Synergistic activity of extracts of three South African alien invasive weeds combined with conventional antibiotics against selected opportunistic pathogens

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Highlights

- Alien invasive plants may contain useful bioactive phytochemicals.
- *Chromolaena odorata*, *Gomphrena celosioides* and *Tithonia rotundifolia* were studied.
- Extracts of the plants synergistically enhanced efficacy of current antibiotics.
- Some extracts had good antioxidant and excellent anti-inflammatory activity.
- The selected weeds may be developed as valuable adjuncts to antimicrobial therapy.

Abstract

Plants are known to play a major role in the treatment of microbial infections, as they contain important bioactive components that may act in synergism with conventional antibiotics, which are presently under threat of microbial resistance. Synergistic activity between dichloromethane, acetone, 70% ethanol, 50% methanol and hot water extracts of three southern African alien invasive plant species, namely *Chromolaena odorata*, *Gomphrena celosioides* and *Tithonia rotundifolia* and gentamicin, streptomycin, rifampicin and amphotericin B was studied using the checkerboard method. Antioxidant activity of the extracts was determined using the quantitative 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) decolorisation methods. The anti-inflammatory activity was determined with the 15-lipoxygense enzyme assay. Synergistic to additive effects were observed among all tested extracts in combination with the conventional antibiotics against most of the tested strains. This was evident when the extracts were combined with gentamicin or amphotericin B against *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Salmonella* Typhimurium, *Cryptococcus neoformans* and *Candida albicans*. The extracts of *C. odorata* had the best antioxidant activity in the ABTS assay with the 70% ethanol extract showing a minimum inhibitory concentration (IC_{50}) of 1.960 µg/mL, better than that of vitamin C (IC_{50} = 2.027 µg/mL). The dichloromethane extract of *T. rotundifolia* displayed promising radical scavenging activity in the DPPH assay with an IC_{50} of 16.231 µg/mL. Most of the extracts of *C. odorata* (except the acetone extract) had anti-inflammatory activity better than that of the positive control quercetin (IC_{50} = 24.601 µg/mL). Results from this study indicate that extracts of these weedy plant species contain bioactive compounds that may act in synergy with conventional antibiotics in the fight against resistant microbial strains. Additionally they may reduce oxidative stress and inflammation.

Keywords: alien invasive; synergistic; antimicrobial; antioxidant; anti-inflammatory; opportunistic

1. Introduction

Infections caused by opportunistic pathogens in humans and animals, especially in immunocompromised individuals, are overwhelming and call for global intervention. Due to the emergence of microbial pathogens resistant to known antibiotics, there is an ongoing search for new antibiotic resistance breakers which can enhance an antibiotic's potency for the successful treatment of microbial infections (Brown, 2015). To prevent a return to the pre-antibiotic era, it is pertinent to enhance useful antibiotics with promising bioactive compounds of natural origin. Plants contain useful antimicrobial compounds that may assist in developing effective treatments against opportunistic pathogens (Balandrin et al., 1993; Tapsell et al., 2006; Rigano et al., 2013). Studies have shown that the combination of plant extracts with known antibiotics can help improve the effectiveness of such drugs against opportunistic pathogens (Mhanna and Adwan, 2009; Farooqu et al., 2015). The use of plants in synergy with antibiotics or other bioactive agents may produce more potent results than when an individual drug is applied. Studies have shown that plants possess synergistic effects with many conventional antibiotics, and in most cases such combinations are more effective than single isolated antibiotics (Wagner and Ulrich-Merzenich, 2009; Chukwujeku and van Staden, 2016; Kumari et al., 2017).

Inflammation is a complex biological response of vascular tissues to microbial infection, which is often characterised by redness, swelling or pain (Palladino et al., 2003; Ferrero-Miliani et al., 2006). This response can be acute or chronic, and in either case presents a health challenge. Chronic inflammation is a major response of the immune system to life-threatening diseases like cancers, kidney or liver infection and heart attack among others (Coussens and Werb, 2002). The long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs results in adverse side effects such as stomach pain and ulcers, as well as damage to human or animal biological systems (Shih and Chang, 2007; Wallace et al., 2007). Oxidative stress is one of the manifestations of inflammation and it induces pathways that generate inflammation mediators (Sommer, 2005). When oxidative stresses occur, the cellular components of the body are altered, leading to several disease states (Nimse and Pal, 2015). Oxidative stress can be neutralised using antioxidants to enhance cellular defence (Sies, 1997; Pal and Nimse, 2006). Plants contain important molecules that have been reported to have good anti-inflammatory activity and act as antioxidants, preventing chain reactions that may lead to the release of free radicals into the body (Kasote et al., 2015). The purpose of this study was to evaluate the synergistic activity of selected extracts of three South African alien invasive

plants: *Chromolaena odorata* (L.) R.M. King & H. Rob. (South African biotype), *Gomphrena celosioides* Mart. and *Tithonia rotundifolia* (Mill.) S.F.Blake in combination with conventional antibiotics used for the treatment of bacterial, mycobacterial and fungal infections against selected opportunistic pathogens. These plants were selected for synergistic study based on promising antimicrobial activity displayed by extracts of the plants against selected opportunistic pathogens, as well as their low cytotoxicity and lack of mutagenicity (Omokhua et al., 2018). Traditional uses of these plant species for the treatment of asthma, worm and kidney infections, bronchitis, liver diseases, coughs, colds, diabetes and dysmenorrhea have also been reported (Omokhua et al., 2018). Further research comprised evaluation of their antioxidant and anti-inflammatory properties as a lead for further investigation on the development of effective treatments against inflammation and other related microbial infections.

2. Materials and methods

2.1 Plant collection, preparation and extraction

The plant material of *C. odorata* was collected from the wild in KwaZulu-Natal while *G. celosioides* and *T. rotundifolia* were collected from the wild in Pretoria North, Gauteng in South Africa. Voucher specimens (Coll. 1 PRU 123725 for *Tithonia rotundifolia*, Coll. No. 4 PRU 123630 for *Gomphrena celosioides* and Coll. No. 5 PRU 123727 for *Chromolaena odorata*) were prepared after being identified with the help of Mrs. Elsa van Wyk and deposited at the HGWJ Schweickerdt Herbarium, University of Pretoria. The leaves were carefully washed, air-dried at room temperature and ground to fine powder. The plant material was stored in glass jars until needed for experimentation. Four grams of each plant material were non-sequentially extracted using 40 mL of 50% methanol, 70% ethanol, hot water, dichloromethane (DCM) and acetone through sonication and centrifugation extraction methods. Resultant extracts were air-dried. A stock concentration of 10 mg/mL of each extract and conventional antibiotics (gentamicin, amphotericin B, streptomycin and rifampicin, obtained from Sigma) were prepared and further diluted according to their minimum inhibitory concentrations multiplied by four. The hot water extracts were reconstituted in sterile water, 50% methanol extracts in 50% methanol and DCM and acetone extracts in acetone. The conventional antibiotics were prepared using sterile water.

2.2 Microbial strains

The microbial strains used for the study included *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Mycobacterium aurum* (NCTC 10437), *M. fortuitum* (ATCC 6841), *M. smegmatis* (ATCC 1441), and clinical isolates *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.* Typhimurium), *Klebsiella pneumoniae*, *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Candida albicans*.

2.3 Synergistic antimicrobial activity

The checkerboard method described by Schelz et al. (2006) was used to determine the effect of a combination of the DCM, acetone, hot water, 70% ethanol and 50% methanol extracts of *C. odorata*, *G. celosioides* and *T. rotundifolia* with gentamicin, streptomycin, rifampicin and amphotericin B. The overnight cultures were prepared by inoculating colonies into sterilized broth (Mueller-Hinton for bacteria, Sabouraud Dextrose for fungi and Middlebrook 7H9 for mycobacteria, all sourced from Sigma). The inoculated cultures were diluted with broth and standardised to McFarland No 1 using a microplate reader (Epoch Biotek). Fifty microliters of sterile water were added to all wells of a 96-well microtitre plate for bacterial and fungal activity testing, while for mycobacteria, Middlebrook 7H9 broth was used. Extracts prepared according to their MICs multiplied by four were added to rows A1 to H1 and two-fold serially diluted down the column to wells A9-H9, before 100 µl was discarded from the final row. For the positive controls (gentamicin, streptomycin, rifampicin and amphotericin B), 100 µL of either sterilised water or broth was added to all wells of a new plate and 100 µL of each control was added to wells A1 to A10. These were serially diluted down the columns before 100 µL was discarded from wells H1 to H10. To A1-A9 wells containing the extracts, 50 µL of the controls were added (positive control wells A1-A9 to A1-A9 wells containing the extracts and finally to G1-G9. Wells A10-G10 positive controls were also transferred to the extract plate A10-G10. Wells A10-G10 were used to determine the MIC of the positive controls, and wells H1-H9 for MIC of extracts. To all wells, except row A12-H12 used as sterile control, 100 µL of the diluted cultures were added and incubated for 24 h at 37°C for bacterial and mycobacterial strains, and 30°C for fungal strains. After incubation, 40 µL (for bacteria and mycobacteria) and 50 µL of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT) was added to all wells except wells A12-H12. Incubation was carried out for another hour for bacterial and mycobacterial strains and results were read. For the fungal strains, plates were further incubated for 24 h and results were read. The fractional inhibitory concentration (FIC) of each extract

was calculated by dividing the concentration of the extract present in a well in combination where growth inhibition of the micro-organism was observed by the MIC of the extract alone. The same was done for the positive controls. The assay was performed in triplicate and was repeated twice. The ∑FIC was calculated for each test sample independently following the equation:

$$\text{FIC (a)} = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) alone}}$$

$$\text{FIC (b)} = \frac{\text{MIC (b) in combination with (a)}}{\text{MIC (b) alone}}$$

Sum of FIC was calculated as:

$$\sum\text{FIC} = \text{FIC (a)} + \text{FIC (b)}$$

2.4 Antioxidant activity

2.4.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Antioxidant activity was carried out through quantifying the radical scavenging activity of the crude extracts (70% ethanol, 50% methanol, acetone, DCM and hot water) estimated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Brand-Williams et al. (1995) with some modifications by Ahmed et al. (2014). Forty microliters of absolute methanol were measured into 96-well microplates followed by the addition of 40 µl of 1 mg/mL plant extracts dissolved in absolute methanol to the first column and serially diluted down the plate. To each well of the microplates, 160 µl of 2,2-diphenyl-1-picrylhydrazyl was added except for wells used as negative controls. Ascorbic acid (Vitamin C) and Trolox (Sigma) at 1 mg/mL were used as positive controls. After incubation for 40 min absorbance was read at 517 nm using a microplate reader. The free radical scavenging activity of the extracts and controls were determined as percentage inhibition. The concentrations of the samples reducing the free radical at 50% (IC_{50}) were also determined. The assay was repeated twice in duplicate.

2.4.2 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The free radical decolourization assay using 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was performed with modification from a test tube format to a 96-well microtitre plate format (Re et al., 1999; Ahmed et al., 2014). To each well of the microplates, 40 µL of absolute methanol was added followed by 40 µL of 1 mg/mL crude extracts dissolved in methanol and serially diluted, Vitamin C and Trolox at the same initial concentration of 1

mg/mL were used as positive controls. One hundred and sixty microliters of ABTS were added to the wells except those used as negative controls. The mixture was allowed to react for 6 min and absorbance was read at 734 nm using a microplate reader. The free radical decolourizing activity of the extracts and controls was determined as percentage inhibition and concentration of the samples reducing free radical at 50% were also determined. The assay was repeated twice in duplicate.

2.4.3 15-Lipoxygenase (15-LOX) enzyme assay

The anti-inflammatory activity of the extracts of selected plants was carried out using the method of Pinto et al. (2007). Twenty microliters of tris-hydrochloric acid buffer with a pH of 7.4 was added to all wells of a 96-well microplate. To the wells, 20 µL of 1 mg/mL extracts were added to row A including the wells used as sample blank and were two-fold serially diluted from row A to H and from row H 20 µl was discarded. Quercetin as the positive control and buffer as negative control were also prepared following the same procedure. To all the wells, 40 µL of freshly prepared 15-lipoxygenase enzyme (Sigma) was immediately added and incubated for 5 min. To the incubated plates, 40 µL of substrate (linoleic acid with a final concentration of 140 µM prepared in 50 mM Tris–HCl buffer) was added to the wells except the wells prepared as blanks. The plates were again incubated for 20 min at room temperature in the dark and then 100 µL of freshly prepared ferrous oxidation–xylenol orange (FOX) reagent (containing 30 mM sulfuric acid, 100 µM xylenol orange, 100 µM iron (II) sulfate in methanol/water ratio 9:1) was added to all the wells. The plates were again incubated for 30 min at room temperature in the dark and 40 µL of substrate was added to the blank wells and absorbance was read at 560 nm using a microplate reader.

2.4.4 Statistical analysis

The data obtained from the synergistic, antioxidant and anti-inflammatory activities were subjected to statistical analysis using one-way ANOVA, Student t-test and Microsoft Excel 2010.

3. Results

3.1 Synergistic activity of selected extracts against tested microbial strains

Tables 1 and 2 show the combination of different extracts of *C. odorata*, *G. celosioid*es and *T. rotundifoli*a combined with gentamicin against bacteria, streptomycin and rifampicin against mycobacteria and amphotericin B against fungal strains. According to Van Vuuren and Viljoen

(2011), ∑FIC ≤0.5 is synergism, >0.5-1 is additive, >1-≤4 is non-interactive and >4 is antagonistic. The sub-minimal inhibitory concentration of the extracts of the three plants in combination with gentamicin showed a 2-fold to 4-fold reduction in MIC (∑FIC = 0.28-0.56) against *K. pneumonia*e, displaying synergism. A 2-fold to 64-fold reduction in MIC (∑FIC 0.16 to 1.02) and (∑FIC 0.31-1.03) ranging from synergism to additive effects against *E. faecalis* and *S.* Typhimurium respectively was also noted. For *M. fortuitum* the extract combinations with streptomycin showed a 2-fold to 64-fold reduction (∑FIC 0.56-2.02) with the 50% methanol extract of *C. odorata* showing a non-interactive effect. The same 2-fold to 64-fold reduction (∑FIC 0.13-0.56) was observed against *M. smegmatis* for the extract combinations with rifampicin.

Regarding the fungal strains tested with the combination of the extracts of the investigated plant species and amphotericin B, a 2-fold to 64-fold (∑FIC 0.31-1.13) and (∑FIC 0.09-1.03) reduction in MIC against *A. fumigatus* and *C. neoformans* was observed with the extracts exhibiting synergistic to additive effects against *A. fumigatus* and acetone extracts of *T. rotundifolia* and *G. celosioides* and DCM extract of *C. odorata* against *C. neoformans* A 2-fold to 4-fold reduction (∑FIC 0.19-0.56) was observed against *C. albicans* with only the acetone extract of *G. celosioides* showing an additive effect.

Table 1. **Synergistic activity of extracts of *C. odorata*, *G. celosioides* and *T. rotundifolia* with gentamicin, streptomycin and rifampicin against bacterial and mycobacterial strains.**

Bacterial strains	Sample	MIC (µg/mL)		[c]∑FIC		Interpretation
		[a]individual	[b]combination	Extract/drug	Index	
E. coli	Coac/gent	40/16	20/2	0.5/0.13	0.63	Additive
	TrHH$_2$O/gent	10/16	0.04/8	0.004/0.5	0.50	Synergy
K. pneumoniae	CoDCM/gent	80/250	10/62.5	0.13/0.25	0.38	Synergy
	Coac/gent	80/250	20/15.6	0.25/0.06	0.31	Synergy
	GcDCM/gent	20/250	5/15.6	0.25/0.06	0.31	Synergy
	Gcac/gent	20/250	10/15.6	0.5/0.06	0.56	Additive
	Tret/gent	80/250	10/125	0.13/0.5	0.63	Additive
	TrDCM/gent	80/250	40/0.41	0.5/0.002	0.50	Synergy
	Trac/gent	40/250	20/0.41	0.5/0.002	0.50	Synergy
E. faecalis	Coac/gent	40/250	10/7.81	0.25/0.03	0.28	Synergy
	GcDCM/gent	80/250	40/0.41	0.5/0.002	0.50	Synergy
	Gcac/gent	40/250	20/0.41	0.5/0.002	0.50	Synergy
	TrDCM/gent	80/250	10/7.81	0.13/0.03	0.16	Synergy
	Trac/gent	80/250	1.25/250	0.02/1	1.02	Additive
	TrHH$_2$O/gent	40/250	20/7.81	0.5/0.03	0.53	Synergy
P. aeruginosa	CoDCM/gent	40/5	20/0.16	0.5/0.03	0.53	Synergy
	Coac/gent	80/10	20/0.16	0.25/0.02	0.27	Synergy
S. aureus	CoHH$_2$O/gent	80/16	0.63/8	0.01/0.5	0.51	Synergy
	Tret/gent	20/16	20/0.25	1/0.016	1.02	Additive
	TrDCM/gent	40/16	0.31/16	0.01/1	1.01	Additive
	Trac/gent	20/16	0.63/8	0.03/0.5	0.53	Synergy
S. Typhimurium	CoDCM/gent	80/10	40/0.16	0.5/0.02	0.52	Synergy
	Coac/gent	80/10	5/5	0.06/0.5	0.56	Additive
	GcDCM/gent	80/10	2.5/10	0.03/1	1.03	Additive
	Gcac/gent	80/10	5/5	0.06/0.5	0.56	Additive
	TrDCM/gent	80/10	40/0.16	0.5/0.02	0.52	Synergy

	Trac/gent	40/10	10/0.63	0.3/0.06	0.31	Synergy
M. aurum	GcDCM/strep	40/2	5/2	0.13/1	1.13	Additive
	TrDCM/strep	20/2	2.5/2	0.13/1	1.13	Additive
	Trac/strep	40/2	10/1	0.25/0.5	0.75	Additive
M. fortuitum	Come/strep	80/7.5	1.25/15	0.02/2	2.02	Non-interactive
	GcDCM/strep	80/7.5	5/3.75	0.06/0.5	0.56	Additive
	TrDCM/strep	20/7.5	5/3.73	0.25/0.5	0.75	Additive
	Trac/strep	20/15	1.25/15	0.06/1	1.06	Additive
M. smegmatis	CoDCM/rif	80/15	1.25/7.5	0.02/0.5	0.52	Synergy
	GcDCM/rif	80/15	5/7.5	0.06/0.5	0.56	Additive
	TrDCM/rif	40/15	2.5/0.94	0.06/0.06	0.13	Synergy
	Trac/rif	40/15	1.25/7.5	0.03/0.5	0.53	Synergy

[a]MIC = Minimum inhibitory concentration for individual sample; [b]MIC =Minimum inhibitory concentration of samples in combination; index = FIC (a) + FIC (b) in combination; CoDCM =*Chromolaena odorata* diclhloromethane extract; Coac = *Chromolaena odorata* acetone extract; Come = *Chromolaena odorata* 50% methanol extract; Coet = *Chromolaena odorata* 70% ethanol extract; $CoHH_2O$= *Chromolaena odorata* hot water extract; extract; GcDCM = *Gomphrena celosioides* dichloromethane extract; Gcac = *Gomphrena celosioides* acetone extract; Gcme = *Gomphrena celosioides* 50% methanol extract; $GcHH_2O$ = *Gomphrena celosiodes* hot water extract; TrDCM = *Tithonia rotundifolia* dichloromethane extract; Trac = *Tithonia rotundifolia* acetone extract; Tret = *Tithonia rotundifolia* 70% ethanol extract; $TrHH_2O$ = *Tithonia rotundifloia* hot water extract; gent = Gentamicin; strep = Streptomycin; rif = Rifampicin

Table 2. **Synergistic activity of extracts of *C. odorata*, *G. celosioides* and *T. rotundifolia* with amphotericin B against fungal strains at 48 h.**

Fungal strains	Sample	MIC (µg/mL)		[c]∑FIC		Interpretation
		[a]Individual	[b]Combination	Extract/drug	Index	
A. fumigatus	CoDCM/ampB	80/2	20/2	0.25/1	1.25	Additive
	Coac/ampB	40/2	1.25/2	0.03/1	1.03	Additive
	Gcme/ampB	10/2	1.25/2	0.13/1	1.13	Additive
	GcDCM/amp B	10/2	2.5/0.25	0.25/0.13	0.38	Synergy
	Gcac/amp B	10/2	2.5/0.25	0.25/0.13	0.38	Synergy
	$GcHH_2O$/ampB	10/2	2.5/0.25	0.25/0.13	0.38	Synergy
	TrDCM/amp B	80/2	20/0.13	0.25/0.06	0.31	Synergy
	Trac/ampB	40/2	5/2	0.13/1	1.13	Additive
C. neoformans	Coet/ampB	40/15	5/0.94	0.13/0.06	0.19	Synergy
	Come/ampB	40/15	10/0.24	0.25/0.02	0.27	Synergy
	CoDCM/ampB	10/15	10/0.24	1/0.02	1.02	Additive
	Coac/ampB	20/15	5/3.75	0.25/0.25	0.50	Synergy
	$CoHH_2O$/ampB	40/15	5/0.94	0.13/0.06	0.19	Synergy
	GcDCM/ampB	40/15	10/0.94	0.25/0.06	0.31	Synergy
	Gcac/ampB	80/15	0.63/15	0.01/1	1.01	Additive
	Tret/ampB	80/15	5/0.47	0.06/0.03	0.09	Synergy
	TrDCM/ampB	40/15	20/0.24	0.5/0.02	0.52	Synergy
	Trac/ampB	40/15	1.25/15	0.03/1	1.03	Additive
C. albicans	CoDCM/ampB	80/15	5/1.88	0.06/0.13	0.19	Synergy
	Coac/ampB	80/15	2.5/7.5	0.03/0.5	0.53	Synergy
	GcDCM/ampB	80/15	10/3.75	0.13/0.25	0.38	Synergy
	Gcac/ampB	80/15	40/0.94	0.5/0.06	0.56	Additive
	TrDCM/ampB	40/15	1.25/7.5	0.31/0.5	0.53	Synergy
	Trac/ampB	40/15	10/0.47	0.25/0.03	0.28	Synergy

[a]MIC = Minimum inhibitory concentration for individual sample; [b]MIC =Minimum inhibitory concentration of samples in combination index = FIC (a) + FIC (b). CoDCM =*Chromolaena odorata* diclhloromethane extract; Coac = *Chromolaena odorata* acetone extract; Come = *Chromolaena odorata* 50% methanol extract; Coet = *Chromolaena odorata* 70% ethanol extract; $CoHH_2O$= *Chromolaena odorata* hot water extract; extract; GcDCM = *Gomphrena celosioides* dichloromethane extract; Gcac = *Gomphrena celosioides* acetone extract; Gcme = *Gomphrena celosioides* 50% methanol extract; $GcHH_2O$ = *Gomphrena celosiodes* hot water extract; TrDCM = *Tithonia rotundifolia* dichloromethane extract; Trac = *Tithonia rotundifolia* acetone extract; Tret = *Tithonia rotundifolia* 70% ethanol extract; ampB = Amphotericin B.

3.2 Antioxidant activity

The antioxidant activity displayed by the extracts of *C. odorata*, *G. celosioides* and *T. rotundifolia* in the DPPH and ABTS assays are presented as percentage inhibition in Figures 1 and 2. Also, the concentration at which 50% inhibition (IC_{50}) was noted are presented in Table 3. Their correlation coefficients (R^2) are presented to show the strength of the relationship between the plant extracts and concentrations used. Following the classification of the antioxidant agents described by Omisore et al. (2005), samples with IC_{50}<50 µg/mL are considered to have good antioxidant activity, and samples with IC_{50}>50 µg/mL as moderate to weak activity. Among all extracts of the plant species tested for the ABTS decolorisation ability, the 70% ethanol, 50% methanol and hot water extracts of *C. odorata* showed low IC_{50} values of 1.96, 3.143 and 4.493 µg/mL and the 50% methanol extract of *T. rotundifolia* IC_{50} 14.580 µg/mL respectively, indicating significantly good antioxidant activity. For the DPPH radical scavenging activity, although most of the extracts showed moderate antioxidant activity IC_{50}>50 µg/mL, only the DCM extract of *T. rotundifolia* and 50% methanol extract of *C. odorata* had good antioxidant activity (IC_{50} = 16.231 and 22.628 respectively). The percentage inhibition values displayed were dose dependent.

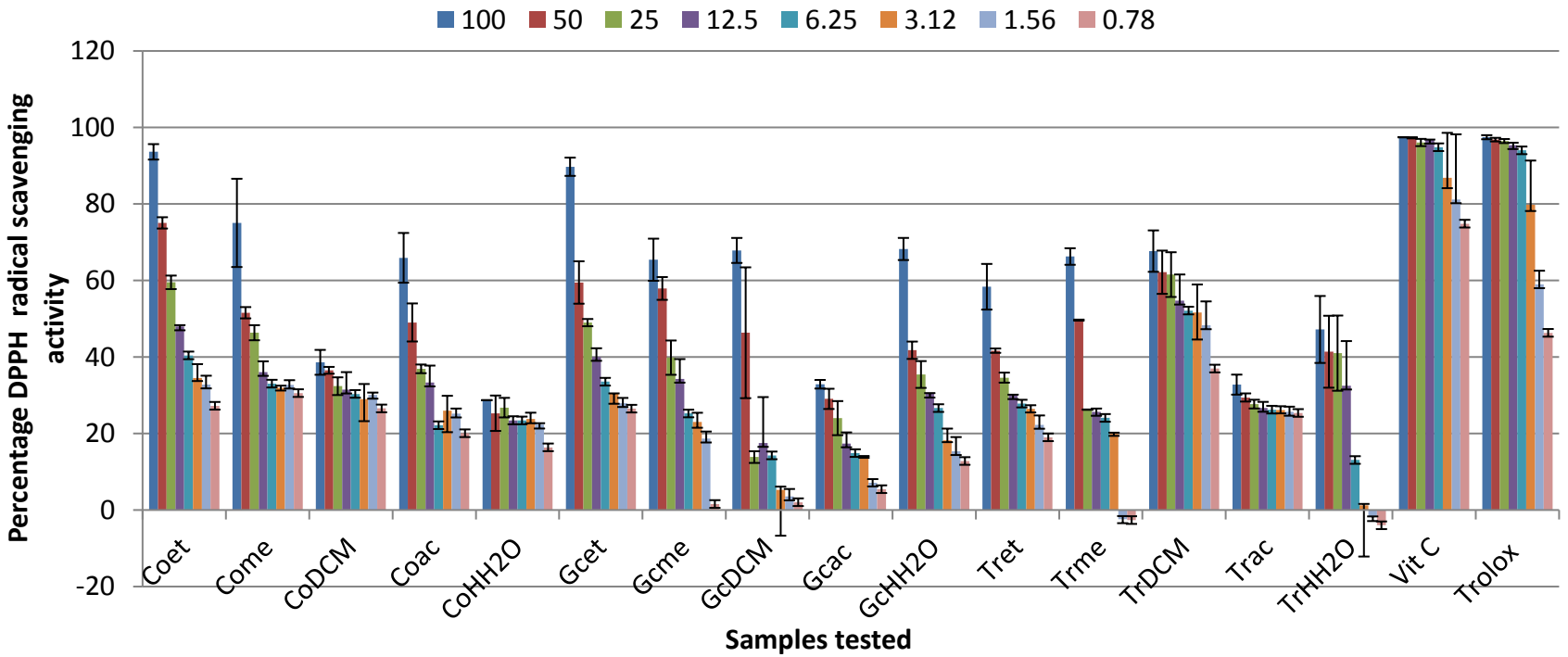

Figure 1: DPPH radical scavenging activity of extracts of *Chromolaena odorata*, *Gomphrena celosioides* and *Tithonia rotundifolia* compared to positive controls. Coet = *C. odorata* 70% ethanol extract; Come = *C. odorata* 50% methanol extract; CoDCM = *C. odorata* dichloromethane extract; *C. odorata* Coac = *C. odorata* acetone extract; $CoHH_2O$ = *C. odorata* hot water extract; Gcet = *G. celosioides* 70% ethanol extract; Gcme = *G. celosioides* 50% methanol extract; GcDCM = *G. celosioides* dichloromethane extract; Gcac = *G. celosioides* acetone extract; $GcHH_2O$ = *G. celosioides* hot water extract; Tret = *T. rotundifolia* 70% ethanol extract; Trme = *T. rotundifolia* 50% methanol extract; TrDCM = *T. rotundifolia* dichloromethane extract; Trac = *T. rotundifolia* acetone extract; $TrHH_2O$ = *T. rotundifolia* hot water extract.

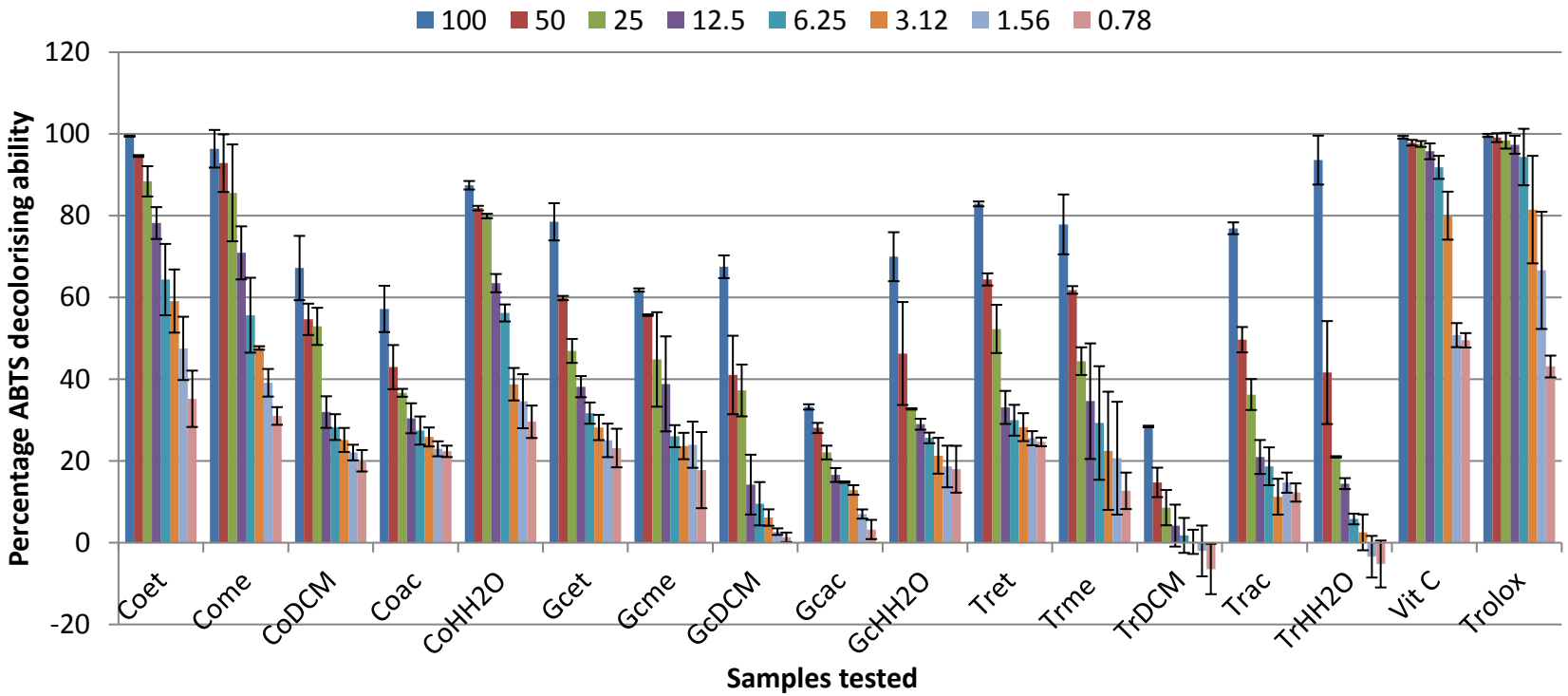

Figure 2*:* ABTS Free radical decolourization activity of invasive *Chromolaena odorata, Gomphrena celosioides,* and *Tithonia rotundifolia* compared to positive controls A: Coet = *C. odorata* 70% ethanol extract; Come = *C. odorata* 50% methanol extract; CoDCM = *C. odorata* dichloromethane extract; *C. odora*ta Coac = *C. odorata* acetone extract; $CoHH_2O$ = *C. odorata* hot water extract; Gcet = *G. celosioides* 70% ethanol extract; Gcme = *G. celosioides* 50% methanol extract; GcDCM = *G. celosioides* dichloromethane extract; Gcac = *G. celosioides* acetone extract; $GcHH_2O$ = *G. celosioides* hot water extract; Tret = *T. rotundifolia* 70% ethanol extract; Trme = *T. rotundifolia* 50% methanol extract; TrDCM = *T. rotundifolia* dichloromethane extract; Trac = *T. rotundifolia* acetone extract; $TrHH_2O$ = *T. rotundifolia* hot water extract.

Table 3. **Antioxidant activities of the plant extracts tested in the DPPH and ABTS assays (IC_{50} in µg/mL ± standard deviation)**

Plant species	Extracts	ABTS		DPPH	
		IC_{50}	**R^2**	**IC_{50}**	**R^2**
C. odorata	Ethanol	**1.96±1.058 ***	0.7154	**22.628±2.953**	0.9248
	Methanol	**3.143±0.444***	0.8002	38.377±2.712	0.9821
	DCM	25.313±1.021	0.9712	183.018±2.111	0.8109
	Acetone	74.212±5.317	0.9192	53.486±2.010	0.9296
	Hot water	**4.493±0.531**	0.9655	116.00±1.923	0.6183
G. celosioides	Ethanol	33.711±0.341	0.8221	37.32±0.99	0.9172
	Methanol	49.767±2.001	0.7993	49.317±2.17	0.8218
	DCM	71.812±0.531	0.527	76.13±1.351	0.8307
	Acetone	151.676±0.582	0.9017	89.22±4.002	0.803
	Hot water	50.719±1.322	0.8207	43.812±1.99	0.9532
T. rotundifolia	Ethanol	36.985±1.908	0.9669	67.81±0.416	0.96295
	Methanol	**14.580±0.00**	0.9123	37.813±1.263	0.8069
	DCM	111.20±2.134	0.798	**16.231±0.121**	0.9349
	Acetone	52.066±0.731	0.6994	152.567±0.312	0.9952
	Hot water	39.440± 0.72	0.9345	33.971±0.215	0.788
Trolox		**0.562±0.415**	0.7933	**0.541 ±0.083**	0.8308
Vitamin C		**2.027± 0.02**	0.9844	**0.162± 0.893**	0.8813

Extracts with good antioxidant activity are highlighted in bold and those with significant activity are marked with an asterisk.

3.3 Anti-inflammatory activity determined using the 15-lipoxygenase enzyme assay

Figure 3 and Table 4 represent the percentage inhibition and IC_{50} values of tested samples. Among the three plant species investigated, only the extracts of *C. odorata* had good anti-inflammatory activity with IC_{50} values lower than that of the positive control, quercetin. The DCM extract had the best activity (IC_{50} = 8.975 µg/mL). The activity was dose dependent.

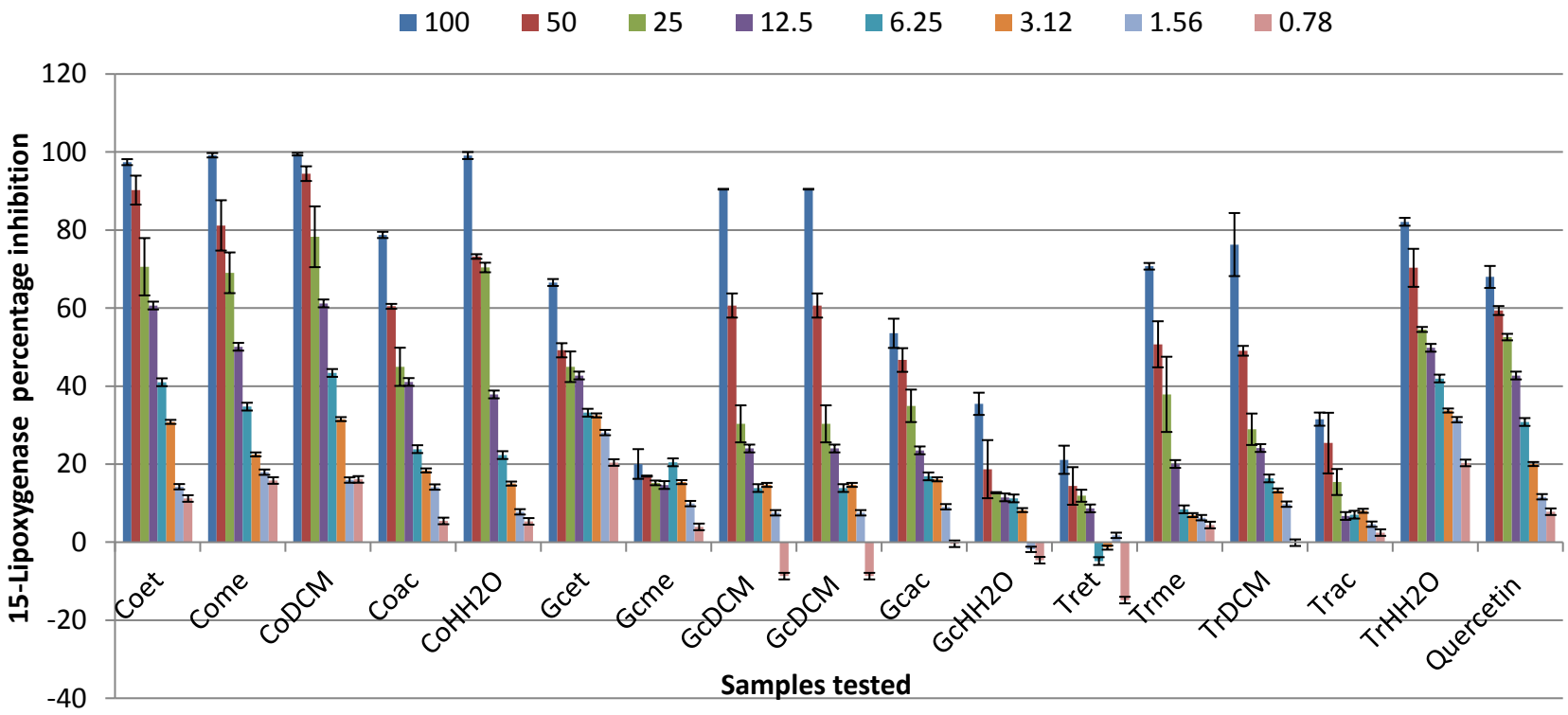

Figure 3: Anti-inflammatory activity in the 15-lipoxygenase inhibition assay of *Chromolaena odorata*, *Gomphrena celosioides* and *Tithonia rotundifolia* compared to the control. Coet = *C. odorata* 70% ethanol extract; Come = *C. odorata* 50% methanol extract; CoDCM = *C. odorata* dichloromethane extract; *C. odorata* Coac = *C. odorata* acetone extract; $CoHH_2O$ = *C. odorata* hot water extract; Gcet = *G. celosioides* 70% ethanol extract; Gcme = *G. celosioides* 50% methanol extract; GcDCM = *G. celosioides* dichloromethane extract; Gcac = *G. celosioides* acetone extract; $GcHH_2O$ = *G. celosioides* hot water extract; Tret = *T. rotundifolia* 70% ethanol extract; Trme = *T. rotundifolia* 50% methanol extract; TrDCM = *T. rotundifolia* dichloromethane extract; Trac = *T. rotundifolia* acetone extract; $TrHH_2O$ = *T. rotundifolia* hot water extract.

Table 4. 15-Anti-inflammatory activity of *C. odorata*, *G. celosioides* and *T. rotundifolia* against 15-lipoxygenase in µg/mL ± standard deviation.

Plant species	extract	IC_{50}	R^2 values
C. odorata	Ethanol	***10.123±0.312**	0.9823 ±0.332
	Methanol	***12.339±1.129**	0.9498 ± 0.052
	DCM	***8.975±1.932**	0.9894 ± 0.712
	Acetone	**26.098±2.966**	0.9306 ± 1.04
	Hot water	***17.201±0.747**	0.8987 ± 0.00
G. celosioides	Ethanol	32.024±0.189	0.9302 ±0.932
	Methanol	119.558±1.437	0.9614 ± 0.732
	DCM	49.037±0.042	0.9571 ± 0.203
	Acetone	124.975±3.078	0.7417 ± 0.993
	Hot water	123.072±1.516	0.6536 ± 0.99
T. rotundifolia	Ethanol	201.828±3.556	0.7224 ±0.09
	Methanol	61.785±3.044	0.8319 ± 0.231
	DCM	53.929±1.152	0.9509 ±0.09
	Acetone	162.870±1.229	0.7401 ± 0.745
	Hot water	**28.581±2.172**	0.9652 ± 0.08
Quercetin (+ve control)		**24.601± 0.700**	0.9829 ±0.01

Extracts with good anti-inflammatory activity are highlighted in bold and those with significant activity are marked with an asterisk.

4. Discussion

From the concentrations tested in combination with the standard antibiotic gentamicin, synergistic activity was observed with the extracts of *C. odorata*, *G. celosioides* and *T. rotundifolia,* especially against *K. pneumoniae* and *E. faecalis,* This indicates that a combination of these extracts with gentamicin may enhance its activity in the fight against antibiotic resistance of the tested strains. Additive to non-interactive effects were observed with the combination of the extracts and streptomycin against *M. aurum* and *M. fortuitum* at the lowest concentrations tested in this study based on their previously calculated MIC values. However, synergistic to additive effects were observed with the extract combination and rifampicin against *M. smegmatis*, which was concentration dependent. This suggests that these extracts in combination with rifampicin may be more effective. Most of the extracts in combination with amphotericin B displayed synergism against *A. fumigatus*, *C. neoformans* and *C. albicans.* Interestingly, none of the extract combinations with any of the antibiotics displayed antagonistic effects against the organisms tested in this study. The synergistic activity observed against most of the tested organisms may be attributed to the ability of the extracts to interact with the conventional antibiotics, enhancing bioavailability of effective substances to inhibit the micro-organisms (Wagner and Ulrich-Merzenich, 2009). Synergistic activity was

observed in a study carried out by Kumari et al. (2017) with a combination of *G. celosioides* extract and streptomycin against *S.* Typhi (*Salmonella enterica* serovar Typhi). These authors also reported synergism of the plant in combination with streptomycin against *S. aureus*. Although information on the synergistic activity of *C. odorata* and *T. rotundifolia* is scarce, reports have shown that plants of the Asteraceae family exhibit synergistic activity in combination with conventional antibiotics (Chovanová et al., 2013; Ghabeish, 2015; Fadila and Alaoui, 2016).

The antioxidant activity of *C. odorata, G. celosioides* and *T. rotundifolia* (Table 3 and Figures 1 and 2) was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and the free radical decolourization assay using 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to estimate possible radical scavenging ability of these plants. Although the investigated plants had varying antioxidant and anti-inflammatory activities, the extracts of *C. odorata* had the most promising activity. Comparing the IC_{50} values and the percentage lipoxygenase inhibition observed in this study, there was a dose dependent pattern with extracts showing good antioxidant activity also displaying anti-inflammatory activity. The extracts of *C. odorata* had better activity than the extracts of *T. rotundifolia* and *G. celosioides*. When comparing the activity of the C. *odorata* extracts, the 70% ethanol extract had the best antioxidant activity while the DCM extract had the best anti-inflammatory activity. Adeoti et al. (2016) reported good antioxidant and anti-inflammatory activities of the ethanol extract of *G. celosioides* in an *in vivo* study on Wistar rats. The authors observed anti-inflammatory activity comparable to the positive control diclofenac, while the antioxidant activity was better than that of vitamin C. However, results of the *in vitro* assay in this study were not comparable to vitamin C. There is a possibility that the anti-inflammatory activity expression of this plant may not be through the 15-LOX pathway. Although information on the antioxidant activity of the southern African biotype of *C. odorata* is limited, the activity exhibited in this study agrees with other findings on the widely spread Asian/West Africa biotype *C. odorata* (AWAB). A recent study carried out by Vijayaraghavan et al. (2018) on the ethanol and water extracts showed that the AWAB is rich in antioxidant activity. This indicates that this weed may be developed as an effective antioxidant agent (Melinda et al., 2010; Bhargava et al., 2011). However, Akinmoladun et al. (2007) reported that the AWAB of *C. odorata* displayed poor radical scavenging activity compared to vitamin C. *In vivo* anti-inflammatory studies on AWAB have shown that the plant has very good anti-inflammatory properties (Owoyele et al., 2005; Taiwo et al., 2008), a finding which was also observed in the

current *in vitro* study on the SAB *C. odorata*. This lends support to the use of *C. odorata* in traditional medicine in the treatment of wounds and inflammation (Omokhua et al., 2016).

5. Conclusions

In vitro synergistic activities of extracts of the problematic southern Africa weeds, *C. odorata*, *G. celosioides* and *T. rotundifolia,* in combination with first line antibiotic treatments against bacterial, mycobacterial and fungal infections were studied. Most of the extracts screened showed synergistic to additive effects at varying concentrations with gentamicin, streptomycin, rifampicin and amphotericin B, and no antagonistic effects were noted. Although antioxidant and anti-inflammatory activities displayed by the extracts of *G. celosioides* and *T. rotundifolia* were moderate to weak, *C. odorata* extracts had very promising activities. The synergistic effects, antioxidant and anti-inflammatory activities displayed by *C. odorata* indicate its potential for the development of effective therapeutic agents against microbial infections. This is the first study to be carried out on these biological activities of both *C. odorata* and *T. rotundifolia*. Combined with the lack of *in vitro* toxicity reported in previous research, these plant species are promising candidates for further development into plant-based remedies. A future study involving the isolation of bioactive compounds from these plants and investigation of the mechanism of action of the bioactive isolated compound(s) is warranted. *In vivo* studies are required on the extracts and isolated compounds from these plants to assist in the development of effective treatments against the investigated opportunistic pathogens. Additional work to evaluate these plant extracts as well as isolated bioactive compounds as efflux pump inhibitors in synergy with known antibiotics and to determine the mechanism through which they work to reduce antibiotic resistance is also advocated.

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Conflict of interest

The authors of this article declare no conflict of interest.

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