12 X	
Urticaceae	*Pouzolzia mixta*	RT, ST, LV	MIC Disk diffusion	12 X	
Vitaceae	*Rhoicissus tridentata*	FR, RT, TB	MIC Disk diffusion	6 X	
Araliaceae	*Cussonia spicata*	BK	MIC	1.25	Luseba et al. (2007)
Asclepiadaceae	*Sarcostemma viminale*	ST	MIC	1.25	
Asphodelaceae	*Aloe marlothii*	LV	MIC	1.25	
Asteraceae	*Schkuhria pinnata*	ST	MIC	1.25	
Euphorbiaceae	*Jatropha zeyheri*	RT	MIC	2.5	
Euphorbiaceae	*Ricinus communis*	LV, ST	MIC	**0.78**	
Fabaceae	*Pterocarpus angolensis*	BK	MIC	2.5	
Pedaliaceae	*Dicerocaryum eriocarpum*	ST	MIC	1.25	
Rhamnaceae	*Ziziphus mucronata*	BK	MIC	1.25	
Vitaceae	*Cissus quandrangularis*	BK	MIC	2.5	
Anacardiaceae	*Rhus lancea* *Sclerocarya birrea*	LF BK	MIC MIC	> 12 > 12	McGaw, Van der Merwe and Eloff (2007)
Apocynaceae	*Secamome filiformis*	AP	MIC	> 12	
Araliaceae	*Cussonia spicata*	RT	MIC	> 12	
Anacardiaceae	*Rhus lancea* *Sclerocarya birrea*	LF, BK BK	MIC MIC	> 12 > 12	
Apocynaceae	*Secamome filiformis*	AP	MIC	> 12	
Araliaceae	*Cussonia spicata*	RT	MIC	> 12	
Asteraceae	*Schkuhria pinnata*	AP	MIC	> 12	
Euphorbiaceae	*Ricinus communis* *Synadenium cupulare*	ST/LF ST/LF	MIC MIC	> 12 > 12	
Fabaceae	*Pterocarpus angolensis* *Schotia brachypetala*	BK/LF BK/LF	MIC MIC	> 12 > 12	
Pandaliaceae	*Dicerocaryum eriocarpum*	WP	MIC	> 12	
Rhammaceae	*Berchemia zeyheri* *Ziziphus mucronata*	BK LF	MIC MIC	> 12 > 12	
Sapindaceae	*Hippobromus pauciflorus*	AP	MIC	> 12	
Sterculiaceae	*Dombeya rotundifolia*	AP	MIC	> 12	
Thymelaeaceae	*Gnidia capitata*	RT	MIC	> 12	
Urticaceae	*Pouzolzia mixta*	LF/ST	MIC	> 12	
Vitaceae	*Cissus quadrangularis*	ST	MIC	> 12	

Table 1 continues on the next page →

TABLE 1 (Continues...): Antibacterial activities of South African medicinal plants screened against Pseudomonas aeruginosa during the period 2000–2020.

Family	Botanical names	Plant part	Bioassay used	Activity	Reference
Cornaceae	*Curtisia dentata*	LV	MIC	**0.6–0.8**	
Meliaceae	*Trichilia emetica*	LV	MIC	**0.4**	Shai et al. (2008)
Bignoniaceae	*Kigelia africana*	LV	MIC	**0.1–0.6**	
Combretaceae	*Terminalia sambesiaca*	LV	MIC	**0.1–0.6**	
Rutaceae	*Vepris reflexa*	LV	MIC	**0.16**	
Combretaceae	*Terminalia phanerophlebia*	LV	MIC	**0.6–0.16**	
Araliaceae	*Cussonia zuluensis*	LV	MIC	1.8	
Guttiferae	*Garcinia kola*	LV	MIC	10	Sibanda and Okoh (2008)
Euphorbiaceae	*Croton megalobotrys*	LV	MIC	**0.06–0.31**	Selowa et al. (2010)
	Croton steenkampianus	LV	MIC	**0.63**	
	Croton silvaticus	LV	MIC	1.25	
Geraniaceae	*Geranium incanum*	WP	Disk diffusion	NA	Babajide et al. (2010)
Verbenaceae	*Lippia javanica*	AP	MIC	**0.42**	Shikanga, Combrinck and Regnier (2010)
	Lippia wilmsii	AP	MIC	**0.63**	
	Lippia rehmanni	AP	MIC	1.3	
	Lippiascaberrima	AP	MIC	1.3	
Fabaceae	*Erythrina caffra*		MIC MBC	0.02 0.04	Olajuyigbe and Afolayan (2011)
Fabaceae	*Senna italica*	LV	MIC	**0.84**	Lekganyane et al. (2012)
Verbenaceae	*Lippia javanica*	LV	MIC	**0.32**	
Verbenaceae	*Lantana camara*	LV	MIC	NA	
Euphorbiaceae	*Ricinus communis*	LV	MIC	NA	
Rhamnaceae	*Ziziphus mucronata*	LV	MIC	NA	
Araceae	*Zantedeschia aethiopica*	LV, ST	MIC MIC	**0.31** 2.5	Mongalo, Opoku and Zobolo (2012)
Fabaceae	*Bauhinia macranthera*	LV	MIC	**0.5**	Mabona et al. (2013)
Amaryllidaceae	*Boophanedisticha*	LV	MIC	1	
Euphorbiaceae	*Bridelia micrantha*	BK, LF	MIC	2	
Chenopodiaceae	*Chenopodium ambrosioides*	LF	MIC	**0.25**	
Menispermaceae	*Cissampelo capensis*	LF	MIC	2	
Crassulaceae	*Cotyledon orbiculata*	LV	MIC	**0.5**	
Asteraceae	*Dicomaanomala*	TB	MIC	8	
Dioscoreaceae	*Dioscoreadregeana*	TB	MIC	2	
Ebenaceae	*Diospyros mespiliformis*	LF	MIC	1	
Sapindaceae	*Dodonaea angustifolia*	LF	MIC	2	
Meliaceae	*Ekebergia capensis*	BK, LV	MIC	**0.75**–1	
Fabaceae	*Elephantorrhizaelephantina*	LF, RT, RZM	MIC	1–2	
Myrsinaceae	*Embeliaruminata*	LF	MIC	**0.75**	
Fabaceae	*Erythrina lysistemon*	LF	MIC	**0.2**	
Myrtaceae	*Eucalyptus camaldulensis*	BK	MIC	2	
Moraceae	*Ficus natalensis*	BK, LF	MIC	4	
Moraceae	*Ficus sur*	LF	MIC	1–2	
Gunneraceae	*Gunneraperpensa*	LF, LF	MIC	1–2	
Scrophulariaceae	*Halleria lucida*	LF, ST	MIC	**0.5**–2	
Anacardiaceae	*Harpephyllumcaffrum*	BK	MIC	**0.25**	
Hypericaceae	*Hypericum perforatum*	LF	MIC	**0.5**	
Aquifoliaceae	*Ilex mitis*	BK, LV	MIC	1.5–2	
Bignoniaceae	*Kigeliaafricana*	FR	MIC	2	
Anacardiaceae	*Lanneadiscolor*	LF	MIC	1	
Verbenaceae	*Lantana rugosa*	LF	MIC	2	
Malvaceae	*Malva parviflora*	LF	MIC	1	
Melianthaceae	*Melianthus comosus*	LV	MIC	**0.1**	
Melianthaceae	*Melianthus major*	LF	MIC	1.25	
Lamiaceae	*Mentha longifolia*	LV	MIC	2	
Cactaceae	*Opuntia ficus-indica*	LF	MIC	4	
Adiantaceae	*Pellaeacalomelanos*	LF	MIC	**0.75**–1	
Rubiaceae	*Pentanisiaprunelloides*	RT-BK	MIC	8	
Pittosporaceae	*Pittosporum viriflorum*	LV, RT	MIC	8	
Apocynaceae	*Rauvolfiacaffra*	LF	MIC	2	
Rubiaceae	*Rothmannia capensis*	LF	MIC	4	
Amaryllidaceae	*Scadoxuspuniceus*	RT, RZM	MIC	2	

Table 1 continues on the next page →

TABLE 1 (Continues...): Antibacterial activities of South African medicinal plants screened against Pseudomonas aeruginosa during the period 2000–2020.

Family	Botanical names	Plant part	Bioassay used	Activity	Reference
Solanaceae	*Solanum incanum*	LF	MIC	**0.5**	
Combretaceae	*Terminalia sericea*	RT	MIC	**0.25**	
Meliaceae	*Trichilia emetica*	LF	MIC	**0.03**	
Asteraceae	*Vernomianatalensis*	LF	MIC	4	
Canellaceae	*Viscum capense*	BK, LV, RT	MIC	8	
Canellaceae	*Warburgiasalutaris*	BK	MIC	**0.1**–1	
Araceae	*Zantedeschia aethiopica*	LF	MIC	**0.5**	
Rhamnaceae	*Ziziphus mucronata*	BK, LF	MIC	**0.5**–1	
Mimosaceae	*Albizia gummifera*	LV	MIC	0.31	Masoko (2013)
Lecythidaceae	*Barringtonia racemosa*	LV	MIC	**0.05–0.52**	
Simaroubaceae	*Kirkia acuminata*	LV	MIC	1.25	
Euphorbiaceae	*Macaranga capensis*	LV	MIC	1.25	
Celastraceae	*Maytenus senegalensis*	LV	MIC	**0.31**	
Celastraceae	*Maytenusundanta*	LV	MIC	**0.63**–1.04	
Fabaceae	*Millettiastuhlmanni*	LV	MIC	**0.3**	
Anacardiaceae	*Sclerocaryabirrea*	LV	MIC	**0.16–1.25**	
Rubiaceae	*Vangueriainfausta*	LV	MIC	**0.63–1.25**	
Fabaceae	*Xanthocerciszambesiaca*	LV	MIC	**1.25–1.67**	
Myricaceae	*Morella serrata*	RT	MIC	**0.09–0.39**	Ashafa (2013)
Asteraceae	*Brachylaenadiscolor*	LV	MIC	**0.31**	Adamu, Naidoo and Eloff (2014)
Rutaceae	*Zanthoxylum capense*	LV	MIC	**0.31**	
Lamiaceae	*Clerodendromglabrum*	LV	MIC	**0.63**	
Apiaceae	*Heteromorpha trifoliata*	LV	MIC	**0.63**	
Icacinaceae	*Apodytesdimidiata*	LV	MIC	**0.31**	
Strychnaceae	*Strychnos mitis*	LV	MIC	**0.16**	
Maesaceae	*Maesa lanceolata*	LV	MIC	**0.02**	
Papilionaceae	*Indigofera frutescens*	LV	MIC	**0.31**	
Rosaceae	*Leucosidea sericea*	LV	MIC	**0.02**	
Meliaceae	*Melia azedarach*	LV	MIC	**0.63**	
Rutaceae	*Clausenaanisata*	LV	MIC	**0.31**	
Cyatheaceae	*Cyathea dregei*	LV	MIC	**0.31**	
Papilionaceae	*Millettia grandis*	LV	MIC	**0.31**	
Fabaceae	*Acacia erioloba*	BK, LV	MIC	2	
Apocynaceae	*Acokantheraoppositifolia*	LV	MIC	1.5	
Xanthorrhoeaceae	*Aloe arborescens*	LV	MIC	1	
Iridaceae	*Aristea ecklonii*	LV, RT	MIC	**0.2**	
Rutaceae	*Agothosemabetulina*	LV	MIC	4	Hübsch et al. (2014)
Asphodelaceae	*Aloe ferox*	LV	MIC	6	
Asteraceae	*Artemisia afra*	LV, TW	MIC	1.5	
Verbenaceae	*Lippiajavanica*	LV	MIC	4	
Geraniaceae	*Pelargonium sidoides*	RT	MIC	1.5	
Fabaceae	*Sutherlandia frutescens*	LV	MIC	4	
Euphorbiaceae	*Antidesmamadagascariense*	LV	MIC	0.25–2	Seebaluck-Sandoram et al.(2017)

AP, aerial part; FL, flower; LV, leaf; MIC, minimum inhibitory concentration; BK, bark; ST, stem; SD, seed; RT, root; RZM, rhizome; TB, tuber, TW, twig; WP, whole plant; X, weak activity; XX, moderate activity; XXX, potent activity. *, Values in bold denotes noteworthy antibacterial activities.

Minimum inhibitory concentration (MIC) values are presented in mg/mL.

KwaZulu-Natal and Mpumalanga (Masika & Afolayan 2002; Masoko 2013; Mongalo et al. 2012; Oyedeji, Afolayan & Eloff 2005; Selowa et al. 2010).

In a study by Samie et al. (2005) on 14 medicinal plants used by the Venda people of Limpopo to manage a wide range of infectious diseases, only *S. cordatum* and *A. johnsonii* demonstrated noteworthy antibacterial activities against *P. aeruginosa* (MIC = 0.35 and 0.62 mg/mL, respectively). Kelmanson, Jäger and Van Staden (2000) examined the antibacterial activities of 14 Zulu medicinal plant species and reported that *Crinumviridis*, *Dioscorea dregeana* and *Vernonia colorata* possess moderate growth inhibitory activities against the bacterium (MIC range: 0.36–0.63 mg/mL). These two were, to the best of our knowledge, the only reports in which the medicinal plants used by a given ethnic group in SA were screened against the pathogen. This could probably stem from a limited number of relevant ethnobotanical surveys available. Generally, the indigenous knowledge gathered through such surveys informs scientists as to which plants to screen for antibacterial or any other pharmacological properties.

Antibacterial investigations of hydro-distilled essential oils

South Africa has a vast array of aromatic plant species some of which are part of the *materia medica* utilised by local

TABLE 2: Antibacterial activity of essential oils extracted from South African medicinal plants screened against *Pseudomonas aeruginosa*.

Family	Botanical names	Plant part	Bioassay used	Activity	Reference
Myrothamnaceae	*Myrothamnus flabellifolius*	AP	Disk diffusion Time-kill	**XXX** X	Viljoen et al. (2002)
Annonaceae	*Annona senegalensis*	LV	MIC Disk diffusion	> 12 X	Samie et al. (2005)
Verbenaceae	*Lippia javanica*	LV	MIC Disk diffusion	6–12 X	
Lamiaceae	*Leonotis leonurus*	LV, FL	MIC	1.25	Oyedeji et al. (2005)
	Leonotis ocymifolia	LV, FL	MIC	**0.31**	
Anacardiaceae	*Schinus terebinthifolius*	LV	Disk diffusion	X–XX	Gundidza et al. (2008)
Myrtaceae	*Callistemon citrinus*	LV	MIC Disk diffusion	2.5 XX	Oyedeji et al. (2009)
	Callistemon viminalis	LV	MIC Disk diffusion	5 XX	
Alliaceae	*Tulbaghia violacea*	RZM	Disk diffusion MIC	XX 2.5–5	Soyingbe et al. (2013)
Lamiaceae	*Lavandula angustifolia*	LV	MIC	**0.3**	De Rapper et al. (2016)

AP, aerial part; FL, flower; LV, leaf; MIC, minimum inhibitory concentration; RZM, rhizome; X, weak activity; XX, moderate activity; XXX, potent activity.
*, Values in bold denotes noteworthy antibacterial activities.
Minimum inhibitory concentration (MIC) values are expressed in mg/mL.

traditional healers. Asteraceae (2300 species), Lamiaceae (235 species) and Rutaceae (290) are classic examples of prominent aromatic families found in the country (Lawrence 2006). Owing to the importance of aromatherapy worldwide, an increasing number of local aromatic plants are being screened for a variety of therapeutic properties.

As shown in Table 2, only a few studies (7) focused on screening essential oils against *P. aeruginosa*. The investigated oils were extracted from different parts of *Annona segegalensis*, *Callistemon citrinus*, *Callistemom viminalis*, *L. angustifolia*, *Leonotis leonurus*, *L.ocymifolia*, *Lippia javanica*, *Myrothamnus flabellifolius*, *Schinus terebinthifolius* and *Tulbaghia violacea*. These plants belonged to seven families namely, Alliaceae, Anacardiaceae, Annonaceae, Lamiaceae, Myrothamnaceae, Myrtaceae and Verbenaceae. However, none of the evaluated oils demonstrated promising antibacterial activities except those from the leaves of *L. angustifolia*, *Leonotis ocymifolia* (MIC: 0.3 mg/mL) and the aerial parts of *M. flabellifolius* (Table 2).

Essential oils are generally volatile and lipophilic making them much more difficult to assess than crude plant extracts (Van Vuuren 2008). This could have possibly resulted in the former being less favoured by researchers than the latter (Tables 1 and 2). It is also important to note that some hydro-distilled essential oils are generally less efficacious than 'full-bodied' crude plant extracts primarily because some bioactive compounds in the purified oils work synergistically with non-volatile phyto-compounds to elicit therapeutic effects. Isolating the oils might therefore disrupt key synergistic interactions. This could possibly explain why most of the essential oils evaluated were not effective against *P. aeruginosa* (Table 2).

Phytochemical analysis

As already alluded to, the empirical basis of herbal medicine lies in the existence of bioactive phyto compounds. The multi-step process of plant-based drug development often starts with the accurate identification of potential sources of therapeutic phyto-compounds. As such, phytochemical analysis has become an important part of ethnopharmacology. In addition to antibacterial screening, some of the plant extracts screened against *P. aeruginosa* were subjected to either qualitative or quantitative phytochemical analysis.

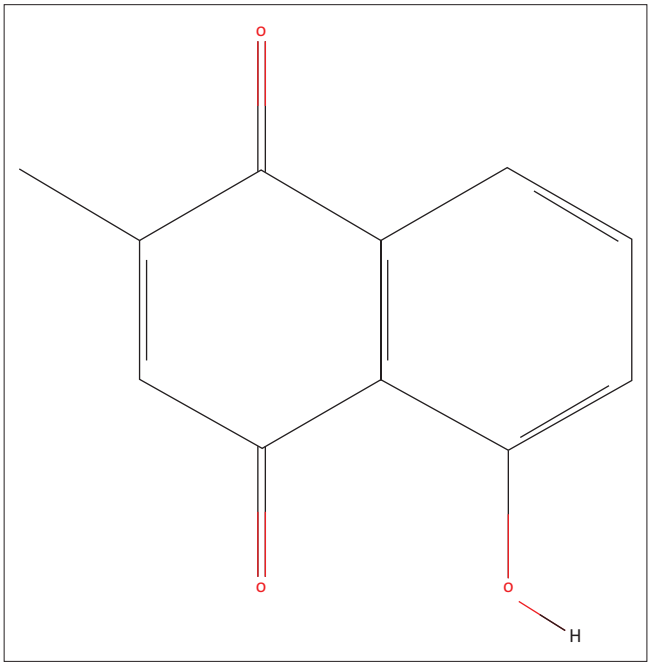

FIGURE 2: Chemical structure of plumbagin courtesy of https://pubchem.ncbi.nlm.nih.gov/

Using bio-guarded isolation techniques, Mabona et al. (2013) managed to isolate and identify a compound known as plumbagin (Figure 2), from the leaves of *Aristea ecklonii* (Asteraceae). Interestingly, the compound displayed potent bactericidal effects against *P. aeruginosa* (8 µg/ mL). To the best of our knowledge, this was the only successful attempt at isolating potent anti-*P. aeruginosa* compounds from South African medicinal plants documented over the past 20 years. It is, however, worth noting that plumbagin was previously isolated from other medicinal plants such as *Plumbago scandens* and *Plumbago zeylanica* (Jeyachandran et al. 2009; Paiva et al. 2003).

TABLE 3: The inhibition of *Pseudomonas aeruginosa* by bioautography of extracts from selected South African medicinal plants.

Plant	Plant species	Plant part	Active bands	Antibacterial activity	Reference
Combretaceae	*Combretum vendae*	Leaves	1	Moderate	Suleiman et al. (2010)
Burseraceae	*Commiphora harveyi*	Leaves	1	Moderate	
Meliaceae	*Khaya anthotheca*	Leaves	1	Moderate	
Anacardiaceae	*Loxo stylisalata*	Leaves	1	Moderate	
Kirkiaceae	*Kirkia wilmsii*	Leaves	1	Moderate	
Ochnaceae	*Ochna natalitia*	Leaves	1	Moderate	
Fabaceae	*Senna italica*	Leaves	4	High	Lekganyane et al. (2012)
Verbenaceae	*Lippia javanica*	Leaves	2	High	
Verbenaceae	*Lantana camara*	Leaves	4	High	
Euphorbiaceae	*Ricinus communis*	Leaves	5	High	
Rhamnaceae	*Ziziphus mucronata*	Leaves	1	High	
Combretaceae	*Terminalia sericea*	Leaves	1	High	Netshiluvhi and Eloff (2016)
Anacardiaceae	*Sclerocarya birrea*	Leaves	3	High	
Combretaceae	*Combretum collinum*	Leaves	1	High	

Based on gas chromatography-mass spectroscopy (GC-MS) data analysis, Gundidza et al. (2008) attributed the antibacterial properties displayed by *S. terebinthifolius* essential oil against *P. aeruginosa* (Table 2) to a wide range of bioactive compounds including camphene, m-cymene, 1-β-pinene, α-pinene and γ-terpinene. Camphene, m-cymene, α-pinene and β-pinene were also putatively detected (GC-MS) in the essential oil extracted from *Myrothamnus flabellifolius* by Viljoen et al. (2002), which, however, demonstrated poor antibacterial properties when assessed using the time-kill assay (Table 2). Other putative antibacterial compounds found in the oils were pinocarvone, myrtenol and trans-pinocarveol. Gas chromatography-mass spectroscopy was also used to determine the phytochemical profiles of antibacterial essential oil extracted from *L. leonurus* and *L. ocymifolia* (Oyedeji et al. 2005). Although a comprehensive phytochemical analysis of the oils was conducted, no mention of possible antibacterial compounds was made in the report.

Using a thin-layer chromatography-based bioautography, some researchers identified medicinal plants with promising anti-*P. aeruginosa* compounds (Table 3). Interestingly, some of the medicinal plants evaluated contained more than one active compound against the pathogen, particularly *Ricinus communis*, *Senna italica* and *Sclerocarya birrea* (5, 4 and 3 active inhibition bands, respectively, Table 3). It is worth noting that even though some of the bands significantly inhibited the growth of the bacterium (Table 3), the principal antibacterial compounds were not isolated and identified. Some preliminary phytochemical studies tested the presence of alkaloids, anthraquinones, flavonoids, phenolics, glycosides, saponins and tannins (Chikoto et al. 2005; Mabona et al. 2013; Shikanga, Combrinck & Regnier 2010). These compounds are known to have a wide range of pharmacological properties and as such could have contributed to the observed antibacterial activities.

Antibacterial combination studies

One effective way to combat drug-resistant pathogens is by using combination therapies. As such, modern antibacterial therapies often include combinations of antibiotics with other antibiotics, plant extracts or different chemical entities.

TABLE 4: Antibacterial synergistic interactions between extracts from South African medicinal plants and conventional antibiotics against *Pseudomonas aeruginosa*.

Plant	Plant species	FICI	Reference
Verbenaceae	***Lippia javanica***		
	Essential oil + Ciprofloxacin	0.56	Hübsch et al. (2014)
	Essential oil + Gentamicin	**0.32**	
	Essential oil + Tetracycline	**< 0.5**	
Lamiaceae	***Lavandula angustifolia***		
	Essential oil + Chloramphenicol	**0.29**	De Rapper et al. (2016)
	Essential oil + Ciprofloxacin	0.74	
	Essential oil + Fusidic acid	1.13	
Euphorbiaceae	***Antidesma madagascariense***		
	Acetone leaf extract + Ciprofloxacin	**0.08–0.11**	Seebaluck-Sandoram et al. (2017)
	Acetone leaf extract + Chloramphenicol	**0.16–0.19**	
	Acetone leaf extract + Streptomycin	**0.08–0.11**	

FICI, Fractional inhibitory concentration index.
*, values in bold indicate noteworthy synergistic interactions.

Combination therapies widen the antibacterial spectrum, improve the efficacy of clinically infective drugs and generally delay the development of antibiotic resistance (Chukwujekwu & Van Staden 2016; Tripodi et al. 2007; Zhao et al. 2002). Systematic evaluation of combination therapies used in folk medicine as well as those involving plant extracts and antibiotics could lead to the discovery of new therapeutic compounds.

It was encouraging to note that some South African medicinal plant extracts interacted synergistically with conventional antibiotics against *P. aeruginosa* (Table 4). Nearly all investigations were conducted using the checkerboard bioassay (Rand et al. 1993) in which the efficacy of each pairwise combination was determined using the fractional inhibitory concentration index (FICI). The FICI was obtained by using the formulae, $FICI = FIC_X + FIC_Y$, where FIC_X was the MIC of antibacterial agent X when used in combination with antibacterial agent Y, divided by the MIC of antibacterial agent X when used alone. The results were interpreted, thus $FICI \leq 0.5$ (synergy), $0.5 < FICI \leq 1.0$ (additive) and $1.0 < FICI \leq 4.0$ (no interaction) and $FICI > 4.0$ (antagonism) according to Van Vuuren and Viljoen (2011).

In a study by Hübsch et al. (2014), *P. aeruginosa* was subjected to combinations of *L. javanica* essential oils with each of the antibiotics ciprofloxacin, gentamicin and tetracycline. Only two of the combinations (*L. javanica* + gentamicin and *L. javanica* + tetracycline) yielded noteworthy antibacterial synergism (FICI range: 0.32–0.5), whilst the combination of ciprofloxacin and *L. javanica* resulted in additive effects (FICI = 0.56, Table 4). In a separate study by De Rapper et al. (2016), the combination of *L. angustifolia* essential oils and chloramphenicol also resulted in notable antibacterial synergism (FICI = 0.29). Further investigations by the same authors using isobolograms reaffirmed the results. Seebaluck-Sandoram et al. (2017) observed a 53-fold decrease in the MIC value of ciprofloxacin when used in combination with essential oils extracted from the leaves of *Antidesma madagascariense* against the pathogen. Combinations of the same oils with chloramphenicol or streptomycin also yielded outstanding synergistic effects (FICI range: 0.08–0.19) against the bacterium.

Overall, these findings seem to suggest that some chemical entities in the evaluated extracts inhibited efflux pumps or beta-lactamase enzymes in *P. aeruginosa* ultimately leading to the intracellular accumulation of antibiotics to lethal levels. This possibly explains the significant reduction in the MICs of antibiotics when used in combination with plant extracts, especially in the pairwise combination of *A. madagascariense* essential oil with either ciprofloxacin or streptomycin (FICI: 0.08–0.11). Another possibility is that the combined bactericidal effect of the antibiotics and some phytocompounds made the pathogen more susceptible to death, especially if the two antibacterial agents used had different target sites.

Future prospects

As advised by the WHO, the search for new and effective anti-*P. aeruginosa* agents must be an utmost research priority, especially in countries endowed with plant species richness like SA (WHO 2017). Local researchers could widen the search for such agents by screening plants (medicinal or non-medicinal) closely related to those who previously exhibited noteworthy activities against the pathogen (Table 1, 2, 3). Such taxonomic-based antibacterial screening should perhaps mainly target plants within the same genus. Future antibacterial screening should also include a wide range of drug-resistant mutants of the pathogen. Currently, the efficacy of South African medicinal plants against antibiotic-resistant pathogens is largely unknown (Van Vuuren 2008). The principal antibacterial compounds in medicinal plants that demonstrated potent antibacterial activities (MIC: 0.02–0.09 mg/mL, Table 1) should be isolated and unequivocally identified. Additionally, as the chemical profiles of antibacterial essential oils were putatively determined by GC-MS, consideration should also be given to identifying the principal antibacterial compounds in the oils using spectroscopic and X-ray data analysis techniques.

Furthermore, precedence should not only be given to the search for bactericidal phyto-compounds, but concerted efforts should also be made to discover effective indigenous plant-based resistance modifying compounds. The use of drug-resistance modifying agents (RMAs) in combination therapies could potentially improve the efficacy and hence allow the possible reintroduction of some clinically ineffective antibiotics. From a financial point of view, this approach seems more appealing than developing novel therapeutic agents which customarily have to undergo extensive efficacy and safety evaluations before approval. In a bid to systematically screen SA medicinal plants for RMAs, synergistic evaluations between conventional antibiotics and plant extracts that possess noteworthy antibacterial activities (Tables 1, 2, 3, 4) should be evaluated against various drug-resistant mutants of *P. aeruginosa*. It should, however, be noted that the checkerboard assay is not always reliable and as such all synergistic interactions detected by the method should be confirmed by other more sensitive techniques such as the time-kill bioassay.

Conclusion

Given the intrinsic drug-resistant nature of *P. aeruginosa*, it was interesting to note that the pathogen has not attracted much attention from local ethnobotanists over the past 20 years. However, empirical evidence from the scant literature available strongly suggests that the SA flora, if fully exploited, could contribute immensely to the discovery of potent anti-*P. aeruginosa* drug candidates with potential use in mono- or combination therapies.

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Competing interests

The authors have declared that no competing interests exist.

Authors' contributions

M.V. conceived the original idea and wrote the first draft; G.D.A., K.N. and R.M.C. edited the manuscript, provided resources and supervised the project.

Ethical considerations

This article followed all ethical standards for a research without direct contact with human or animal subjects.

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Data availability

Data created and analysed in the present study were included in this manuscript.

Disclaimer

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