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Croton gratissimus Leaf Essential Oil Composition, Antibacterial, Antiplatelet Aggregation, and Cytotoxic Activities

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ABSTRACT

Essential oil hydrodistilled from the leaves of *Croton gratissimus* was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) to identify the constituents by comparison of their mass spectra (MS) data and linear retention indices (LRI) with literature. Forty constituents corresponding to 96.7% of the total oil content were identified from the oil sample. The major compounds were sabinene (14.6%), *α*-phellandrene (12.3%), *β*-phellandrene (10.7%), *α*-pinene (6.05%), and germacrene D (5.9%). The essential oil exhibited stronger antibacterial activity with minimum inhibitory concentration (MIC) of 0.6 $mg.mL^{-1}$ (*Staphylococcus aureus*), 0.2 $mg.mL^{-1}$ (*S. faecalis*), and 1.3 $mg.mL^{-1}$ (*Escherichia coli* and *Bacillus cereus*). In the antiplatelet aggregation inhibitory activity, the oil displayed action against the induced platelet aggregation in the order collagen (IC_{50} < 1) > thrombin (IC_{50} 1.18) > Adenosine diphosphate (IC_{50} 2.32) > epinephrine (IC_{50} 3.65). The lethal concentration (LC_{50}) of the oil at 8.52 $mg.mL^{-1}$ was toxic to *Artemia salina*.

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Croton gratissimus; Euphorbiaceae; terpenes; antibacterial; anti-platelet aggregation; cytotoxic activity

Introduction

Croton gratissimus (Euphorbiaceae) is a deciduous shrub (3 m tall), or a tree (~20 m tall) (8). The plant is used to treat cough, fever, abdominal disorders, respiratory disorders, skin inflammation, earache, and malarial and chest complaints (29). *C. gratissimus* extracts had anti-staphylococcal, anti-HIV (23), antimicrobial (30), antioxidant, and acetylcholinesterase inhibitory (2) effects. The antioxidant and acetylcholinesterase inhibitory effects were attributed to tiliroside, isovitexin, and kampferol (2). An alkaloid, (+)-nuciferine, was shown to be responsible for the pyrogenic activity of *C. gratissimus* (29). Previous phytochemical investigation on extracts of *C. gratissimus*

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yielded cembranolides, some of which exhibited moderate activity against the progressive external ophthalmoplegia 1 (PEO1) and PEO1TaxR ovarian cancer cell lines as well as *in vitro* antiplasmodial screening against *P. falciparum* (CQS) D10 strain (15, 24). Other phytochemicals lupeol, 4(15)-eudesmene-1β,6α-diol, 24-ethylcholesta-4,22-dien-3-one, α-glutinol, (+)-(1R*,10R*)-cembra-2E,4E,7E,11Z-tetraen-20,10-olide, and (+)-(1R*,4S*,10R*)-4-hydroxycembra-2E,7E,11Z-trien-20,10-olide have been isolated from this plant. *C. gratissimus* oil may have potential as a nonedible feedstock for industrial-scale synthesis of high-quality biodiesel (5).

C. gratissimus var. *subgratissimus* contained α-phellandrene (20.7%), germacrene D (8.6%), and 1,8-cineole (8.35%), and had antimicrobial property (31). This study examined the chemical composition and biological activities of essential oil of the leaf of *C. gratissimus* grown in South Africa.

Materials and methods

Chemicals

All chemicals and reagents were of analytical grade, purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA).

Animals

Sprague-Dawley rats (8 weeks, 220–250 kg) of either sex were collected from the animal house in the Department of Biochemistry and Microbiology, University of Zululand. The animals were maintained under standard conditions (temperature 23 ± 2°C and 12-h light/dark cycle) and had free access to standard pellet feed and drinking water as needed. Ethical clearance certificate (UZREC 171110-030 PGD 2014/53) was obtained from the Research Animal Ethical Clearance Committee (RAEC), University of Zululand.

Plant material

Fresh plant materials of *C. gratissimus* were collected from the University of Zululand, KwaDlangezwa campus, South Africa, in September 2014, identified by its Department of Botany, and a voucher specimen (OAL-51-UZ) deposited in the Herbarium of the University.

Hydrodistillation of essential oil

Three hundred grams of fresh and squeezed leaves of *C. gratissimus* were carefully introduced into a 5-L flask and distilled water was added until it

covered the sample completely. Hydrodistillation was carried out in an all-glass Clevenger-type distillation unit for 3 h (6). The volatile oils distilled over water were collected separately in the receiver arm of the apparatus into clean and previously weighed sample bottles, and refrigerated until further analyses.

Analysis of the oil

GC analyses was carried out on a Hewlett Packard Gas Chromatograph (HPGC) HP 6820 equipped with a flame ionization detector and HP-5MS column (60 m × 0.25 mm i.d.), 0.25-μm film thickness, and split ratio of 1:25. The oven temperature was programmed from 50°C (after 2 min) to 240°C at 5°C.min^{-1} and was held for 10 min. Injection and detector temperatures were 200°C and 240°C, respectively. Hydrogen was the carrier gas at a flow rate of 1 mL.min^{-1}. A quantity of 0.5 μL of the diluted oil was injected into the GC. Peaks were measured by electronic integration. *n*-Alkanes were run at the same condition for retention indices determination.

GC-MS analyses of the oils were performed on a HPGC-HP 6890 interfaced with a Hewlett Packard 5973 mass spectrometer system equipped with a HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 70 to 240°C at the rate of 5°C.min^{-1}. The ion source was set at 240°C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL.min^{-1}. Scanning range was 35–425 amu. One microliter of diluted oil in hexane was injected into the GC/MS.

Identification of essential oil constituents

The identities of the oil components were assigned by comparison of their retention indices with the authentic samples and matching of their mass spectra with the Wiley 275 library mass spectra database as well as with published data (1, 25).

Antibacterial activity

The antibacterial activity of *C. gratissimus* essential oil was evaluated by paper disc diffusion and dilution methods against eight reference bacterial strains obtained from the Department of Biochemistry & Microbiology, University of Fort Hare, Alice, South Africa. The gram-positive bacteria were *Bacillus cereus* (ATCC 10702), *S. aureus* (ATCC 6538), and *S. faecalis* (ATCC 29212), while the gram-negative strains include *Enterobacter cloacae* (ATCC 13047), *E. coli* (ATCC 4983), *Kiebsiella pneumoniae* (ATCC 10031), *Proteus vulgaris* (ATCC 6830), *Proteus vulgaris* (CSIR 0030), and *Pseudomonas aeruginosa* (ATCC19582). The stock cultures were maintained at 4°C in Mueller-Hinton agar (MHA) (Oxoid).

Disc diffusion method

The microorganisms were grown overnight at 37°C in 20 mL of Mueller-Hinton broth (Oxoid). The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 5 standard (1.0×10^8) CFU.mL^{-1} (10). Ninety-millimeter Petri dishes (Merck, South Africa) containing 12 mL of sterilized Mueller-Hinton agar (Oxoid) were inoculated with the microbial suspensions. Sterile Whatman No. 1 (6-mm) filter discs were individually placed on the surface of the seeded agar plates and 10 μL of essential oils in hexane/DMSO mixture (5 mg.mL^{-1}) were applied to the filter paper disk. The plates were incubated at 37°C for 24 h and the diameter of the resulting zones of inhibition (mm) of growth was recorded. All tests were done in triplicate using chloramphenicol (25 μg) and gentamycin (5 μg) as positive controls.

Minimum inhibitory concentration (MIC) by microtiter plate dilution method

The bacterial cultures were incubated in Müller-Hinton (MH) broth overnight at 37°C and a 1:1 dilution of each culture in fresh MH broth was prepared prior to use in the microdilution assay. Stock solution of the essential oil was obtained by adding 4 mg of essential oils (11) to 100 μL of DMSO and 100 μL of hexane to obtain of 40 mg.mL^{-1}. Sterile water (100 μL) was pipetted into all wells of the microtiter plate, before transferring 100 μL of stock solutions of essential oils to the wells of row A. Serial dilutions were made from the first well vertically in the plate, and volumes in excess of 100 μL were discarded from the wells in row H to obtain concentrations ranging from 10 mg.mL^{-1} to 0.078 mg.mL^{-1}. One hundred microliters of bacterial culture of approximate inoculum size of 1.0×10^8 CFU.mL^{-1} was added to all wells and incubated at 37°C for 24 h. After incubation, 40 μL of 0.2 mg.mL^{-1} *p*-iodo-nitrotetrazolium violet (INT) solution was added to each well and incubated at 37°C for about 30–60 min. Microbial growth is indicated by the presence of a reddish color which is produced when *p*-iodo-nitrotetrazolium violet, a dehydrogenase activity-detecting reagent, is reduced by metabolically active microorganisms to the corresponding intensely colored formazan. The MIC is the lowest concentration that produces an almost complete inhibition of visible microorganism growth in liquid medium. Chloramphenicol (25 μg) and gentamycin (5 μg) were used as positive controls.

Preparation of blood platelets

The rats were sacrificed by a blow on the head and blood was immediately collected by cardiac puncture (28). The blood was mixed (5:1 v:v) with an anticoagulant (acid-dextrose anticoagulant, 0.085 M trisodium citrate, 0.065

citric acid, 2% dextrose). The platelets were obtained by a series of centrifugation at 1,200 rpm for 15 min and at 2,200 rpm for 3 min consecutively. The supernatant was collected and centrifuged at 3,200 rpm for 15 min. The resulting supernatant was discarded, the sediment (platelets) was resuspended in 5 mL washing buffer (pH 6.5), centrifuged again at 3,000 rpm for 15 min, after which the supernatant was discarded and the platelets were finally suspended in a small volume of resuspending buffer (pH 7.4; containing 0.14 M NaCl, 15 mM Tris-HCl, 5 mM glucose). The platelets were further diluted with the resuspending buffer (1:10) and mixed with calcium chloride (0.4 ml:10 μL $CaCl_2$).

Antiplatelet aggregation evaluation

The oil was solubilized in dimethyl sulfoxide (DMSO) before making up the volume with 50 MmTris-HCl buffer [pH 7.4; containing 7.5 mM ethylenediaminetetraacetic acid (EDTA) and 175 mM (NaCl) to a final 1% DMSO concentration]. Different concentrations (1, 3, 5, and 10 $mg.mL^{-1}$) of the oil was used in the assay. The platelet aggregation inhibitory activity of the oil was separately evaluated on ADP (5 mM), collagen (5 mM), epinephrine (10 mM), and thrombin (5 $U.mL^{-1}$) induced aggregation (20). The platelets (100 μL) were preincubated for 5 min with different concentrations of the oil before introduction of platelets agonist (20 μL) to the mixture. Aggregation was determined with the Biotek plate reader (ELx 808 UI, Biotek Instrument Supplies) using Gen5 software by following change in absorbance at 415 nm. DMSO (1%) was used as negative control and aspirin was used as positive control.

Brine shrimp assay

Ten shrimps (counted on a slide) were introduced into the beakers (30 mL) containing 50 μL of different concentrations of *C. gratissimus* essential oil in DMSO solution and made up to 5 mL volume with the artificial seawater (21). The concentrations were 10, 20, 50, 100 250, 500, 750, and 1,000 $\mu g.mL^{-1}$ in DMSO solution. Each beaker was shaken lightly to ensure a homogeneous test solution and was left at room temperature. The controls were prepared with 24.95 mL of degassed distilled water and 50 μL of DMSO solution without essential oil to which shrimp larvae were added. Each test was performed in triplicate. A drop of dry yeast suspension (3 mg in 5 mL artificial seawater) was added as food to each test tube. The beakers were maintained under illumination and the survivors were counted after 24 h and expressed as percentage death. Gallic acid was used as the standard. Criterion for death was loss of locomotive action of *nauplii*.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA, $p \leq 0.05$) using Microsoft Excel 2003 and Origin 6.0. The lethal concentrations (LC_{50}) values for the antiplatelet aggregation and cytotoxic activities were calculated using the U.S. Environmental Protection Agency (EPA) probit analysis, Version 1.5.

Results

The yield of the essential oil was 0.32% (v:w) calculated on a fresh-weight basis (Table 1). The oil sample was light yellow in color. Monoterpene hydrocarbons (54.9%), sesquiterpene hydrocarbons (22.9%), and oxygenated monoterpenes (10.4%) represented the major classes of compounds in the oil. The main constituents of the oil were monoterpenes represented by sabinene (14.6%), α-phellandrene (12.3%), and β-phellandrene (10.7%). The major sesquiterpene compounds were germacrene D (5.9%) and β-caryophyllene (4.2%).

The oil demonstrated weak to considerable bactericidal or bacteriostatic activity against the microorganisms and was more effective on gram-positive than on gram-negative bacteria (Table 2). The essential oil exhibited stronger activity against *S. aureus* (ZI, 21.6 ± 0.6 mm, and MIC, 0.6 $mg.mL^{-1}$), *S. faecalis* (ZI, 20.0 ± 0.6 mm, and MIC, 0.2 $mg.mL^{-1}$), *E. coli* (ZI, 23.0 ± 0.6 mm, and MIC, 1.3 $mg.mL^{-1}$), and *B. cereus* (ZI, 17.3 ± mm, and MIC, 1.3 $mg.mL^{-1}$). The essential oil displayed moderate inhibition to the growth of *P. vulgaris* (CSIR 0030; ZI, 11.4 ± 0.6 mm, and MIC 2.5, $mg.mL^{-1}$), *P. aeruginosa* (ZI, 11.0 ± 0.6 mm, and MIC, 2.5 $mg.mL^{-1}$), and *K. pneumoniae* (ZI, 21.2 ± 0.6 mm, and MIC, 5.0 $mg.mL^{-1}$). The most resistant were *P. vulgaris* (ATCC 6830) and *E. cloacae*, with ZI < 10 mm and MIC > 10 $mg.mL^{-1}$. In some cases the oil showed higher antibacterial activity than the standards.

The percentage platelet aggregation inhibition activity of the oil of *C. gratissimus* was concentration-dependent (Table 3). At the highest concentration (10 $mg.L^{-1}$), *C. gratissimus* essential oil had platelet aggregation inhibitory activity of 69.0%, 69.4%, 54.2%, and 78.6% for ADP, collagen, epinephrine, and thrombin, respectively, compared to the percentages of platelet aggregation inhibitory activity of aspirin (61.0%, 69.1%, 57.6%, and 53.3% for ADP, collagen, epinephrine, and thrombin). It may be postulated that the aggregation induced by collagen, thrombin, and ADP were better than that of aspirin. In addition, the lethal concentration (IC_{50} < 1 $mg.mL^{-1}$) indicated that *C. gratissimus* essential oil possessed higher strength of collagen-induced platelet aggregation than aspirin (IC_{50} 4.20 $mg.mL^{-1}$). Although epinephrine had the lowest ability to induced platelet aggregation (IC_{50} 3.65 $mg.mL^{-1}$) against the oil, its activity showed better antiplatelet

Table 1. Chemical constituents of essential oil of *Croton gratissimus* leaves.

Compounds[a]	RI[b]	RI[c]	Percent composition
α-Pinene	935	932	6.0
Sabinene	972	969	14.6
Myrcene	989	988	2.4
α-Phellandrene	1,002	1,002	12.3
α-Terpinene	1,015	1,014	1.5
β-Phellandrene	1,029	1,028	10.7
(*E*)-*β*-Ocimene	1,041	1,044	2.8
γ-Terpinene	1,066	1,065	2.7
(*E*)-Sabinene hydrate	1,073	1,072	1.5
Terpinolene	1,089	1,091	1.8
Linalool	1,099	1,103	4.1
1-Octenyl-3-acetate	1,113	1,114	0.4
p-Menth-2-en-1-ol	1,136	1,136	0.3
Camphor	1,141	1,145	0.5
Borneol	1,165	1,168	0.5
α-Phellandrene epoxide	1,175	1,175	0.4
Terpinen-4-ol	1,177	1,179	1.9
α-Terpineol	1,189	1,191	0.8
α-Cubebene	1,345	1,343	0.2
α-Copaene	1,372	1,376	2.5
β-Elemene	1,388	1,389	0.5
β-Cubebene	1,390	1,390	0.4
β-Bourbonene	1,393	1,395	0.7
Cyperene	1,398	1,389	1.0
β-Caryophyllene	1,426	1,417	4.2
α-Humulene	1,461	1,452	1.1
Pentyl benzoate	1,469	1,472	0.5
allo-Aromadendrene	1,476	1,477	2.0
Germacrene D	1,481	1,484	5.9
β-Selinene	1,489	1,489	0.4
Bicyclogermacrene	1,493	1,495	1.6
α-Amorphene	1,501	1,501	0.8
δ-Cadinene	1,519	1,522	1.1
Elemol	1,546	1,548	0.8
Caryophyllene oxide	1,581	1,583	2.4
Humulene epoxide II	1,602	1,601	0.9
Selina-1,3,7(11)-trien-8-one	1,632	1,635	0.4
β-Eudesmol	1,644	1,647	0.3
Total			93.0
Monoterpene hydrocarbons			56.3
Oxygenated monoterpenes			8.5
Sesquiterpene hydrocarbons			22.4
Oxygenated sesquiterpenes			4.9
Non-terpenes			0.9

[a]Elution order on HP-5MS column; [b]Retention indices on HP-5MS column; [c]Literature retention indices.

aggregation than aspirin (IC_{50} 8.18 mg.mL^{-1}). Thus, the essential oil of *C. gratissimus* had better antiplatelet aggregation inhibitory activity compared to asprin.

The degree of brine shrimp lethality was directly proportional to the concentrations of the oil, with LC_{50} of 8.52 mg.mL^{-1}. It is apparent that the essential oil of *C. gratissimus* was very toxic when compared to the standard gallic acid (LC_{50} = 11.45 mg.mL^{-1}).

Table 2. Antibacterial activity of essential oils of *Croton gratissimus* leaves.[a]

	*Cg*EO		Chloramphenicol		Gentamycin	
Microorganisms	IZ[b]	MIC[c]	IZ[d]	MIC[e]	IZ[d]	MIC[e]
Bacillus cereus	17.3 ± 0.6	1.3	23.7 ± 1.3	0.08	14.0 ± 2.0	0.6
Staphylococcus aureus	21.6 ± 0.6	0.6	16.7 ± 1.3	0.3	17.3 ± 0.9	0.3
Staphylococcus faecalis	20.0 ± 0.6	0.2	20.3 ± 1.3	0.2	16.0 ± 1.6	1.3
Escherichia coli	23.0 ± 0.6	1.3	23.7 ± 1.3	0.08	21.3 ± 1.3	0.2
Proteus vulgaris (CSIR)	11.4 ± 0.6	2.5	21.0 ± 2.0	0.6	21.3 ± 0.9	0.6
Pseudomonas aeruginosa	11.0 ± 0.6	5.0	22.7 ± 1.7	0.3	20.7 ± 0.9	0.6
Kiebsiella pneumoniae	21.2 ± 0.6	5.0	20.0 ± 1.4	0.6	23.7 ± 1.3	0.08
Proteus vulgaris (ATCC)	6.7 ± 0.6	>10	6.0 ± 0.0	ND	6.0 ± 0.0	ND
Enterobacter cloacae	6.0 ± 0.0	>10	13.3 ± 1.3	5.0	17.7 ± 0.5	2.5

[a]Essential oil (10 $\mu g.mL^{-1}$); [b]Methanolic solutions of gentamycin (25 $\mu g.mL^{-1}$) and chloramphenicol (25 $\mu g\ mL^{-1}$); [c]ZI, zones of inhibition (mm) including diameter of sterile disc (6 mm), values are mean ± SD (3 replicates); [d]MIC, minimum inhibitory concentration, values are ($mg.mL^{-1}$); ATCC. American Type Culture Collection, USA; CSIR, Council for Scientific and Industrial Research, South Africa; ND, not determined.

Table 3. Antiplatelet aggregation and cytotoxic activities of essential oil of *Croton gratissimus* leaves.[a]

		Antiplatelet aggregation[b]				Cytotoxicity
Sample		ADP	Collagen	Epinephrine	Thrombin	Brine shrimp
*Cg*EO	1	16.6 + 6.6	52.0 + 1.2	35.5 + 2.1	45.3 + 1.8	-
	3	58.2 + 1.0	61.3 + 2.5	50.1 + 2.8	59.1 + 1.2	-
	5	63.1 + 5.3	67.6 + 1.2	53.6 + 1.9	72.5 + 4.4	-
	10	69.0 + 2.6	69.4 + 1.0	54.2 + 4.8	78.6 + 2.4	-
	IC_{50}[c]	2.32	> 1	3.65	1.18	8.52 (5.86–17.04)
Aspirin	1	36.6 + 0.4	27.0 + 0.7	12.2 + 0.6	46.1 + 3.1	-
	3	55.2 + 0.2	37.0 + 3.2	37.1 + 2.1	75.3 + 2.0	-
	5	58.0 + 0.7	59.0 + 5.0	39.6 + 1.6	77.6 + 3.4	-
	10	61.0 + 0.5	69.1 + 1.1	57.6 + 1.0	53.3 + 1.2	-
	IC_{50}[c]	2.34	4.20	8.18	1.88	-
Gallic acid (IC_{50})[c]		—	—	—	—	11.45 (6.63–38.15)

[a]Values are mean ± SD (3 replicates); [b]Percentage platelet aggregation inhibition; [c]IC_{50} values ($mg.mL^{-1}$) with 95% confidence intervals (95% CI); *Cg*EO, *C. gratissimus* essential oil.

Discussion

The compositions of essential oils of *C. gratissimus* were different from those reported for a related species, *C. gratissimus* var. *subgratissimus* (31), although both oils contained high contents of α-phellandrene and moderate quantity of germacrene D. However, 1,8-cineole reported in the latter was not identified in the former. The observed antimicrobial activity of the *C. gratissimus* oil was in agreement with previous studies on the antimicrobial potentials of essential oils from *C. gratissimus* var. *subgratissimus* (31) and *C. oblongifolius* (3). A previous analysis indicated that the oils of *C. matourensis* and *C. micans* have moderate cytotoxicity (9). The bioactivities of the crude extracts and fractions from different organs of *C. gratissimus*, which strongly enhance its utilization in traditional medicines, have been extensively reported (2, 15, 23, 24, 30). The potency of the essential oil of *C. gratissimus*

may be due to its major phytochemicals and the synergistic effects of the minor constituents, some of which have been reported to possess several biological activities (16, 17).

Among the identified compounds, germacrene D show antifungal activity (7, 27) and insecticidal activity against mosquitoes (13); α-pinene contributes to antimicrobial effects of some essential oils (16, 17). The positive analgesic effects of α-pinene (12) has also been reported. α-Phellandrene, a monoterpene, promoted immune responses (18) and acted as an anticancer agent (19). The contribution of α-phellandrene and sabinene to the antioxidant activity of an essential oil has been documented (22), while α-phellandrene inhibited the feeding of caterpillars (14). Sabinene displayed anti-inflammatory activity by inhibiting nitric oxide production in lipopolysaccharide (LPS)–triggered macrophages (4), while β-phellandrene showed insecticidal activity (26). Thus, a combination of phytochemicals with reported bioactivity in the essential oil of *C. gratissimus* may contribute to its antibacterial, antiplatelet aggregation, and cytotoxic activities observed in this study.

References

1. Adams, R. P. 2007. *Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry*, 4th ed. Allured Publishing, Carol Stream, IL.
2. Ashwell, R. N., M. A. Aderogba, N. Bhekumthetho, and J. van Staden. 2013. Antioxidative and cholinesterase inhibitory effects of leaf extracts and their isolated compounds from two closely related *Croton* species. *Molecules* 18:1916–1932.
3. Athikomkulchai, S., S. Tadtong, N. Ruangrungsi, and T. Hongratanaworakit. 2015. Chemical composition of the essential oil from *Croton oblongifolius* and its antibacterial activity against *Propionibacterium acnes*. *Nat. Prod. Commun*. 10:1459–1460.
4. Arunkumar, R., S. A. Nair, K. B. Rameshkumar, and A. Subramoniam. 2014. The essential oil constituents of *Zornia diphylla* (L.) Pers, and anti-inflammatory and antimicrobial activities of the oil. *Rec. Nat. Prod*. 8:385–393.
5. Bahadur, I., D. Ramjugernath, F. Bux, A. Guldhe, B. Singh, et al. 2014. Assessment of potentials of *Croton gratissimus* oil for macroscale production of biodiesel based on thermophysical properties. *Energy Fuels* 28:7576–7581.
6. *British Pharmacopoeia Specification*. Vol. II. 1980. H. M. Stationary Office.
7. Cheng, S. S., H. Y. Lin, and S. T. Chang. 2005. Chemical composition and antifungal activity of essential oils from different tissues of Japanese cedar (*Cryptomeria japonica*). *J. Agric. Food Chem*. 53:614–619.
8. Coates, P. M. 2002. *Croton gratissimus Burch Keith Coates Palgrave Trees of Southern Africa*. Struik Publishers, South Africa, vol. 3, pp. 185–187.
9. Compagnone, R. S., K. Chavez, E. Mateu, G. Orsini, F. Arvelo, and I. S. Alírica. 2010. Composition and cytotoxic activity of essential oils from *Croton matourensis* and *Croton micans* from Venezuela. *Rec. Nat. Prod*. 4:101–108.
10. Dorman, H. J. D., and S. G. Deans. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J. Appl. Microbiol*. 88:308–316.

11. Eloff, J. N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med*. 64:711–713.
12. Him, A., H. Ozbek, I. Turel, and A. C. Oner. 2008. Antinoceceptive activity of alpha-pinene and fenchone. *Pharmacol. Online* 3:363–369.
13. Kiran, S. R., and P. S. Devi. 2007. Evaluation of mosquitocidal activity of essential oil and sesquiterpenes from leaves of *Chloroxylon swietenia DC*. *Parasitol. Res*. 101:413–418.
14. Kordan, B., and B. Gabryś. 2013. Feeding deterrent activity of natural monoterpenoids against larvae of the large white butterfly *Pieris brassicae* (L.). *Pol. J. Nat. Sci*. 28:63–69.
15. Langat, M. K., N. R. Crouch, P. J. Smith, and D. A. Mulholland. 2011. Cembranolides from the leaves of *Croton gratissimus. J. Nat. Prod*. 74:2349–2355.
16. Lawal, O. A., I. A. Ogunwande, A. R. Opoku, A. A. Kasali, and A. O. Oyedeji. 2015. Chemical composition, antibacterial and cytotoxicity activities of essential oil from the leaves of *Helichrysum odoratissimum* from South Africa. *J. Essent. Oil Bear. Plants*. 18:236–241.
17. Lawal, O. A., I. A. Ogunwande, A. F. Salvador, A. A. Sanni, and A. R. Opoku. 2014. *Pachira glabra* Pasq. essential oil: Chemical constituents, antimicrobial and insecticidal activities. *J. Oleo Sci*. 63:629–635.
18. Lin, J. J., J. W. Lin, S. C. Hsu, S. W. Weng, Y. P. Huang, et al. 2013. Alpha-phellandrene promotes immune responses in normal mice through enhancing macrophage phagocytosis and natural killer cell activities. *In Vivo* 27:809–814.
19. Lin, J. J., K. W. Lu, Y. S. Ma, N. Y. Tang, P. P. Wu, et al. 2014. Alpha-phellandrene, a natural active monoterpene, influences a murine WEHI-3 leukemia model in vivo by enhancing macrophague phagocytosis and natural killer cell activity. *In Vivo* 28:583–588.
20. Mekhfi, H., E. I. Haouari, and A. Legssyer. 2004. Platelet anti-aggregant property of some Moroccan medicinal plants. *J. Ethnopharmacol*. 94:317–322.
21. Meyer, B. N., N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols, and J. L. McLaughlin. 1982. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Med*. 45:31–34.
22. Mohammadi, A., T. S. Ahmadzadeh, A. A. Ameri, M. Imani, E. Golmakani, and H. Kamali. 2015. Seasonal variation in the chemical composition, antioxidant activity, and total phenolic content of *Artemisia absinthium* essential oils. *Pharmacogn. Res*. 7:329–334.
23. Mthethwa, N. S., O. A. Oyedeji, L. C. Obi, and O. A. Aiyegoro. 2014. Anti-staphylococcal, anti-HIV and cytotoxicity studies of four South African medicinal plants and isolation of bioactive compounds from *Cassine trasvaalensis* (Burtt. Davy) cood. *BMC Comp.l Alter. Med*. 14:512. doi: 10.1186/1472-6882-14-512
24. Mulholland, D. A., M. K. Langat, N. R. Crouch, H. M. Coley, E. M. Mutambi, and J. C. Nuzillard. 2010. Cembranolides from the stem bark of the southern African medicinal plant, *Croton gratissimus* (Euphorbiaceae). *Phytochemistry* 71:1381–1386.
25. *National Institute of Science and Technology*. 2011. Chemistry Web Book. Data from NIST Standard Reference Database 69. 2011. http://www.nist.gov.
26. Prieto, J. A., O. J. Patiño, W. A. Delgado, J. P. Moreno, and L. E. Cuca. 2011. Chemical composition, insecticidal, and antifungal activities of fruit essential oils of three Colombian *Zanthoxylum* species. *Chil. J. Agric. Res*. 7:73–82.
27. Sahin, F., M. Güllüc, D. Daferera, A. Sökmen, M. Sökmen, et al. 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the eastern Anatolia region of Turkey. *Food Control*. 15:549–557.

28. Tomita, T., K. Umgegaki, and E. Hayash. 1983. Basic aggregation of properties of washed rat platelets: Correlation between aggregation, phospholipids degradation, malondialdehyde, and thromboxane formation. *J. Pharmacol. Meth.* 10:31–44.
29. van Vuuren, S. F., and A. M. Viljoen. 2008. *In vitro* evidence of phyto-synergy for plant part combinations of *Croton gratissimus* (Euphorbiaceae) used in African traditional healing. *J. Ethnopharmacol.* 119:700–704.
30. van Vureen, S. F., and D. Naidoo. 2010. An antimicrobial investigation of plants used traditionally in southern Africa to treat sexually transmitted infections. *J. Ethnopharmacol.* 130:552–558.
31. van Vuuren, S. 2007. The antimicrobial activity and essential oil composition of medicinal aromatic plants used in African traditional healing. Ph.D. thesis, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg.