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Antimicrobial and antimalarial activity of *Cussonia* species (Araliaceae)

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ABSTRACT

Ethnopharmacological relevance: Cussonia species are used in African traditional medicine mainly against pain, inflammation, gastro-intestinal problems, malaria and sexually transmitted diseases.
Aim of the study: To summarise ethnomedicinal uses of *Cussonia* and to find scientific evidence in support of selected main uses.
Materials and methods: Using the minimum inhibitory concentration (MIC) method, leaves of 13 Cussonia species, *Schefflera umbellifera* and *Seemannaralia gerrardii* were tested against pathogens associated with diarrhoea (*Enterococcus faecalis* and *Escherichia coli*), sexually transmitted infections (*Neisseria gonorrhoeae* and *Trichomonas vaginalis*) and general infectious diseases (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). Antimalarial sensitivity was studied using *Plasmodium falciparum* and the [^{3}H]-hypoxanthine incorporation assay. Cytotoxic effects on a T-cell leukaemia (Jurkat) cell line were determined using the tetrazolium-based cellular toxicity assay.
Results: Methanolic extracts were active against *Pseudomonas aeruginosa* (MIC of 1.0–1.5 mg/mL), *Trichomonas vaginalis* (MIC of 0.8–1.3 mg/mL) and *Staphylococcus aureus* (*Cussonia arborea*, 1.8 mg/mL). All samples were active against *Neisseria gonorrhoeae* (MIC of 0.02–0.7 mg/mL). The methanol extract of *Cussonia arborea* was the most active against *Plasmodium falciparum* (13.68 μg/mL) and showed anticancer properties (5.60 μg/mL).
Conclusions: The traditional use of *Cussonia* species to treat sexually transmitted diseases and *Plasmodium* infections appears to have a scientific basis.

1. Introduction

Africa has a long and rich history of traditional plant use, the diversity of which varies with local cultures and tradition (Watt and Breyer-Brandwijk, 1962). Indigenous plants are still a main source of medicine, craft materials and tools in Africa (Teklehaymanot and Giday, 2007). It was estimated, in the late 1980s, that up to 65% of Africa's population rely solely or partially on traditional herbal medicines (Farnsworth et al., 1985). Recording the traditional uses of plants also aids in the conservation of indigenous culture, knowledge and belief systems (Grace et al., 2003). Governments of most countries in Africa have come to recognize the importance of recording traditional knowledge for further development, such as Ethiopia (Teklehaymanot and Giday, 2007), Tanzania (Gessler et al., 1995) and South Africa (Grace et al., 2003). Traditional medicinal plants are also a rich source of inexpensive and novel biologically active compounds (Hostettmann et al., 2000), which are of great interest to both the developed and developing countries (McGaw et al., 2000). The evaluation of the efficacy, safety and dosage of traditional medicines is crucial, due to the reliance of the African population on plants as sources of medicines (Masika and Afolayan, 2002).

Cussonia Thunb. (Araliaceae) consists of 21 described species (Frodin and Govaerts, 2003) that occurs throughout the grasslands and woodlands of sub-Saharan Africa and Yemen (Arabian Peninsula). The uses of *Cussonia* species (and the related genus *Schefflera* J.R. and G. Forst.) in African traditional medicine are well documented and are summarized in Table 1. Of the 21 *Cussonia* species, 11 have recorded uses and these include applications for magical purposes, the making of implements and the treatment of diseases. There are recurrent uses between the species and also for the same species in different parts of the African continent. The main uses of *Cussonia* are as an analgesic, antimalarial and anti-inflammatory medicine, as well as a treatment against mental illness, sexually transmitted diseases and diarrhoea (Table 1). Based

Abbreviations: ATCC, American type culture collection; CFU, colony forming units; DCM, dichloromethane; DMSO, dimethyl sulfoxide; GC, gonococcal; MeOH, methanol; MIC, minimum inhibitory concentration; MTT, 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide; NCCLS, National Committee on Clinical Laboratory Standards; NHLS, National Health Laboratory Services; WHO, World Health Organization; STI, sexually transmitted infection.

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Table 1
A summary of the traditional medicinal uses of *Cussonia* and *Schefflera* species as recorded in the literature. Abbreviations for species listed—*Cussonia arborea*: Ca; *Cussonia bancoensis*: Cb; *Cussonia holstii*: Ch; *Cussonia natalensis*: Cna; *Cussonia nicholsonii*: Cni; *Cussonia paniculata*: Cp; *Cussonia ostinii*: Co; *Cussonia spicata*: Cs; *Cussonia zimmermannii*: Czi; *Cussonia zuluensis*: Czu; *Schefflera umbellifera*: Su.

Traditional use	Species	References
Analgesic, anti-inflammatory and wound healing	Ca, Cb, Ch, Cp, Cs, Su	Phillip, 1917; Watt and Breyer-Brandwijk, 1962; Sandberg, 1965; Lindsay and Hepper, 1978; Haxaire, 1979; Gelfand et al., 1985; Iwu, 1993; Hutchings et al., 1996; Neuwinger, 2000; Shin et al., 2004; Abubakar et al., 2007; Teklehaymanot and Giday, 2007.
Antidote to poisons	Ca, Cs	Quimby and Persinos, 1964; Kokwaro, 1976; Haxaire, 1979; Boulesteix and Guinko, 1979; Mabogo, 1990; Neuwinger, 2000; Lovett et al., 2006.
Blood problems	Ca, Czi	Quimby and Persinos, 1964; Haxaire, 1979; Gelfand et al., 1985; Baerts and Lehmann, 1989; Lovett et al., 2006; Konadu, 2007.
Malaria	Ca, Cp, Cs, Czi, Su	Watt and Breyer-Brandwijk, 1962; Hardi, 1964; Quimby and Persinos, 1964; Haxaire, 1979; Chhabra et al., 1984; Burkill, 1985; Gelfand et al., 1985; Hutchings, 1989; Baerts and Lehmann, 1989; Mabogo, 1990; Gessler et al., 1994, 1995; Hutchings et al., 1996; Neuwinger, 2000.
Fever	Ca	Kerharo and Bouquet, 1950; Kokwaro, 1976.
Infectious diseases	Ca, Cs, Czi	Kerharo and Bouquet, 1950; Bouquet and Debray, 1974; Ferry et al., 1974; Boulesteix and Guinko, 1979; Burkill, 1985; Malgras, 1992; Neuwinger, 2000.
Eye problems	Ca	Haxaire, 1979; Malgras, 1992.
General tonic	Ca, Co	Sillans, 1954; Bouquet and Debray, 1974; Burkill, 1985; Neuwinger, 2000, Amenu, 2007.
Gastro-intestinal problems	Ca, Can, Cni Cs	Watt and Breyer-Brandwijk, 1962; Bouquet and Debray, 1974; Haxaire, 1979; Burkill, 1985; Heine and König, 1988; Mabogo, 1990; Neuwinger, 2000; Amusan et al., 2002; Arbonnier, 2004; Kisangau et al., 2007.
Gynaecological conditions	Ca, Cb, Ch	Gelfand et al., 1985; Neuwinger, 2000; Fassil, 2004; Konadu, 2007; Lovett et al., 2006.
Internal and external parasites	Ca	Haxaire, 1979; Ladikpo, 1981; Bizimana, 1994; Neuwinger, 2000.
Magic	Ca, Cp, Cs, Czi, Czu	Phillip, 1917; Krige, 1968; Burkill, 1985; Baerts and Lehmann, 1989; Malgras, 1992; Lovett et al., 2006.
Mental illness	Ca, Cp, Cs, Czi	Watt and Breyer-Brandwijk, 1962; Hardi, 1964; Chhabra et al., 1984, 1987; Burkill, 1985; Gelfand et al., 1985; Iwu, 1993; Neuwinger, 2000; Moshi et al., 2005.
Respiratory problems	Ca, Ch, Co	Kerharo and Bouquet, 1950; Ferry et al., 1974; Iwu, 1993; Koné et al., 2004.
Sexually transmitted diseases	Ca, Cb, Cs, Czi	Bally, 1937; Watt and Breyer-Brandwijk, 1962; Hardi, 1964; Kokwaro, 1976; Hutchings, 1989; Ndubani and Höjer, 1999; Neuwinger, 2000; Lovett et al., 2006
Skin problems	Cs	Watt and Breyer-Brandwijk, 1962

on the recorded ethnobotanical uses, it was decided to investigate the efficacy of *Cussonia* extracts against gonorrhoea, malaria and various pathogens that cause infection.

Gonorrhoea is one of the most common venereal diseases in Africa, which is spread primarily by sexual intercourse (Silva et al., 2002). The infection is caused by the bacterium *Neisseria gonorrhoeae* and it affects the mucous membrane of the vagina or the urethra of the male. Symptoms can include painful inflammation, leukorrhea (a white or green discharge), itching and painful passing of urine (Roberts and Janovy, 1996; Atlas, 1997). It is estimated that more than 340 million new episodes of curable sexually transmitted diseases occur each year and that gonorrhoea is one of the most prevalent infections (WHO, 2008a). The *Neisseria gonorrhoeae* infection is treated with *Cussonia* by either applying a water decoction or maceration of *Cussonia arborea* root or bark topically, by drinking a root tea or by bathing in a vapour bath made of the root (Bally, 1937; Watt and Breyer-Brandwijk, 1962; Kokwaro, 1976; Ndubani and Höjer, 1999). Macerations can be made of *Cussonia zimmermannii* leaves and applied topically (Hardi, 1964). It has been documented that gonorrhoea is treated with an unknown *Cussonia* species in Tanzania (Brenan and Greenway, 1949), which is probably *Cussonia holstii* or *Cussonia spicata* (the two most common and widely distributed species in Tanzania). Authors have mentioned *Cussonia holstii* and *Cussonia spicata* root for the treatment of venereal diseases and have only speculated that it could be gonorrhoea (Hutchings, 1989). Even though the genus is widespread throughout Africa, no anti-gonorrhoeal investigations have been performed.

Diarrhoea is another recorded condition treated with *Cussonia* (Table 1). It can be caused by various pathogens and is also one of the symptoms of a malaria infection (Gessler et al., 1995). The traditional treatment of diarrhoea, with *Cussonia*, is to drink either a root decoction of *Cussonia arborea* (Heine and König, 1988; Kisangau et al., 2007) or a decoction of leaves from *Cussonia arborea*, *Aframomum latifolium* (Afzel.) K. Schum. (Zingiberaceae), *Eriosema psoraleoides* (Lam.) G. Don. (Fabaceae), *Euphorbia hirta* L. (Euphorbiaceae), *Ipomoea batatas* (L.) Lam. (Convolvulaceae), *Markhamia sessilis* Sprague (Bignoniaceae), *Terminalia laxiflora* Engl. (Combretaceae) and *Vernonia glaberrima* Welw. (Asteraceae) (Haxaire, 1979). Watt and Breyer-Brandwijk (1962) also reported the use of *Cussonia* for gastro-intestinal complaints.

The WHO estimated that at least 250 million clinical cases of malaria occurred in 2006, which led to a million deaths (WHO, 2008b). The infection is caused either by the *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* or *Plasmodium ovale* parasite (in humans) and is transmitted by *Anopheles* mosquitoes (Roberts and Janovy, 1996). An increase in the resistance of the parasite (mainly *Plasmodium falciparum*) to prophylaxis and chemotherapy has resulted in the search for new and safe compounds or drugs (Muthaura et al., 2007). The traditional use of *Cussonia arborea* is to drink a decoction made from the bark, together with *Swartzia madagascariensis* Desv. (Fabaceae) bark and *Aframomum latifolium* leaves (Haxaire, 1979; Burkill, 1985) or a root decoction (Neuwinger, 2000). An infusion of *Cussonia spicata* bark or root (Watt and Breyer-Brandwijk, 1962; Hutchings, 1989; Hutchings et al., 1996) or the leaves (Chhabra et al., 1984; Burkill, 1985; Mabogo, 1990; Lovett et al., 2006) are used to treat malarial infections. A root infusion (Hardi, 1964; Gessler et al., 1994, 1995) or a root decoction (Neuwinger, 2000) of *Cussonia zimmermannii* can be taken orally. *Cussonia paniculata* subsp. *sinuata* (Reyneke & Kok) De Winter (parts unknown) (Hutchings et al., 1996) and the bark of *Schefflera umbellifera* (Watt and Breyer-Brandwijk, 1962) can also be used to treat malaria.

Watt and Breyer-Brandwijk (1962) reported that the roots are poisonous, even though it can be used as a source of water. The leaves of *Cussonia spicata* are used as a fish poison in Tanzania. Luseba et al. (2007) showed that *Cussonia spicata* does not have any mutagenic or anti-mutagenic effects on *Salmonella typhimurium*. These conflicting reports encourage the investigation into the safety of *Cussonia* as a traditional medicine.

The uses of *Cussonia* species against such ailments as malaria and gonorrhoea suggest that their efficacy and safety should be tested. Polyacetylenes, flavonoids and saponins occur in several *Cussonia* species (Dadoun and Cave, 1972; Gunzinger et al., 1986; Haruna

Table 2
Voucher specimens of the material of *Cussonia*, *Seemannaralia* and *Schefflera* used for antimicrobial, antimalarial and cytotoxicity studies.

Taxa	Voucher specimen	Locality	Date collected
Cussonia arborea Hochst. ex A. Rich.	*M. Coates Palgrave s.n.* (JRAU)	Mukuvisi Woodland Park, Harare, Zimbabwe	03-2009
Cussonia arenicola Strey	*B.J. de Villiers, P.M. Tilney and A.A. Oskolski 78* (JRAU)	Lowveld Botanical Garden, Nelspruit, Mpumalanga	16-09-2006
Cussonia gamtoosensis Strey	*B.J. de Villiers, C. de Villiers, A.R. Magee and M.M. le Roux 51* (JRAU)	Gamtoos River, Jeffrey's Bay, Eastern Cape	20-01-2006
Cussonia natalensis Sond.	*B.J. de Villiers, P.M. Tilney and A.A. Oskolski 72* (JRAU)	near Ohrigstad, Mpumalanga	15-09-2006
Cussonia nicholsonii Strey	*B.J. de Villiers, P.M. Tilney and A.A. Oskolski 77* (JRAU)	Lowveld Botanical Garden, Nelspruit, Mpumalanga	16-09-2006
Cussonia paniculata Eckl. and Zeyh. subsp. *paniculata*	*B.J. de Villiers and C. de Villiers 49* (JRAU)	Walter Sisulu Botanical Garden, Johannesburg, Gauteng	17-07-2004
Cussonia paniculata subsp. *sinuata* (Reyneke et Kok) De Winter	*B.J. de Villiers and B-E. van Wyk 110* (JRAU)	Sani Pass, KwaZulu-Natal	13-09-2008
Cussonia sphaerocephala Strey	*B.J. de Villiers, P.M. Tilney and A.A. Oskolski 81* (JRAU)	Lowveld Botanical Garden, Nelspruit, Mpumalanga	16-09-2006
Cussonia spicata Thunb. 1	*B.J. de Villiers 1* (JRAU)	Weltevreden Park, Johannesburg, Gauteng	07-04-2004
Cussonia spicata 2	*B.J. de Villiers and C. de Villiers 37*	ARC Elsenburg Research Station, Stellenbosch, Western Cape	07-09-2006
Cussonia thyrsiflora Thunb.	*B.J. de Villiers and C. de Villiers 64* (JRAU)	Harold Porter Botanical Garden, Betty's Bay, Western Cape	06-09-2006
Cussonia transvaalensis W.F. Reyneke	*B.J. de Villiers 34* (JRAU)	University of Pretoria Botanical Garden, Pretoria, Gauteng	22-08-2005
Cussonia zuluensis Strey	*B.J. de Villiers and C. de Villiers 43* (JRAU)	Kirstenbosch Botanical Gardens, Rondebosch, Western Cape	30-08-2004
Seemannaralia gerrardii (Seem.) Harms	*B.J. de Villiers, A.A. Oskolski and B-E. van Wyk 97* (JRAU)	Roselands Farm, Richmond, KwaZulu-Natal	23-04-2007
Schefflera umbellifera (Sond.) Baill.	*B.J. de Villiers, P.M. Tilney and A.A. Oskolski 75* (JRAU)	Lowveld Botanical Garden, Nelspruit, Mpumalanga	16-09-2006

et al., 1994; Senn et al., 2007) and our own investigations have shown that the triterpenoid and flavonoid patterns are conservative. It therefore stands to reason to also explore additional species, other than those used ethnobotanically. The aim of this study is to identify the main medicinal uses of *Cussonia* and to investigate the efficacy and toxicity of methanolic and aqueous leaf extracts against selected pathogens.

2. Materials and methods

2.1. Materials studied

The biological activity tests were done with leaf samples from 13 species of *Cussonia* and one sample each of the closely related *Schefflera umbellifera* and *Seemannaralia gerrardii* (Seem.) Harms. We decided to use leaves because the sampling of root and bark material would have been too destructive. Voucher specimens of all the tested extracts (listed in Tables 3 and 4) are given in Table 2. The specimens are housed in the University of Johannesburg Herbarium (JRAU) and were authenticated by B.J. de Villiers and B-E. van Wyk, both from the Department of Botany and Biotechnology, University of Johannesburg. A taxonomic revision of the genus *Cussonia* is currently in progress in this department.

2.2. Methods

2.2.1. Extract preparation

Leaves were air-dried and macerated before extraction. For each species, two samples of 10 g were extracted, the one with 250 mL methanol and the other with 250 mL double distilled water. In the case of the geographically variable *Cussonia spicata*, four samples were used. The samples were agitated by hand for 10 min and left to extract for 24 h. The extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper. To dry the samples, the methanolic extracts were passed over gaseous nitrogen and the aqueous extracts were freeze dried using a Virtis freeze drier with a RV5 BOC Edwards pump. The percentage yield of each extract is listed in Table 3.

2.2.2. Micro-organisms and human cell line

The choice of test organisms was based on a consideration of the recorded ethnobotanical uses (Table 1). *Enterococcus faecalis* (ATCC 29212) and *Escherichia coli* (ATCC 8379) were included on the basis of its causative association with gastro-intestinal infections. Due to its association with wound and skin infections, *Pseudomonas aeruginosa* (ATCC 27853) was studied. *Staphylococcus aureus* (ATCC 6538) is the cause of various infections, which include wound, skin and gastro-intestinal (diarrhoea) and was included in the study. Two sexually transmitted micro-organisms, *Trichomonas vaginalis* (a clinical strain was obtained from the STI Reference Centre at NHLS as no reference strain is available) and *Neisseria gonorrhoea* (ATCC 19424), were also investigated.

Plasmodium falciparum was studied based on the fact that it causes malignant tertian, subtertian or estivoautumnal malaria, is the most virulent of all the *Plasmodium* species and is responsible for 50% of the malarial infections worldwide (Roberts and Janovy, 1996). The chloroquine-sensitive (3D7) malaria protozoan, *Plasmodium falciparum*, was used to evaluate the *in vitro* antiplasmodial properties of the 30 extracts. The cytotoxic properties were determined using the human T-cell leukaemia (Jurkat) cancer line.

2.2.3. Minimum inhibitory concentrations

The microplate bioassay minimum inhibitory concentrations (MIC) were determined using the microplate method (Eloff, 1998; NCCLS, 2003). The cultures (*Escherichia coli*, *Enterococcus faecalis, Pseudomonas aeruginosa*, and *Staphylococcus aureus*) were subcultured from stock Mueller-Hinton agar plates and grown in Tryptone Soya broth overnight. *Trichomonas vaginalis* was subcultured from blood agar (Mueller-Hinton broth supplemented with 5% sheep's blood) and grown in Diamonds medium (NHLS). The bacterial culture of *Neisseria gonorrhoeae* was subcultured from stock agar plates (GC agar base supplemented with 5% sheep's blood and GC selectavial (LCAT) supplement (Davies Diagnostics). Distilled, sterile water (100 μL) were placed into each well of aseptically prepared microtitre plates. The dried methanolic extracts (100 μL) were reconstituted in acetone and the dried aqueous extracts (100 μL) were reconstituted in distilled sterile water to

Table 3
The mean minimum inhibitory concentrations (mg/mL) for methanolic (MeOH) and aqueous (H_2O) extracts of the leaves of *Cussonia* species, *Schefflera umbellifera* and *Seemannaralia gerrardii*. NI: no inhibition activity shown at the highest concentration tested.

Test samples	% yield		*Staphylococcus aureus*		*Escherichia coli*		*Enterococcus faecalis*		*Pseudomonas aeruginosa*		*Trichomonas vaginalis*		*Neisseria gonorrhoeae*	
	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O
Cussonia arborea	31.6	16.0	1.8	6.7	4.0	6.0	12	8.0	3.0	NI	1.3	NI	0.5	0.3
Cussonia arenicola	9.2	14.2	6.0	NI	4.0	16	16	NI	1.0	8.0	0.5	12	0.4	0.4
Cussonia gamtoosensis	6.2	14.1	4.0	NI	8.0	NI	8.0	4.0	1.0	6.0	0.3	NI	0.4	0.3
Cussonia natalensis	6.9	9.9	6.0	4.0	4.0	8.0	8.0	8.0	1.0	NI	1.0	NI	0.6	0.3
Cussonia nicholsonii	4.4	4.1	6.0	4.0	8.0	4.0	NI	NI	2.0	4.0	1.0	NI	0.02	0.7
Cussonia paniculata subsp. paniculata	10.2	6.2	6.0	NI	4.0	10.7	8.0	16	2.0	4.0	1.0	NI	0.4	0.1
Cussonia paniculata subsp. sinuata	16.9	13.4	NI	2.0	4.0	NI	NI	10.7	1.0	NI	1.3	NI	0.3	0.3
Cussonia sphaerocephala	8.1	8.6	8.0	8.0	4.0	6.0	8.0	NI	8.0	NI	1.0	NI	0.2	0.5
Cussonia spicata 1	22.5	17.5	4.0	NI	7.0	NI	8.0	4.0	1.4	NI	1.3	NI	0.4	0.3
Cussonia spicata 2	30.2	22.3	2.0	2.0	4.0	8.0	4.0	16	1.0	NI	0.8	13.3	0.3	0.3
Cussonia thyrsiflora	16.3	12.5	8.0	NI	6.0	13.3	12	4.0	2.0	4.0	1.0	NI	0.3	0.3
Cussonia transvaalensis	6.0	8.4	4.0	NI	4.0	NI	8.0	4.0	1.5	1.5	0.8	8.0	0.5	0.3
Cussonia zuluensis	15.2	7.8	4.0	1.5	4.0	9.3	8.0	8.0	4.0	8.0	0.8	NI	0.2	0.3
Seemannaralia gerrardii	7.5	9.8	8.0	3.0	NI	10.7	8.0	6.0	6.0	8.0	4.0	NI	0.1	0.3
Schefflera umbellifera	18.4	16.3	4.0	NI	6.7	NI	NI	NI	1.5	NI	1.5	4.5	0.23	0.8
Control—ciprofloxacin			0.00063		0.00078		0.00047		0.00012		0.00119		0.00017	

Voucher specimens were deposited in the University of Johannesburg Herbarium (JRAU).

a starting concentration of 32 or 64 mg/mL. The extracts were subsequently transferred into the first rows of a microtitre plate and serial dilutions were performed. Twenty four hour stock cultures of *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were diluted in fresh Tryptone Soya broth, while *Trichomonas vaginalis* was diluted in Diamond medium and *Neisseria gonorrhoeae* was diluted in a Mueller-Hinton broth with 5% sheep's blood and GC selectavial (LCAT) supplement (Davies diagnostic). The dilutions were standardised to a 0.5 McFarland standard (approximate inoculum size of 1×10^6 CFU/mL) and 100 μL was added to all wells (NCCLS, 2003). To confirm antimicrobial susceptibility, ciprofloxacin was included as the positive control at a starting stock concentration of 0.01 mg/mL. Incubations at optimal conditions (37 °C for 24 h) followed for *Escherichia coli*, *Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Trichomonas vaginalis,* while *Neisseria gonorrhoeae* was incubated at 35 °C for 24 h in a candle jar (5% CO_2-enriched atmosphere with humidity). A 0.2 mg/mL *p*-iodonitrotetrazolium violet solution was transferred into the inoculated wells of *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa, Trichomonas vaginalis* and *Staphylococcus aureus* (40 μL). After 6 h the colour change (for each well) was examined to determine the relation in concentration to the microbial growth. For *Neisseria gonorrhoeae,* organism growth was indicated by a change to a milky colour. Tests were performed in duplicate (or in triplicate, where resulting MIC values did not show congruency after the second replicate). Any activities equivalent to or greater than 16 mg/mL were omitted from the data (Table 3).

The MIC values for *Neisseria gonorrhoeae* were confirmed by spreading randomly selected serial extract dilutions on GC agar plates with selectavial (LCAT) supplement and incubating for 24 h at 35 °C in a candle jar. The agar plates were examined for microbial growth to confirm MIC results.

2.2.4. Antiplasmodial activity

The tritiated hypoxanthine incorporation assay was used to determine the sensitivity of the chloroquine-sensitive (3D7) strain of *Plasmodium falciparum* to the extracts (Desjardins et al., 1979; Van Zyl et al., 2008). The parasite suspension, consisting of predominately the ring stage, was adjusted to 0.5% parasitaemia and 1% haematocrit and exposed to the various concentrations of extracts for a single cycle of parasite growth. All assays were carried out using untreated parasites and uninfected red blood cells as controls. Labelled ^{3}H-hypoxanthine (0.5 μCi/well) was added after 24 h and the parasitic ^{3}H-DNA harvested after a further 24 h incubation period. The concentration that inhibits 50% of parasite growth (IC_{50} value) was determined from the log sigmoid dose–response curve generated by GraphPad Prism™. To confirm antimalarial susceptibility, quinine was included as the positive control and all the tests were done in triplicate.

2.2.5. Cytotoxicity test

The cytotoxicity of the extracts was determined against the human T-cell leukaemia (Jurkat) cell line using the MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) cellular viability assay (Mosmann, 1983; Van Zyl et al., 2008). A cell suspension of 45,000 cells/mL (counted with a haemocytometer) was plated out along with the extracts for 44 h at 37 °C in 5% CO_2 at which time, 2 mM MTT was added and incubated for a further 4 h. DMSO was then added to stop the reaction and to dissolve the formazan crystals. The absorbance was read at the test wavelength of 540 nm and reference wavelength of 690 nm. The percentage cellular viability was calculated with appropriate controls taken into account. The concentration that inhibits 50% cellular growth (IC_{50} value) was determined from the log sigmoid dose response curve generated by GraphPad Prism™. To confirm cytotoxicity suscep-

Table 4
The antimalarial and cytotoxic activity for methanol (MeOH) and aqueous (H_2O) extracts of the leaves of *Cussonia* species, *Schefflera umbellifera* and *Seemannaralia gerrardii*. The IC_{50} values ± standard deviations (μg/mL) are shown for the active samples. The safety index is the IC_{50} for T-cell leukemia cytotoxicity/IC_{50} for antimalarial activity. ND: not determined.

Test samples	*Plasmodium falciparum*		T-cell leukemia (Jurkat)		Safety index (MeOH)
	MeOH	H_2O	MeOH	H_2O	
Cussonia arborea	13.68 ± 1.08	>50	5.60 ± 1.09	>50	0.41
Cussonia arenicola	>50	>50	>50	>50	
Cussonia gamtoosensis	>50	>50	>50	>50	
Cussonia natalensis	>50	>50	>50	>50	
Cussonia nicholsonii	>50	>50	>50	>50	
Cussonia paniculata subsp. *paniculata*	>50	>50	>50	>50	
Cussonia paniculata subsp. *sinuata*	>50	>50	26.48 ± 1.12	>50	>0.53
Cussonia sphaerocephala	32.31 ± 1.19	>50	>50	>50	>1.55
Cussonia spicata 1	28.20 ± 1.23	>50	27.69 ± 1.09	>50	0.98
Cussonia spicata 2	20.20 ± 1.19	>50	23.89 ± 1.07	>50	1.18
Cussonia thyrsiflora	>50	>50	>50	>50	
Cussonia transvaalensis	>50	>50	>50	>50	
Cussonia zuluensis	>50	>50	36.98 ± 1.05	>50	>0.74
Seemannaralia gerrardii	ND	>50	ND	>50	
Schefflera umbellifera	>50	>50	>50	>50	
Control-quinine	0.037 ± 0.00052				
Control-(S)-(+)-camptothecin			0.0730 ± 0.01616		

Voucher specimens were deposited in the University of Johannesburg Herbarium (JRAU).

tibility, (S)-(+)-camptothecin was included as the positive control and all the tests were done in triplicate.

3. Results

The antimicrobial MIC results are given in Table 3. The highest activities noted against the pathogen *Staphylococcus aureus* was 1.8 mg/mL (methanolic extract of *Cussonia arborea*) and 1.5 mg/mL (aqueous extract of *Cussonia zuluensis*). Other *Cussonia* and related species indicated moderate sensitivity against this pathogen. Similar poor to moderate sensitivities were noted for all the *Cussonia* and related species against *Escherichia coli* and *Enterococcus faecalis*. Six *Cussonia* species [*Cussonia arenicola*, *Cussonia gamtoosensis*, *Cussonia natalensis*, *Cussonia paniculata* subsp. *sinuata* *Cussonia spicata* (both extracts) and *Cussonia transvaalensis*] displayed good antimicrobial activity (1.0–1.5 mg/mL) when tested against *Pseudomonas aeruginosa*. The methanol extracts of the *Cussonia* species displayed mostly noteworthy sensitivity with MIC values ranging from 0.3 to 1.5 mg/mL against the STI pathogen *Trichomonas vaginalis*. Similarly, the STI pathogen *Neisseria gonorrhoeae* was also particularly sensitive to the *Cussonia* and related species. The anti-gonorrhoeal activity of the species tested had a minimum inhibition concentration between 0.02 and 0.7 mg/mL. The related species *Seemannaralia gerrardii* and *Schefflera umbellifera* indicated similar noteworthy sensitivities (0.1–0.8 mg/mL) towards *Neisseria gonorrhoeae*. The methanolic extract of *Cussonia nicholsonii* (methanol extract) had the most noteworthy activity with a MIC value of 0.02 mg/mL.

The methanol extracts possessed more favourable antimalarial properties than the aqueous extracts, with *Cussonia arborea*, *Cussonia sphaerocephala* and *Cussonia spicata* inhibiting 50% parasite growth at concentrations ranging from 13.68 to 32.31 μg/mL (Table 4). *Cussonia paniculata* subsp. *paniculata*, *Cussonia paniculata* subsp. *sinuata*, *Cussonia gamtoosensis*, *Cussonia transvaalensis*, *Cussonia nicholsonii*, *Cussonia natalensis* and *Schefflera umbellifera* inhibited on average 90.87 ± 9.32% parasite growth at 100 μg/mL and 0% at 50 μg/mL. This indicates that the IC_{50} value for these species lay between 50 and 100 μg/mL. Of the 15 aqueous extracts evaluated, all but *Cussonia thyrsiflora* displayed negligible antimalarial or cytotoxic properties at 50 or 100 μg/mL, where 100 μg/mL of methanolic extract of *Cussonia thyrsiflora* resulted in 44.75 ± 3.37% parasite growth inhibition. Unfortunately the inhibitory effect towards the parasite was also observed when the human T-cell leukaemia cell line was exposed to these latter extracts. *Cussonia arborea* was the most cytotoxic with a safety index of 0.41. *Cussonia paniculata* subsp. *sinuata* and *Cussonia zuluensis* also inhibited leukaemia cell growth (Table 4).

4. Discussion

Papajewski et al. (1998, 2001) showed that the polyacetylenes isolated from *Cussonia arborea* have antibacterial activity (against *Bacillus subtilis* and *Pseudomonas fluorescens*), antifungal activity (against *Cladosporium cucumerin*), anti-molluscicidal activity (against *Biomphalaria glabrata*) and haemolytic activity. McGaw et al. (2000) reported that ethanolic and ethyl acetate extracts of the bark and roots of *Cussonia spicata* showed *Staphylococcus aureus* inhibition in disc-diffusion assays and MIC's of 12.5 mg/mL. Tetyana et al. (2002) reported disc diffusion inhibition for *Cussonia spicata* where the ethyl acetate and ethanol extract showed highest inhibitory activity against *Staphylococcus aureus*. Our results showed MIC values ranging between 4.0 and 8.0 mg/mL for the methanolic extracts when tested against *Staphylococcus aureus*. The extracts tested here did not show any significant activity against *Escherichia coli* which is in agreement with the study by Tetyana et al. (2002). Shai et al. (2008) investigated the possible use of *Cussonia zuluensis* against bacterial and fungal infections, but the negative results supported the lack of traditional use against infections for this species. *Cussonia holstii* showed significant biological activity against *Trichomonas* species (He et al., 2003). The dichloromethane extracts of bark of this species contain a pentacyclic triterpenoid that exhibited an IC_{50} of 2.8 μM (He et al., 2003). Calzada et al. (2007) tested 23 Mexican medicinal plants against *Trichomonas vaginalis* and found that the IC_{50} ranged between 0.03 and 0.9 mg/mL. Cos et al. (2002) tested the minimum inhibition concentration of 45 different plants from Rwanda against *Pseudomonas aeruginosa*, which ranged between 0.16 and 0.5 mg/mL. Atawodi (2004) and Biapa et al. (2007) have shown that *Cussonia arborea* has a potential use as an anti-oxidant. Anti-oxidative flavonoids and tannins have been reported to be responsible for the anti-diarrhoeal activity (Biapa et al., 2007), hence the possible traditional use of *Cussonia arborea*.

Various *Cussonia* species are used in Africa to treat gonorrhoea. *Cussonia arborea* is the most frequently reported species. Previous anti-gonorrhoeal studies on herbal medicines include the study of Silva et al. (2002)—*Terminalia macroptera* ethanolic

extracts displayed MIC values of 0.1–0.2 mg/mL. Ruddock et al. (2005) obtained an MIC value of 10 mg/mL when crude garlic was tested against gonorrhoea. Kambizi and Afolayan (2008) demonstrated an MIC value of 0.5 mg/mL for methanolic extracts of *Aloe ferox*, and *Withania somnifera* also showed excellent activity of 0.5 mg/mL (methanolic extract) and 10 mg/mL (aqueous extract). Both the methanolic and aqueous extracts of the three genera tested in this report compares favourably to that of other studies. There appear to be no consistent differences in the anti-gonorrhoeal activity between the species tested, between the samples within the same species and that of the related genera. The chemical compound or compounds responsible for the high activities are as yet unknown.

This study provides an overview of the antimalarial and cytotoxic properties of 13 of the 21 species of *Cussonia*, as well as *Schefflera umbellifera* and *Seemannaralia gerrrardii* (Table 4). Previous studies have also indicated variable activity of *Cussonia* species with the aqueous extracts displaying no antimalarial activity, regardless of the fact that this is the preferred method of preparing the plants when used in traditional medicines (Tetyana et al., 2002; Clarkson et al., 2004). The roots of *Cussonia spicata* and *Cussonia zimmermannii* and the bark of *Schefflera umbellifera* are traditionally used for the successful treatment of malaria (Gessler et al., 1994; Tetyana et al., 2002). The methanolic leaf extracts of *Cussonia arborea*, *Cussonia sphaerocephala* and *Cussonia spicata* displayed moderate activity (IC_{50}: 13–32 μg/mL). Clarkson et al. (2004) reported that the methanol leaf extracts of *Cussonia spicata* and *Schefflera umbellifera* also possessed moderate activity (27.5 and 49.5 μg/mL, respectively). It was shown that the methanol root extract of *Cussonia spicata* showed moderate activity with an IC_{50} value of 45.1 μg/mL (Kraft et al., 2003). Recently, Mthembu et al. (2010) reported that the antimalarial activity of *Schefflera umbellifera* can be ascribed to a lupine-type compound, betulin (IC_{50}: 3.2 μg/mL). These findings support this study, even though, in some of the studies, a different *Plasmodium falciparum* strain and methodology was used. It is interesting to note that those solvents which extracted more lipophilic compounds from the extracts yielded more potent inhibitory activity than the polar solvents. The petroleum ether root bark extract of *Cussonia zimmermannii*, dichloromethane leaf extract of *Schefflera umbellifera* and dichloromethane/methanol leaf extract of *Cussonia spicata* had IC_{50} values of 3.3, 3.7 and 13 μg/mL, respectively (Gessler et al., 1994; Clarkson et al., 2004; Senn et al., 2007). The former promising activity of *Cussonia zimmermannii* and isolated compounds was also noted for other protozoal species, namely trypanosomes and leishmania (Senn et al., 2007). A few authors have identified saponins in the genus, for example Dubois et al. (1986) and Papajewski et al. (1998). Kougan et al. (2009) found that the isolated saponins from *Cussonia arborea* have the potential to cause haemolysis, which would result in parasite death. However no haemolysis was observed at 50 μg/mL in this study.

The low safety index is indicative of the high *in vitro* cytotoxicity of the extracts and the lack of selectivity in inhibiting the malaria parasite (Table 4). *Cussonia arborea* was the most cytotoxic (safety index of 0.41), but the low IC_{50} value (5.60 μg/mL) against T-cell leukaemia warrants further research to determine if this promising inhibitory activity is selective to cancer cells or to "normal" cells. Fouche et al. (2008) reported that the dichloromethane/methanol leaf extract of *Cussonia paniculata* inhibited 100% cell growth at concentrations of 1.0 (leukaemia RPMI-8226), 1.45 (colon HCT116), 2.69 (colon KM12), 5.25 (renal TK10), 15.0 (melanoma UACC62) and 55.68 μg/mL (breast MCF7). In contrast, Reid et al. (2006) reported that the methanol and dichloromethane leaf extracts of *Cussonia* species (not named in the publication) possessed no mutagenic or antimutagentic properties. A saponin known from *Cussonia* species (23-hydroxyursolic acid) has been reported to induce apoptosis via activation of caspases in human cervical squamous carcinoma HeLa cells (Takaya et al., 2009). The compound was isolated from *Cussonia arborea* (Kougan et al., 2009), *Cussonia bancoensis* (Tapondjou et al., 2002, 2003; Takaya et al., 2009) and *Cussonia paniculata* (Dovgii et al., 2005; Grishkovets et al., 2005) and its presence may explain the observed cytotoxic activity of *Cussonia arborea* and *Cussonia paniculata*.

5. Conclusions

Biological investigations indicated that certain species of *Cussonia* displayed promising antimicrobial activity against *Pseudomonas aeruginosa*. Methanolic extracts of the leaf of *Cussonia* species, *Schefflera umbellifera* and *Seemannaralia gerrardii* have a promising or mostly noteworthy activity against both STI pathogens (*Trichomonas vaginalis* and *Neisseria gonorrhoeae*) tested. The moderate antimalarial activity of *Cussonia arborea*, *Cussonia sphaerocephala* and *Cussonia spicata* species justifies their use in the treatment of malaria, although the cytotoxicity of the extracts warrants further investigation. Thus, the antimicrobial and antimalarial activities observed in *Cussonia* species provide a scientific basis for the use of the plants in traditional medicine.

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