BCSIR

Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **54(2)**, 139-146, 2019

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

In-vitro and *in-vivo* antibacterial effect of *Croton lobatus* Linnaeus L. on two days post surgical wounds in rats

M. A. Kilani[1]*, A. Z. Hassan[1], S. T. Fadason[1], A. M. Obalowu[2], A. Aliyu[3] and H. M. Badmus-Kilani[1]

[1]*Department of Veterinary Surgery and Radiology, Ahmadu Bello University, P. M. B. 1096, Zaria, Kaduna State, Nigeria*
[2]*University of Ilorin Veterinary Teaching Hospital, Ilorin, Kwara State, Nigeria*
[3]*Department of Veterinary Surgery and Radiology, University of Ilorin, Kwara State, Nigeria*
[4]*Ministry of Health, General Hospital Hunkuyi, Kaduna State, Nigeria*

Abstract

Phytochemical constituents of *Croton lobatus* L. (*C. lobatus*) water extracts and quantitative analysis were carried out following standard procedures. The antibacterial activity against *Staphylococcus aureus* (ATCC 33591); *Streptococcus Spp*; *Pseudomonas aeruginosa* (ATCC 9028); *Proteus vulgaris*; *Escherichia coli* (ATCC 43895); and *Salmonella Spp* (ATCC 4932) was carried out at the concentration of 0.5g/mL, 0.05 g/mL and 0.00 5g/mL of water. *In vivo* antimicrobial assay was carried out by creating four wounds of 0.5 by 0.5 cm on dorsal surface of a male albino rat under anesthesia. The wounds were left for 48 hrs, after which they were accessed and samples were collected for culture, identification and colony forming unit counts (CFU). Respective treatment using dried *C. lobatus*, *C. lobatus* (water extract), Physiological saline solution and Cicatrin powder was carried out and samples were collected at day one, three, five and seven after initiation of treatments for CFU counts on nutrient and MacConkey agar. The phytochemical studies revealed that *C. lobatus* contains carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids, alkaloids and tannins. *Croton lobatus* L. showed a dose dependent activity against micro organisms with *C. lobatus* 0.5 inhibited the growth of most bacteria at the zone of inhibition ≤ 21mm. This was also supported by *in vivo* antimicrobial assay. Secondary metabolite tannins, triterpenoids, flavonoids, crotonic acids and saponin were responsible for its antimicrobial activity against the tested microorganisms thereby supporting its usage by the traditional medicine practitioner in Nigeria to treat chronic wounds.

Received: 29 March 2018
Revised: 25 Novembe 2018
Accepted: 03 December 2018

DOI: https://doi.org/10.3329/bjsir.v54i2.41670

Keywords: Phytochemical screening; Antibacterial; *Croton lobatus* L.; Surgical wounds; *Astraea lobata*

Introduction

One thousand three hundred and thirty species of trees, shrubs and herbs belonging to Croton were distributed in the Northern and Southern Hemispheres (Block *et al.* 2004). Majority of which were popularly used for various purposes due to their medicinal qualities in different continents (Yibralign, 2007). *Croton lobatus* L. is commonly known as Rusffoil; Croton; Lobed croton (Malpighiales of North America Update, 2011). In Nigeria, Burkill reported that the plant is known in three major languages which are: *Gaásàyaá* (*Hausa)*; *Òkwè*-one a kind of bean (*Igbo)*; *Àjẹ*ìof*òlé* or *ẹ*ìru (*Yoruba)*. The old name was *Astraea lobata* Klotzsch (Global Biodiversity Information Facility, 2016; Malpighiales of North America Update, 2011). *Croton lobatus* L. is native of Caribbean and American but introduced in different part of African countries (Gaikwad *et al.,* 2012; Malpighiales of North America Update, 2011).

*Corresponding author e-mail: kilanimuhyideen.kma@gmail.com

In Africa, *C. lobatus* is commonly used by Indigenous people to treat different diseases such as malaria, diarrhea and problems due to pregnancy (Aké, 1975). The crude extracts from the aerial and roots of *C. lobatus* have anti-plasmodia activity (Weniger *et al.* 2004). Heated leaves are rubbed on to areas of costal and rheumatic pain, and a leaf-decoction by mouth, or a bark-decoction by enema is given as a purgative (Burkill, 1985). The plant is used generally as healing leaf medicine, pain-killer, laxative, cutaneous and subcutaneous parasitic infection, treatment for menstrual cycle disorders, antidotes (venomous stings and bites), treatment for paralysis, epilepsy, convulsions, spasm and topically to treat ulcers, sores, and headache (Burkill, 1985). Odukoya *et al.* (2012) reported that *C. lobatus* is usually boiled with water and the decoction is taken to treat wounds that refuse to heal while Bouquet and Debray, (1974) reported the whole plant infusion to be used as a topical bath to treat skin diseases.

Croton lobatus L. has recently been reported by traditional medicine practitioner in Nigeria as having a good therapeutic index on chronic wounds by applying its fresh leaves or dried powder on wound surface while the leaf-decoction is used to aid development of fetus. Hence, it is necessary to screen for the phytoconstituents of *C. lobatus* and evaluate its antimicrobial activities against common microorganisms that infect wounds.

Methods and materials

Sample Collection

Croton lobatus L. was collected from Obokun Local Government Area of Osun state Nigeria and was confirmed in the Botanical Garden, Biological Science department of Ahmadu Bello University, Zaria, with voucher specimen number of 913.

Preparation of crude extract

Leave of *C. lobatus* was harvested and air dried at normal temperature. The dried powder of the plant was extracted with distilled for 24 h. The extract was filtered through Whatmann filter paper No 1 and it was thereafter, centrifuged at 5000G for 15 min. The supernatant were further filtered through Millipore filter before storage at 4° C as described by (Prashanthi *et al.,* 2012).

Preliminary phytochemical test

Screening for the phyto-constituents such as saponins, alkaloids, flavonoids, steroids, tannins, cardiac glycosides, glycosides, and proteins was carried out as described by (Evans and Trease, 1996; Harborne, 1973; Sofowara, 1993).

Table I. Concentration of *C. lobatus* water extracts and standard antibiotics used for both *in vitro* and *in vivo* antimicrobial studies

Antibiotic disk cartridges (Treatment)		Code	Disk Potency (treatment dose)
1	C. lobatus	CL – P (A)	Dried Paste (Liberal)
2	C. lobatus	CL – 0.5 (B)	0.5 g/mL of water (2 drops)
3	C. lobatus	CL – 0.05	0.05 g/mL of water
4	C. lobatus	CL – 0.005	0.00 5g/mL of water
5	Ceftriaxone	CRO 30	30 µg
6	Erythromycin	E 15	15 µg
7	Sulphamethoxazole	STX 25	25 µg
8	Cloxacillin	OB 5	5 µg
9	Oxacillin	OX 1	1 µg
10	Ceftazidime	CAZ 10	10 µg
11	Gentamycin	CN 10	10 µg
12	Amoxycillin	AML 25	25 µg
15	Physiological saline solution (PSS)	C	2 drops
16	Cicatrin powder	D	Liberal

KEY: CL – P (*C. lobatus* Paste), CL – 0.5 (*C. lobatus*: 0.5g/mL of water), CL – 0.05 (*C. lobatus*: 0.05g/mL of water), CL – 0.005 (*C. lobatus*: 0.005g/mL of water), CRO 30 (Ceftriaxone), E 15 (Erythromycin), STX 25 (Sulphamethoxazole), OB 5 (Cloxacillin), OX 1 (Oxacillin), CAZ 10 (Ceftazidime), CN 10 (Gentamycin), AML 25 (Amoxycillin).

Quantitative analysis of the phytochemicals

Flavonoids, alkaloid saponin and tannin were determined as describes by Bohm and Kocipai-Abyazan (1994), Harborne (1973), Obadoni and Ochuko (2001) and Van-Burden and Robinson (1981) respectively. While the spectrophotometric method was used to estimate total phenol contents of the plant.

Examinations of Antimicrobial activity of the C. lobatus

Microbial isolate used were *Staphylococcus aureus* (ATCC 33591)*; Streptococcus Spp* (By Dr Raji bacteria zoonosis laboratory, A.B.U)*; Pseudomonas aeruginosa* (ATCC 9028)*; Proteus vulgaris* (isolate identified from bacteria zoonosis laboratory VPH, A.B.U)*; Escherichia coli* (ATCC 43895)*; Salmonella Spp* (ATCC 4932).

In vitro antimicrobial assay

A method by Irobi *et al.* (1994) and Shinwari *et al.* (2009) was adopted. The prepared *C. lobatus* water extract was reconstituted with sterile distilled water similar to what was done by Ongsakul *et al.* (2009) to obtain a concentration of 0.5g/mL. Lower concentrations of 0.05 g/mL and 0.00 5g/mL of water were then prepared from the concentration of 0.5g/mL (Table I). Broth cultures with turbidity equivalent of 0.5 McFarland standards of known bacteria strains as listed earlier were prepared. Using a sterile cotton swab stick, the Mueller Hinton Agar (MHA) Plates were inoculated. Sterile cork borer was used to create wells of 5 mm diameter in the medium and 100 µl of *C. lobatus* 0.5g/mL, 0.05 g/mL and 0.005 g/mL extracts were filled into each of the wells. The plates were incubated at 37°C for 24 hrs. The diameters of the growth inhibition formed in mm were recorded. The zone of inhibition was compared with standard antibiotics (Table I).

In vivo antimicrobial assay

A male albino rat of 22 weeks old, weighing about 200g was selected from an inbred colony obtained from Jos, Plateu State. It was housed in a steal cage under normal temperature, humidity and light. The rat was fed with commercial rat feed (prepared from vital feed grower mash) and adequate water was provided. Ethical permission was granted by the Ahmadu Bello University Committee on Animal Use and Care. The permission number was ABUCAUC/2016/027. The animal was allowed to acclimatize for two weeks before the experiment. It was anesthetized by intra muscular injection of Rompun(R) (xylazine hydrochloride) at the dosage of 5 mg/kg and ketamine hydrochloride at the dosage of 40 mg/kg. The whole body of the animal was cleaned with diluted Purit(R). Its dorsal region was surgically prepared for aseptic surgery and indelible marker was used to make a sterile square shape (0.5 by 0.5 centimeter) in four different places (Fig. 1). Full-thickness skin incision was carried out to create the wounds. The wounds were left for 48 hrs to mimic the clinical condition, after which they were accessed and samples were collected for culture, identification and CFU. Respective treatment using *C. lobatus* (paste), *C. lobatus* (water extract), Physiological saline solution (PSS), Cicatrin powder was carried out after 48 hrs of wound creation (Table I) and samples were collected at day one, three, five and seven after initiation of treatments for CFU counts in nutrient agar and MacConkey agar.

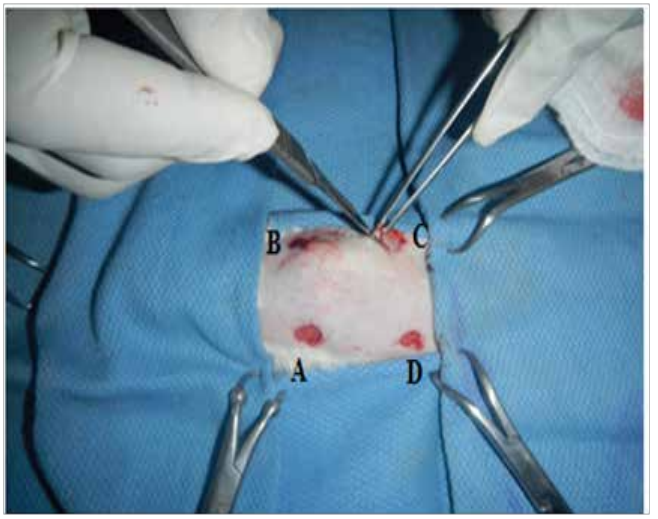

Fig. 1. Surgical creation of four wounds on the dorsal surface of the rat. Wound treated with *C. lobatus* paste (A), *C. lobatus* water extract (B), normal saline (C) and Cicatrin powder (D)

Data analysis

The results were presented inform of tables and charts, with bars representing standard error of means.

Results and discussion

Phytochemical Studies

The qualitative studies on the leaves of *C. lobatus* water extracts revealed that it contains carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids, alkaloids and tannins (Table II). The percentage composition of the alkaloids, flavonoids, saponins, phenols and tannins in the leaves of *C. lobatus* water extract is presented in the Table II. This study revealed that *C. lobatus* contains carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids and

Table II. Phytochemical constituents of *C. lobatus*

S/N	Chemical Constituents/ Test reagent	Qualitative test	Quantitative test (%)
1	Carbohydrates		
	Molisch's test	+	
	Fehling's test	+	
2	Cardiac glycoside		
	Kella-killiani test	+	
	Kedde's test	+	
3	Free Anthraquinones		
	Borntrager's test	-	
4	Combined Anthracene		
	Modified Borntrager's test	-	
5	Saponins		3.04
	Frothing test	+	
	Hemolysis test	+	
6	Steroids and Triterpenes		
	Leiberman-Burchards test	+	
7	Flavonoids		8.60
	Shinoda test	+	
	Sodium hydroxide test	+	
8	Tannins		3.80
	Lead sub-acetate test	+	
	Ferric chloride test	+	
9.	Alkaloids		5.22
	Mayer's test	+	
	Dragendorff's test	+	
10.	Phenols		3.40

Key: +: Present, - : Absent

tannins as contained in other species of Croton (Attioua, 2005; Burkill, 1985; Chabert *et al.,* 2006; Farnsworth, 1969; Willaman and Li, 1970). *Croton lobatus* L. contained 5.22 % alkaloid that was classified into five different types by Barthelemy *et al.* (2012). The study also revealed that *C. lobatus* contains steroids and triterpenes, flavonoids, alkaloids, and saponin similar to other species of croton. Similar studies have revealed the presence of triterpenes (Barbosa *et al.,* 2003), steroids, flavonoids (Cai *et al.,* 1991; Salatino *et al.,* 2007) alkaloids (Aboagye *et al.,* 2000; Amaral and Barnes, 1998; Milanowski *et al.,* 2002; Yibralign, 2007) triterpenoids, either pentacyclic or steroidal (Nath *et al.* 2013) and saponin (PROTA, 2011).

Antibacterial activities

Croton lobatus L. showed a dose dependent activity against micro organisms. *Croton lobatus* paste and *C. lobatus* 0.5 retarded the growth of *Staphylococcus aureus; Streptococcus Spp; Pseudomonas aeruginosa; Proteus vulgaris; E. coli* and *Salmonella Spp* at the zone of inhibition ≤ 21mm. The *C. lobatus* 0.05 inhibited the growth of *Proteus vulgaris* at 34mm while *C. lobatus* 0.005 inhibited the growth of *Staphylococcus aureus* at 18mm (Fig. 2). CFU counted on nutrient agar reduced drastically after initiation of treatment (day 2) in all the treated wounds (A, B and D) except with physiological normal saline treated wounds (Fig. 3). On the MacConkey agar, CFU counted significantly reduced with days on cicatrin treated wounds, slightly reduces with days on the wounds treated with *C. lobatus* paste while persistent high number of CFU

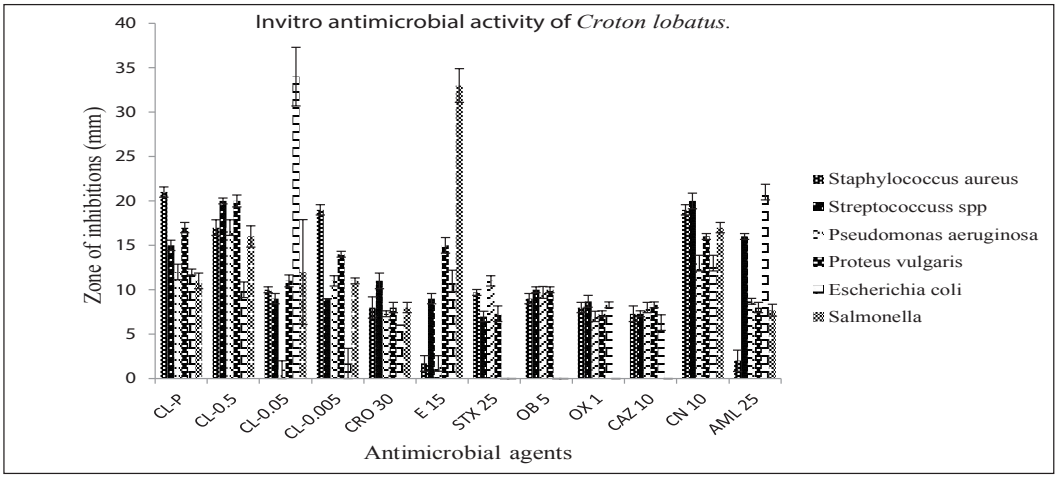

Fig. 2. The clear zone of inhibitions (mm) of various concentration of *C. lobatus* plant extract compared with standard antibiotic drugs

KEY : CL – P (*C. lobatus* Paste), CL – 0.5 (*C. lobatus*: 0.5g/mL of water), CL – 0.05 (*C. lobatus*: 0.05g/mL of water), CL – 0.005 (*C. lobatus*: 0.005g/mL of water), CRO 30 (Ceftriaxone), E 15 (Erythromycin), STX 25 (Sulphamethoxazole), OB 5 (Cloxacillin), OX 1 (Oxacillin), CAZ 10 (Ceftazidime), CN 10 (Gentamycin), AML 25 (Amoxycillin)

count was recorded with physiological normal saline treated wounds and *C. lobatus* water extract (Fig. 4).

The *in vitro* antimicrobial assay of *C. lobatus* plant extracts showed a dose depended activity against the tested microorganisms while the *in vivo* antimicrobial studies reveals that *C. lobatus* is very active against organisms that grow best in nutrient agar like *Bacillus spp, Streptococcus spp, E. coli, Pseudomonas* and *Staphylococcus spp.* Hence, it is active against microbial wound infections thereby preventing delayed wound healing. The antimicrobial property can be attributed to tannins (Christian *et al.,* 2016;

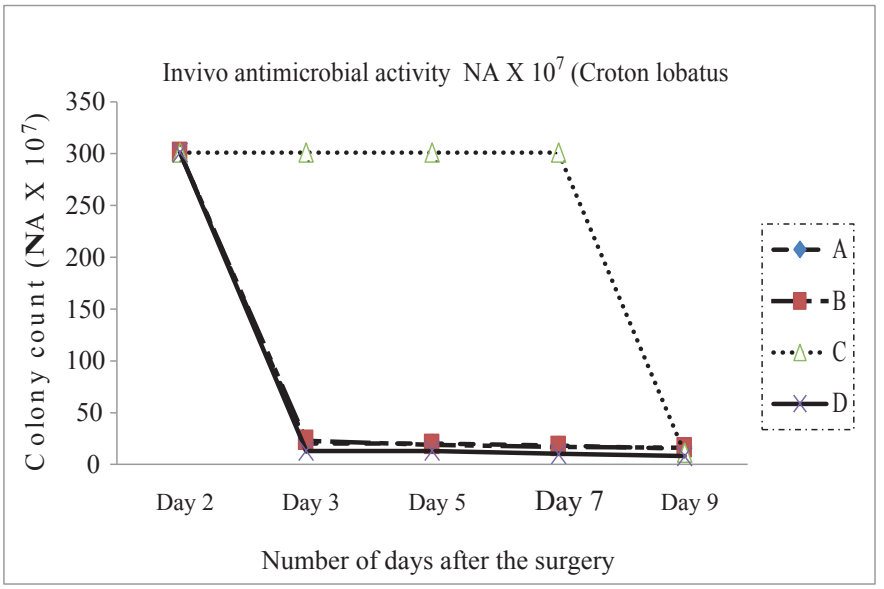

Fig. 3. Colony forming units (CFU) counted on nutrient agar (NA) inoculated with swaps collected from wounds treated with different agents: A (*C. lobatus* paste); B (*C. lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder)

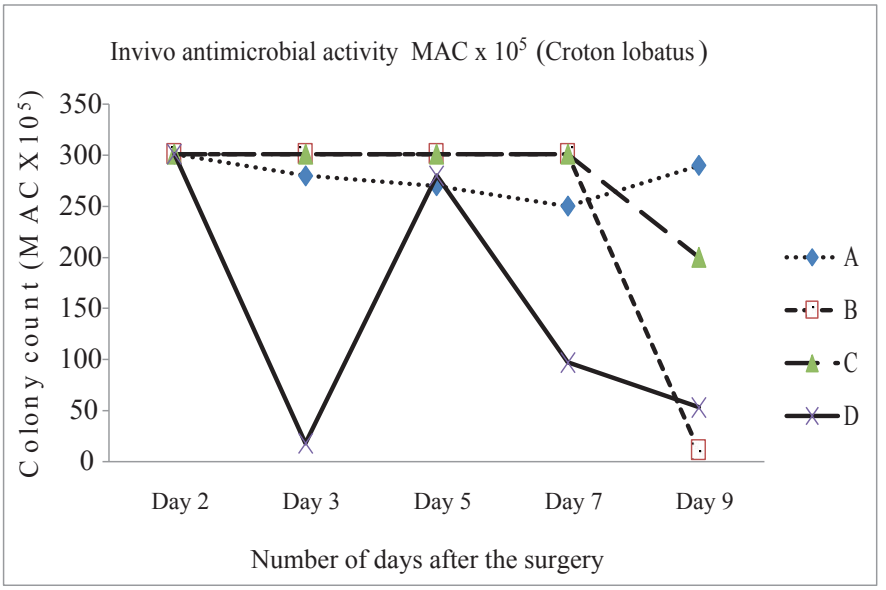

Fig. 4. Colony forming units (CFU) counted on MacConkey (MAC) agar inoculated with swaps collected from wounds treated with different agents: A (*C. lobatus* paste); B (*C. lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder)

Fakhim *et al.,* 2015), triterpenoids and flavonoids (Akpalo *et al.,* 2015; Thakur *et al.,* 2011) crotonic acids (Goldstein *et al.,,* 2003; Michalik and Wahli, 2007) and saponin (Thakur *et al.,* 2011) that is present in the leaves. This finding is supported by several other scientists who reported the antimicrobial activities of other species of croton (Abo *et al.,* 1999; Dadson *et al.,* 2012; McChesney *et al.,* 1991; Peres *et al.,* 1997; Salatino *et al.,* 2007).

Conclusion

This study has revealed that *C. lobatus* contains secondary metabolites such as carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids and tannins, which are responsible for its antimicrobial property. Hence, this explains its usage by the local medicine practitioner in Nigeria and various part of the world as alternative therapy in the management of diseases whose symptoms involve microbial infections. Further research is required on this plant.

Acknowledgment

Authors acknowledge the effort of the department of the Veterinary Public Health and Preventive Medicine; and Department of Pharmacognosis, Faculty of Pharmacy, Ahmadu Bello University Zaria. Dr Raji (bacteria zoonosis laboratory, A.B.U) is also acknowledged for kind donation of *Streptococcus* sp. of bacteria used.

References

Abo KA, Ogunleye VO and Ohidi JS (1999), Antimicrobial potential of *Spondias mombin*, *Croton zambesicus* and *Zygotritonia crocea*, *Phytotherapy Research* **13**(6): 494-7.

Aboagye FA, Sam GH, Massiot GL and Julocrotine CA (2000), Glutarimide alkaloid from *Croton membranaceus Fitoterapia* **71:** 461-462. DOI:org/10.1016/S0367-326X (00)00141-6

Aké AL (1975), Contribution aux études ethnobotaniques et floristiques au Togo, Collection "Médecine traditionnelle et pharmacopée, *Agence de Coopération Culturelle et Technique* 153.

Akpalo E, Tete-Benissan A, Awaga K and Akpagana K (2015), Review of twelve West Africa Medicinal Plants: Active phytochemical combinations in direct biochemically wound healing process, *Journal of Medicinal Plant Research* **9**(34): 908-917.

Amaral ACF and Barnes RA (1998), A tetrahydroprotoberberine alkaloid from *Croton hemiargyreus*, *Phytochemistry* **47:** 1445. DOI:org/10.1016/S0031-9422(97)00764-4

Attioua B (2005), *Contribution à l'étude phytocimique des feuilles et tiges de Croton lobatus* Linn. *(Euphorbiaceae),* Ph.D. Dissertation, University of Strasbourg, p 135.

Barbosa PR, Fascio M, Martins DS, Guedes ML and Roque F (2003), Triterpenes of *Croton betulaster* (Euphorbiaceae), *Biochemical Systematics and Ecology* **313:** 07–308.

Barthelemy KA, Ramiarantsoa H, Jean BB, Vincent AA, Félix ZT and Léon AD (2012), Isolation and identification of alkaloids from *C. lobatus*, *International Journal of Pharmaceutical Sciences* **13**(2): 001

Block S, Baccelli C, Tinant B, Meervelt LC, Rozenberg R, Jiwan JLH, Llabrès G, Pauw-Gillet MC and Quetin-Leclercq J (2004), Effects of *Croton rhamnifolioides* Essential Oil on Aedes, *Phytochemistry* **65:** 1165.

Bohm BA and Kocipai-Abyazan R (1994), Flavonoids and condensed tannins from the leaves of *Vaccinum raticulation* and *Vaccinum calcyimium*, *Pacific Science* **48:** 458-463.

Bouquet A and Debray M (1974), Medicinal plants of the Ivory Coast, *Travaux Ed Qocumentes de I'orstom* **32:** 232.

Burkill HM (1985), *Croton lobatus* Linn, family Euphorbiaceae ***In:*** *The Useful Plants of West Tropical Africa* (2), Royal Botanic Gardens, Kew (K),

Cai Y, Evans F, Roberts M, Phillipson JD, Zenk M and Gleba Y (1991), Effects of *Sangre de Drago* from *Croton lechleri* Muell, *Phytochemistry* **30:** 2033. DOI:org/10.1016/S0378- 8741(97)00087-1

Chabert P, Attioua B, Weniger B and Brouillard R (2006), *Croton lobatus* Linn., an African Medicinal plant: Spectroscopic and chemical elucidation of its many constituents, *Bio Factors* **27:** 69-78.

Christian A, Bekoe EO, Boakye YD, Dapaah SO, Appiah T and Bekoe SO (2016), Medicinal Plants and Natural Products with Demonstrated Wound Healing Properties ***In:*** Wound Healing - New insights into Ancient Challenges, *INTECH* 483-531.

Dadson YP, Tikkanen-Kaukanen C and Miettola J (2012), Antibacterial properties of *Croton species*. Institute of Public Health and Clinical Nutrition, Faculty of Health Sciences, University of Eastern Finland.

Evans WC and Trease GE (1996), Trease and Evan Pharmacognosy, 14th Ed. WB, Sounders Co. Ltd, London, pp 542-578.

Fakhim SA, Babaei H, Nia AK and Ashrafi J (2015), Wound Healing Effect of Topical Grape Seed Extract (*Vitis Vinifera*) on rat palatal mucosa, *International Journal of Current Research and Academic Review* **3**(6): 477–489.

Farnsworth (1969), *Croton lobatus* Linn. Linn. family Euphorbiaceae ***In:*** *The Useful Plants of West Tropical Africa (2),* Burkill HM, Royal Botanic Gardens, Kew, UK.

Gaikwad SP, Gore RD and Garad KU (2012), *Astraea lobata* (Euphorbiaceae), a new record for India, *Rheedea* **22**(2): 131-132.

Global Biodiversity Information Facility, GBIF (2016), *Backbone Taxonomy*. Available at https://www.gbif.org. **20:** 17.

Goldstein JT, Dobrzyn A, Clagett-Dame M, Pike JW and DeLuca HF (2003), Isolation and characterization of unsaturated fatty acids as natural ligands for the retinoid-X receptor, *Archives of Biochemistry and Biophysics* **420**(1): 185-193. DOI:org/10.1016/j.abb.2003.09.034

Harborne JB (1973), Phytochemcial methods, London, Chapman and Hall Ltd., pp 49-188.

Irobi ON, Moo-Young M, Anderson WA and Daramola SO (1994), Antimicrobial Activity of the Bark of *Bridelia fermginea* (Euphorbiaceae), *Interational Journal of Pharmacology* **34:** 87-90. DOI:org/10.1016/0378-8741 (94)90041-8

Malpighiales of North America Update, database (2011), Retrieved from http://www.itis.gov/servlet/SingleRpt/SingleRpt? On August 10, 2016 at 22:30 GMT

McChesney JD, Clark AM and Silveira ER (1991), Antimicrobial diterpenes of *Croton sonderianus.* II. ent-Beyer-15-en-18-oic acid, *Pharmaceutical Research* **8**(10): 1243-7. DOI: 10.1021/np50078a021

Michalik L and Wahli W (2007), Peroxisome proliferator-activated receptors (PPARs) in skin health, repair and disease, *Biochim Biophys Acta (BBA) Molecular and Cell Biology of Lipids* **1771**(8): 991–998. DOI:org/10.1016/j.bbalip.2007.02.004

Milanowski DJ, Winter REK, Elvin-Lewis MPF and Lewis WH (2002), Molecular Cloning and Characterization of CYP80G2, *Journal Natural Product* **65:** 814.

Nath R, Roy S, De B and Choudhury MD (2013), Anticancer and Antioxidant activity of Croton: A Review, *International Journal of Pharmacy and Pharmaceutical Sciences* **5**(2): 63-70.

Obadoni BO and Ochuko PO (2001), Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria, *Global journal of Pure and Applied Sciences* **8:** 203-208. DOI:org/10.4314/gjpas.v8i2.16033

Odukoya OA, Sofidiya MO, Samuel AT, Ajose I, Onalo M and Shuaib B (2012), Documentation of Wound Healing Plants in Lagos-Nigeria: Inhibition of Lipid Peroxidation as In-vivoPrognostic Biomarkers of Activity, *Annals of Biological Research* **3**(4): 1683-1789

Ongsakul M, Jundarat A and Rojanaworant C (2009), Antibacterial Effect of Crude Alcoholic and Aqueous Extracts of six medicinal plants against *Staphylococcus aureus* and *Escherichia coli*, *Journal of Health Research* **23**(30): 153-156.

Peres MT, Monache DF, Cruz AB, Pizzolatti MG and Yunes RA (1997), Chemical composition and antimicrobial activity of *Croton urucurana* Baillon (Euphorbiaceae), *Journal of Ethnopharmacology* **56**(3): 223-6. DOI:org/10.1016/S0378-8741(97)00039-1

Prashanthi R, Mohan N and Siva GV (2012), Wound healing property of aqueous extract of seed and outer layer of *Momordica charantia* L. on albino rats, *Indian Journal of Science and Technology* **5**(1): 1936- 1940.

PROTA (Plant Resources of Tropical Africa) (2011), *Croton macrostachyus* Hochst. ex Delile. Available at http://www.prota4u.org/protav8.asp?h=M4&t=Croton&p=Croton+macrostachyus#Synonyms. Accesed on 07.012.2015 at 22:00GMT.

Salatino A, Salatino MLF and Negri G (2007), Traditional uses, Chemistry and Pharmacology of Croton species

(Euphorbiaceae), *Journal of Brazilian Chemical Society* **18**(1): 11-33. DOI:org/10.1590/S0103-50532007 000100002

Shinwari ZKI, Khan S and Hussain NA (2009), Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases, *African Journal of Biotechnology* **8**(24): 7082-7086.

Sofowara A (1993), Medicinal plants and Traditional medicine in Africa, Spectrum Books Ltd, Ibadan, Nigeria, p 289.

Thakur R, Jain N, Pathak R and Sandhu SS (2011), Practices in Wound Healing Studies of Plants, *Evidence-Based Complementary and Alternative Medicine,* 438056. DOI:org/10.1155/2011/438056

Van-Burden TP and Robinson T (1981), The biochemistry of alkaloids. 2nd Ed. Springer, Heidelberg, Newyork.

Weniger B, Lagnika L, Vonthron-Sénécheau C, Adjobimey J, Gbenou J, Moudachirou M, Brun R, Anton R and Sanni A (2004), Evaluation of ethnobotanically selected Benin medicinal plants for their *in vitro* antiplasmodial activity, *Journal of Ethnopharmacology* **90:** 279-284. DOI:org/10.1016/j.jep.2003.10.002

Willaman and Li (1970), *C. lobatus* Linn. family Euphorbiaceae ***In:*** *The Useful Plants of West Tropical Africa (2),* Burkill HM, Royal Botanic Gardens, Kew (K),

Yibralign Z (2007), *Phytochemical Investigation on the stem bark of Croton macrostachyuss (Bisana),* Master's Thesis. Addis Ababa University, Addis Ababa.