

Cytotoxicity and anti-HIV activity of solvent fractions of Kenyan *Croton megalocarpus* In Vitro and Mechanisms in In Silico Models

Ermias Mergia Terefe^{1*}, Faith Apolot Okalebo², Solomon Derese³, Joseph Muriuki⁴, Arabinda Ghosh⁵, Gaber El-Saber Batiha⁶

¹Department of Pharmacology and Pharmacognosy, School of Pharmacy and Health Sciences, United States International University-Africa, eterefe@usiu.ac.ke

²Department of Pharmacology and Pharmacognosy, College of Health Sciences, University of Nairobi, faith.okalebo@uonbi.ac.ke

³Department of Chemistry, University of Nairobi, sderese@uonbi.ac.ke

⁴Centre for Virus Research, Kenya Medical Research Institute JMuriuki@kemri.org

⁵Microbiology Division, Department of Botany, Gauhati University, Guwahati, Assam-781014, India; dra.ghosh@gauhati.ac.in

⁶Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, AlBeheira, Egypt; gaberbatiha@gmail.com

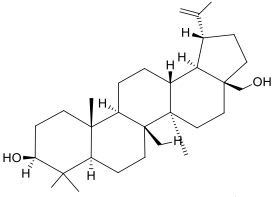
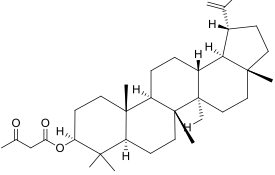
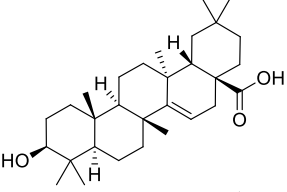
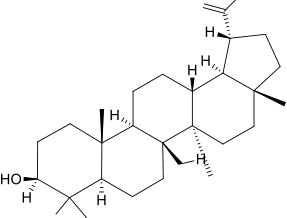
*Corresponding Author P.O. BOX 14634 - 00800 Nairobi, KENYA, eterefe@usiu.ac.ke; mergiae@gmail.com, +254746272742

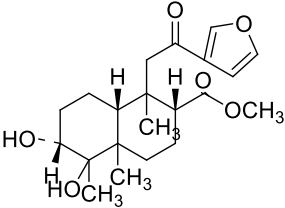
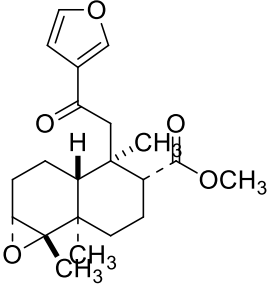
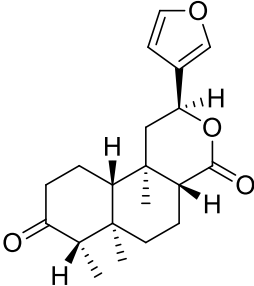
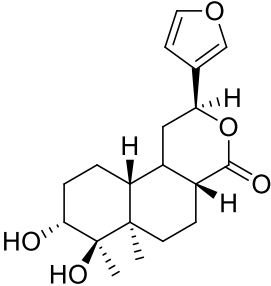
Contents

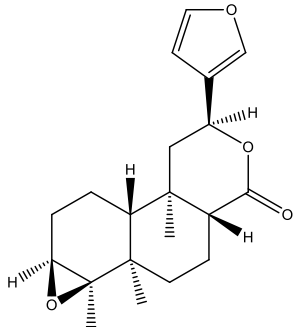
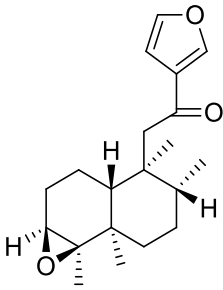
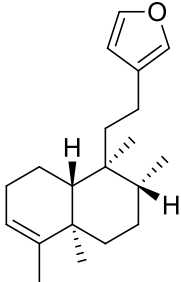
Table S1 Description of Phytochemicals isolated from <i>Croton megalocarpus</i>	2
Table S2 Phytochemical screening of Solvent fractions of <i>C. megalocarpus</i>	7
Table S3 Area under curve (AUC) graph for validation of docking	8
Table S4. Interactions and binding energies of top 10 conformations of <i>Croton megalocarpus</i> ligands with HIV-1 Reverse transcriptase enzyme (PDB: 1REV)	9
Table S5. Lipinski properties of phytochemicals isolated from <i>Croton megalocarpus</i>	12

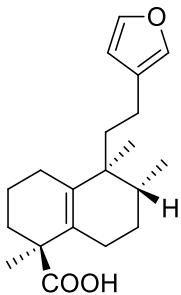
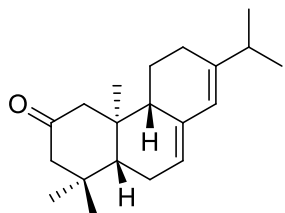
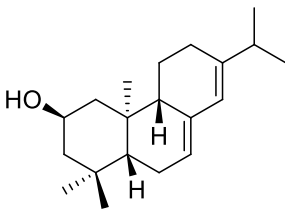
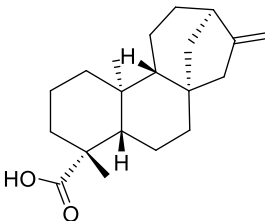
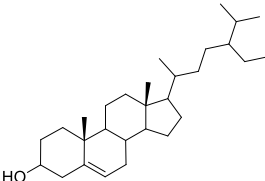
Table S6 Predicted ADMET properties of phytochemical compounds isolated from *Croton megalocarpus* and FDA approved drugs..... 14

Table S1 Description of Phytochemicals isolated from *Croton megalocarpus*

Ser. No	Code	Name of phytochemical	Chemical structure	Reference
1.	L62	Betulin		[1–4][3,5–7]
2.	L63	3-β-O-Acetoacetyl lupeol		[5,8,9]
3.	L12	Aleuritolic acid		[8–13]
4.	L9	Lupeol		[10,14,15]

Ser. No	Code	Name of phytochemical	Chemical structure	Reference
5.	L46	Chiromodine		[1,2,9,16,17]
6.	L60	Epoxychiromodine		[5,8,9,16,18]
7.	L104	Crotonolide E		[19,20]
8.	L105	Furocrotinsulolide A		[19,21]

Ser. No	Code	Name of phytochemical	Chemical structure	Reference
9.	L106	3 β ,4 β :15,16-diepoxy-13(16),14-ent-clerodadien-17,12S-olide		[19]
10.	L107	3 β ,4 β :15,16-diepoxy-13(16),14-ent-clerodadiene		[19,22,23]
11.	L108	Trans-annonene		[19,24]

Ser. No	Code	Name of phytochemical	Chemical structure	Reference
12.	L109	Crotohalimaneic acid		[19,25,26]
13.	L110	7,13-abietadien-2-one		[19]
14.	L111	7,13-abietadien-2-ol		[19]
15.	L68	Ent-kaur-16-en-19-oic acid		[8]
16.	L61	Beta sitosterol		[3,5]

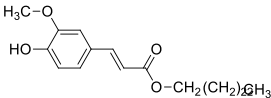
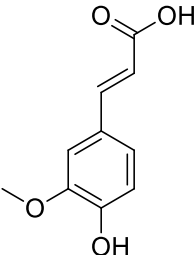
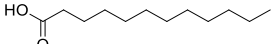
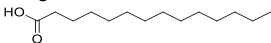
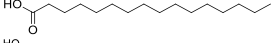
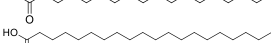
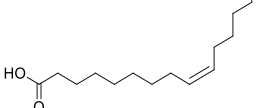
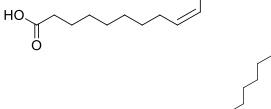
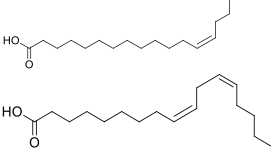
Ser. No	Code	Name of phytochemical	Chemical structure	Reference
17.	L64	Esters of ferulic acid		[8]
18.	L75	Ferulic acid		[9]
19.	L76	Lauric acid		[27,28]
20.	L77	Myristic acid		[27,28]
21.	L78	Palmitic acid		[27,28]
22.	L79	Stearic acid		[27,28]
23.	L80	Arachidic acid		[27,28]
24.	L81	Palmitoleic acid		[27,28]
25.	L82	Oleic acid		[27,28]
26.	L83	Erucic acid		[27,28]
27.	L84	Linoleic acid		[27,28]

Table S2 Phytochemical screening of Solvent fractions of *C. megalocarpus*

	Hexane		Dichloromethane		Ethyl acetate		Methanol	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
Alkaloids	+	-	+	+	+	+	+	-
Anthraquinones	-	-	-	-	+	-	-	-
Flavonoids	-	-	-	-	+	+	+	+
Glycoside	+	+	-	+	+	+	-	-
Saponins	+	+	-	-	+	+	+	+
Steroids	+	+	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+	+	+
Tannins	-	+	-	-	+	+	-	-
Terpenes	+	+	+	+	+	+	+	+

Table S3 Area under curve (AUC) graph for validation of docking

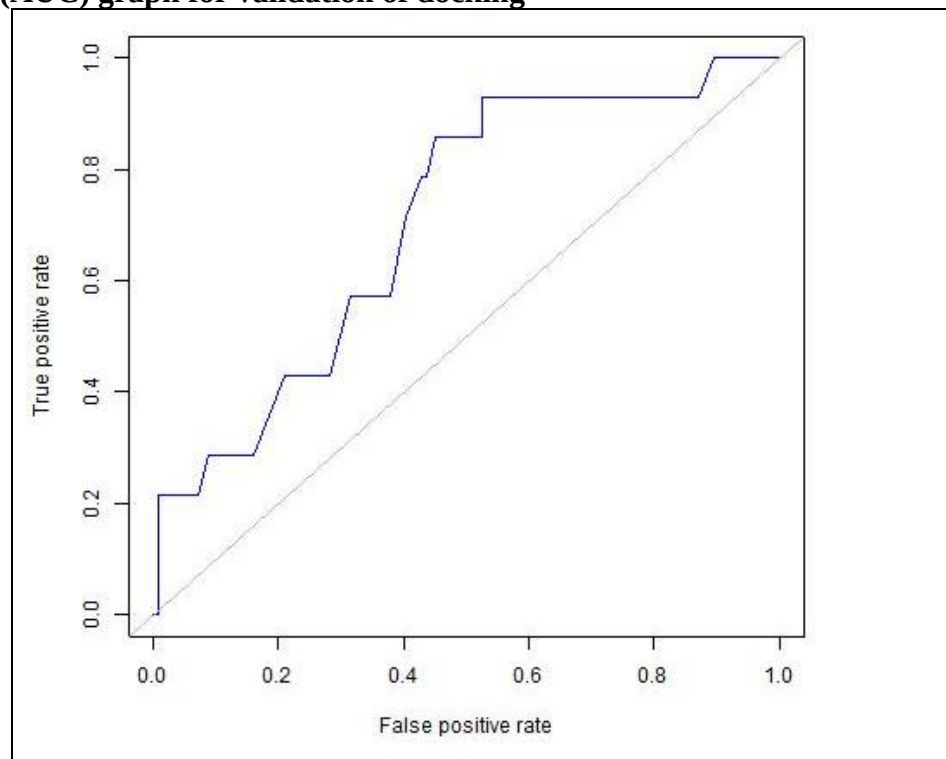


Figure 1 Area under curve (AUC) graph for validation of docking

Table S4. Interactions of top 10 conformations of *Croton megalocarpus* ligands with HIV-1 Reverse transcriptase enzyme (PDB: 1REV)

Ser. No.	Code	Name of Phytochemical	Interacting amino acids residues	Number of Hydrogen bonds	Hydrogen bond interactions
1.	L12	Aleuritolic acid	ASN 265, GLN 269 (3.65 Å), GLU 378, GLY 352, HIS 96, ILE 382 (4.00 Å), ILE 94 (3.60 Å), LYS 350, LYS 353, LYS 374, PRO 95 , SER 268, TYR 232, TYR 339, VAL 381,	2	LYS 353 (2.77 Å), LYS 353 (3.62 Å),
2.	L9	Lupeol	ASN 265, GLN 269, GLU 378, GLY 352, HIS 96, ILE 382 (3.26 Å), ILE 94 (3.35 Å), LYS 350, LYS 353, LYS 374, SER 268, TRP 266, TYR 232, TYR 339, VAL 381 (3.80 Å),	2	LYS 353 (2.74 Å) LYS 353 (3.00 Å)
3.	L62	Betulin	ASN 265, GLN 269, GLU 378, GLY 352, HIS 96, ILE 382 (3.04 Å), ILE 94 (3.39 Å), LYS 350, LYS 353, LYS 374, SER 268, TRP 266, TYR 232, TYR 339, VAL 381 (3.65 Å),	2	LYS 353 (2.66 Å), LYS353 (3.11 Å)
4.	L63	3-β-O-Acetoacetyl lupeol	ASN 265, GLN 269, GLU 378, GLY 352, GLY 93, HIS 96 (3.24 Å), ILE 382, ILE 94 (3.51 Å), LEU 92, LYS 350, LYS 353, LYS 374, SER 268, TRP 266 (3.99 Å), TYR 232, TYR 339 (3.69 Å), VAL 381,	1	ILE 94 (3.07 Å)
5.	L46	Chiromodine	ASN 265, GLN 269 (3.19 Å), GLU 378, HIS 96 (3.61 Å), ILE 382 (3.57 Å), ILE 94 (3.72 Å), LYS 350, MET 230, PRO 95 TRP 266,	4	LYS 350 (2.85 Å), TYR 232 (2.84 Å), TRP 266, ASN 265

Ser. No.	Code	Name of Phytochemical	Interacting amino acids residues	Number of Hydrogen bonds	Hydrogen bond interactions
6.	L104	Crotonolide E	GLU 378, GLY 99, HIS 96 (2.94 Å), ILE 382 (3.95 Å), ILE 94 (3.29 Å), PRO 95, TRP 266 (3.82 Å), TYR 232, VAL 381 (3.84 Å),	1	TYR 232 (2.79 Å)
7.	L109	Crotohalimaneic acid	ASN 265, GLN 269 (2.88 Å), GLY 352, HIS 96, LYS 350, LYS 353 (3.80 Å), LYS 374, SER 268, THR 351, TRP 266 (3.29 Å), TYR 232, TYR 339,	2	LYS 374 (3.62 Å), TYR 232,
8.	L60	Epoxychiromodine	GLY 93, HIS 96 (3.03 Å), HIS 96, ILE 382, ILE 94 (3.49 Å), LEU 92, MET 230, PRO 95, TRP 266 (3.93 Å), TYR 232 (3.30 Å), VAL 381,	1	ILE 94 (3.01 Å)
9.	L105	Furocrotinsulolide A	ASN 265, GLN 269 (3.10 Å), GLY 262, GLY 352, LYS 353, LYS 374, SER 268, TRP 266 (3.67 Å), TRP 266, TYR 339 (3.81 Å),	3	LYS 353 (2.83 Å), LYS 353 (3.38 Å), LYS 374 (2.69 Å)
10	L111	7,13-abietadien-2-ol	ASN 265, GLN 269 (3.32 Å), HIS 96 (3.08 Å), ILE 382, ILE 94 (3.22 Å), LYS 350, PRO 95, SER 268, TRP 229 TRP 266 (3.28 Å), TRP 266, TYR 232, VAL 381,	0	-
11	L75	Ferulic acid	ALA 355, ARG 356 (3.24 Å), ARG 358, GLN 373, GLU 370, LYS 374, TYR 354 (2.95 Å), TYR 354,	6	ALA 355 (2.79 Å), ARG 356 (3.14 Å) ARG 356 (3.24 Å) ARG 358 (2.81 Å), TYR354 (3.18 Å) LYS 374 (2.61 Å)
12	L110	7,13-abietadien-2-one	ASN 265, GLN 269, GLU 378 HIS 96, ILE 382, ILE 94 (3.42 Å), LYS 350, SER 268, TRP 266 (3.80 Å), TYR 232,	0	-

Ser. No.	Code	Name of Phytochemical	Interacting amino acids residues	Number of Hydrogen bonds	Hydrogen bond interactions
1.	DLV	Delaviridine	ASN 265 (3.49 Å), ASN 265, GLN 269, GLY 262, HIS 96 (3.58 Å), ILE 382 (3.78 Å), ILE 94, LYS 350, TRP 266 (3.33 Å), TYR 232,	2	ILE 94 (2.08 Å), LYS 350 (2.30 Å)
2.	AZT	Zidovudine	TRP266 (3.45 Å), TRP 266 (3.89 Å), GLN 269 (3.67 Å),	6	ILE 94 (3.05 Å), PRO 95 (2.69 Å), TYR 232 (2.20 Å), GLN 269 (3.19 Å), LYS 350 (1.91 Å), GLU 378 (3.34 Å),
3.	NVP	Nevirapine	GLN 269 (3.77 Å), GLU 378, HIS 96 (3.48 Å), ILE 382 (3.32 Å), ILE 94 (3.24 Å), LYS 350, PRO 95, PRO 97, TRP 229, TRP 266 (3.37 Å), TYR 232 (3.53 Å), VAL 381 (3.38 Å),	3	ILE 94, LYS 350 (3.13 Å), TYR 232 (2.31 Å),
4.	ABC	Abacavir	HIS 96 (3.96 Å), TRP 266 (3.31 Å), VAL 381 (3.27 Å), ILE 382 (3.43 Å),	4	PRO 95 (2.50 Å), TYR 232 (1.71 Å), TYR 232 (1.99 Å), ASN 265 (2.56 Å),

Table S5. Lipinski properties of phytochemicals isolated from *Croton megalocarpus*

Ser. No.	Code	Name of phytochemicals	Molecular weight (gm/mol) (<500)	LogP (<5)	H-Donors (<5)	H-Acceptors (<10)	MR molar refractivity (<130)
1.	L62	Betulin	442.72	6.7202	2	2	136.3
2.	L63	3- β -O-Acetoacetyl lupeol	510.79	7.9873	0	3	154.69
3.	L12	Aleuritolic acid	456.7	6.0643	2	3	136.65
4.	L9	Lupeol	426.72	7.6469	1	1	135.14
5.	L46	Chiromodine	378.46	2.0711	2	6	99.91
6.	L60	Epoxychiromodine	360.44	2.6255	0	5	96.55
7.	L104	Crotonolide E	330.42	3.0554	0	4	90.4
8.	L105	Furocrotilinsulolide A	334.41	1.7568	2	5	88.02
9.	L106	3 β ,4 β :15,16-diepoxy-13(16),14-ent-clerodadien-17,12S-olide	330.42	2.6427	0	4	89.21
10.	L107	3 β ,4 β :15,16-diepoxy-13(16),14-ent-clerodadiene	316.43	3.4739	0	3	90.46
11.	L108	Trans-annonene	286.45	5.3788	0	1	90.56
12.	L109	Crotohalimaneic acid	316.43	4.2734	1	3	92.33
13.	L110	7,13-abietadien-2-one	286.45	4.8677	0	1	90.64
14.	L111	7,13-abietadien-2-ol	288.47	4.724	1	1	91.61
15.	L68	Ent-kaur-16-en-19-oic acid	302.45	4.1175	1	2	90.32
16.	L61	Beta sitosterol	414.71	7.8552	1	1	133.23
17.	L64	Esters of ferulic acid-1	530.82	11.889	1	4	166.51

Ser. No.	Code	Name of phytochemicals	Molecular weight (gm/mol) (<500)	LogP (<5)	H-Donors (<5)	H-Acceptors (<10)	MR molar refractivity (<130)
18	L75	Ferulic acid	194.18	1.0582	2	4	51.63
19	L76	Lauric acid	200.32	4.2449	1	2	61.57
20	L77	Myristic acid	228.37	5.1537	1	2	71.18
21	L78	Palmitic acid	256.42	6.0625	1	2	80.8
22	L79	Stearic acid	284.48	6.9713	1	2	90.41
23	L80	Arachidic acid	312.53	7.8801	1	2	100.03
24	L81	Palmitoleic acid	254.41	5.8103	1	2	80.32
25	L82	Oleic acid	282.46	6.7191	1	2	89.94
26	L83	Erucic acid	338.57	8.5367	1	2	109.17
27	L84	Linoleic acid	280.45	6.4669	1	2	89.46

Table S6 Predicted ADMET properties of phytochemical compounds isolated from *Croton megalocarpus* and FDA approved drugs

Properties	L12	L9	L62	L63	L46	L104
PSA= TPSA SWISS	57.53	20.23	40.46	43.37	96.97	56.51
AlogP98=WLOGP SWISS	7.23	8.02	7	8.55	2.97	3.95
Absorption						
Water solubility (logmol/L)	-4.757	-6.33	-5.923	-6.167	-3.763	-4.8
Caco-2 permeability (log Papp in 10 ⁻⁶ cm/s)	1.369	1.323	1.326	1.267	0.917	1.447
Intestinal absorption (human) (%) absorbed)	97.287	100	97.928	100	91.755	96.989
Skin permeability (log Kp)	-2.73	-2.687	-2.788	-2.773	-4.176	-3.129
P-Glycoprotein substrate	No	No	No	No	Yes	No
P-Glycoprotein I inhibitor	No	Yes	Yes	Yes	Yes	Yes

References

1. Addae-Mensah I, Achenbach H, Thoithi GN, Waibel R, Mwangi JW. Epoxychiromodine and other constituents of *Croton megalocarpus*. *Phytochemistry*. 1992;
2. Maroyi A. Ethnopharmacological Uses, Phytochemistry, and Pharmacological Properties of *Croton macrostachyus* Hochst. Ex Delile: A Comprehensive Review. *Evidence-based Complement Altern Med*. 2017;
3. Tala MF, Tan NH, Ndontsa BL, Tane P. Triterpenoids and phenolic compounds from *Croton macrostachyus*. *Biochem Syst Ecol*. 2013;
4. Tene M, Ndontsa B, Tane P, De Dieu Tamokou J, Kuiate J-R. Antimicrobial diterpenoids and triterpenoids from the stem bark of *Croton macrostachys*. *Int J Biol Chem Sci* [Internet]. 2009 Aug 25 [cited 2021 Aug 8];3(3). Available from: <https://www.ajol.info/index.php/ijbcs/article/view/45331>
5. Maroyi A. Ethnomedicinal uses and pharmacological activities of *Croton megalobotrys* Müll Arg: A systematic review. *Trop J Pharm Res*. 2017;16(10):2535–43.
6. Addae-Mensah, Muriuki, Karanja W, Waibel and A. Constituents of the stem bark and twigs of *Croton macrostachyus*. *Fitoterapia*, vol. 63, no. 1,; 1992. p. 81.
7. Tene M, Ndontsa BL, Tane P, Tamokou JDD, Kuiate J. Antimicrobial diterpenoids and triterpenoids from the stem bark of *Croton macrostachys*. *Int J Biol Chem Sci*. 2009;
8. Addae-Mensah I, Achenbach H, Thoithi GN, Waibel R, Mwangi JW. Epoxychiromodine and other constituents of *Croton megalocarpus*. *Phytochemistry*. 1992;31(6):2055–8.
9. Addae-Mensah I, Waibel R, Achenbach H, Muriuki G, Pearce C, Sanders JKM. A clerodane diterpene and other constituents of *Croton megalocarpus*. *Phytochemistry*. 1989;
10. Yannick Stephane FF, Dawe A, Angelbert Fusi A, Jean Jules BK, Ulrich KKD, Lateef M, et al. Crotoliganfuran, a new clerodane-type furano-diterpenoid from *Croton oligandrus* Pierre ex Hutch. *Nat Prod Res* [Internet]. 2019;0(0):1–9. Available from: <https://doi.org/10.1080/14786419.2019.1613399>
11. Maroyi A. *Croton megalocarpus* Hutch. in *Tropical Africa: Phytochemistry, Pharmacology and Medicinal Potential*. *Res J Med Plants*. 2017;11(4):124–33.
12. Aldhafer A, Langat M, Ndunda B, Chirchir D, Midiwo JO, Njue A, et al. Diterpenoids from the roots of *Croton dichogamus* Pax. *Phytochemistry* [Internet]. 2017;144:1–8. Available from: <http://dx.doi.org/10.1016/j.phytochem.2017.08.014>
13. Langat MK, Crouch NR, Pohjala L, Tammela P, Smith PJ, Mulholland DA. Ent-kauren-19-oic acid derivatives from the stem bark of *Croton pseudopulchellus* Pax. *Phytochem Lett*. 2012;
14. De Araújo-Júnior VT, Da Silva MS, Leita da-Cunha EV, Agra MDF, Da Silva-Filho RN, Barbosa-Filho JM, et al. Alkaloids and diterpenes from *Croton moritibensis*. *Pharm Biol*. 2004;
15. Burns D, Reynolds WF, Buchanan G, Reese PB, Enriquez RG. Assignment of ¹H and ¹³C

- spectra and investigation of hindered side-chain rotation in lupeol derivatives. *Magn Reson Chem* [Internet]. 2000 Jul 1 [cited 2020 Sep 21];38(7):488–93. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/1097-458X%28200007%2938%3A7%3C488%3A%3AAID-MRC704%3E3.0.CO%3B2-G>
16. Addae-mensah I, Achenbach H, Muriuki G, Waibel R. Chiromodin a Novel Clerodane-Type Diterpene from *Croton megalocarpus*. 1988;(1):572–3.
 17. Kokwaro OJ. Medicinal Plants of East Africa. *Contemp Sociol*. 2009;
 18. Markó IE, Wiaux M, Warriner SM, Giles PR, Eustace P, Dean D, et al. Towards the total synthesis of clerocidin. Efficient assembly of the decalin subunit. *Tetrahedron Lett*. 1999;
 19. Alqahtani A. Phytochemical investigation of members of the Asparagaceae and Euphorbiaceae families. Univ Surrey, PhD dissertation. 2015;1(Volume 1).
 20. Liu CP, Xu JB, Zhao JX, Xu CH, Dong L, Ding J, et al. Diterpenoids from *croton laui* and their cytotoxic and antimicrobial activities. *J Nat Prod* [Internet]. 2014 Apr 25 [cited 2020 Oct 14];77(4):1013–20. Available from: <https://pubs.acs.org/doi/abs/10.1021/np500042c>
 21. Graikou K, Aligiannis N, Chinou I, Skaltsounis A-L, Tillequin F, Litaudon M. Chemical Constituents from *Croton insularis*. *Helv Chim Acta* [Internet]. 2005 Oct 1 [cited 2020 Oct 14];88(10):2654–60. Available from: <http://doi.wiley.com/10.1002/hlca.200590206>
 22. Roengsumran S, Petsom A, Kuptiyanuwat N, Vilaivan T, Ngamrojnvanich N, Chaichantipyuth C, et al. Cytotoxic labdane diterpenoids from *Croton oblongifolius*. *Phytochemistry*. 2001 Jan 5;56(1):103–7.
 23. Harinantenaina L, Asakawa Y. Malagasy Liverworts, Source of New and Biologically Active Compounds. *Nat Prod Commun* [Internet]. 2007 Jun 25 [cited 2020 Sep 30];2(6):1934578X0700200. Available from: <http://journals.sagepub.com/doi/10.1177/1934578X0700200616>
 24. McCrindle R, Nakamura E, Anderson AB. Constituents of *Solidago* species. Part VII. Constitution and stereochemistry of the cis-clerodanes from *Solidago arguta* ait. and of related diterpenoids. *J Chem Soc Perkin Trans 1* [Internet]. 1976 Jan 1 [cited 2020 Sep 30];(15):1590–7. Available from: <https://pubs.rsc.org/en/content/articlehtml/1976/p1/p19760001590>
 25. Roengsumran S, Pornpakakul S, Muangsins N, Sangvanich P, Nhujak T, Singtothong P, et al. New Halimane Diterpenoids from *Croton oblongifolius*. *Planta Med* [Internet]. 2004 Jan 1 [cited 2020 Sep 30];70(1):87–9. Available from: <http://www.thieme-connect.de/DOI/DOI?10.1055/s-2004-815466>
 26. Ndunda B. Phytochemistry and bioactivity investigations of three Kenyan *Croton* species. PhD Thesis, University of Nairobi, Nairobi; 2014. p. 307.
 27. Kafuku G, Mbarawa M. Biodiesel production from *Croton megalocarpus* oil and its process optimization. *Fuel*. 2010;
 28. Wu D, Roskilly AP, Yu H. *Croton megalocarpus* oil-fired microtrigeneration prototype for remote and self-contained applications: Experimental assessment of its performance and

gaseous and particulate emissions. *Interface Focus*. 2013;

