Journal of Pharmacognosy and Phytotherapy Vol. 1(4) pp. 047-051, October, 2009
Available online at http://www.academicjournals.org/jpp

Full Length Research Paper

The antimicrobial activity of *Croton membranaceus*, a species used in formulations for measles in Ghana

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Accepted 3 September, 2009

The antimicrobial activity of the methanolic root extract and compounds isolated from *Croton membranaceus* against the bacteria; *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and fungi; *Aspergillus niger* and *Candida albicans*, using the agar diffusion and broth dilution techniques was studied in order to establish its possible usefulness in the treatment of measles. The methanolic extract showed a significant ($p < 0.01$) antibacterial and antifungal activity against the test organisms used with minimum inhibitory concentrations (MICs) ranging from 0.53 - 1.43 mg/ml. Out of the six compounds tested (crotomembranafuran, gomojoside H, julocrotine, β-sitosterol, β-sitosterol-3-D-glucoside, and DL-threitol), gomojoside H, showed significant antibacterial activity against; *S. aureus, B. subtilis and P. aeruginosa* (MICs < 10 µg/ml). The other compounds exhibited no significant antimicrobial activity (MICs > 200 µg/ml). The activities of gomojoside H were found to be similar to the effects of gentamicin on these organisms. The results therefore indicated that this compound is a potent antibacterial and its presence appears to explain in part the antimicrobial activity exhibited by the *C. membranaceus* root extract and supports its usefulness in treating secondary bacterial infection in measles.

Key words: *Croton membranaceus*, gomojoside H, antibacterial activity, measles.

INTRODUCTION

The root extract of *Croton membranaceus* Mull. Arg. (Euphorbiaceae) is used in formulations for the treatment and management of prostate and related cancers and measles in Ghana (Mshana et al., 2000).

Our earlier investigations revealed significant cytotoxic activity of the extract on human cancer cells (Bayor et al., 2007). We later isolated six compounds from the active ethyl acetate fraction of this extract, including; a new furano-clerodane diterpenoid, crotomembranafuran, in addition to the known glutarimide alkaloid, julocrotine; β-sitosterol; β-sitosterol-3-D-glucoside; the labdane diterpenoid, gomojoside H and DL-threitol.

Further investigations revealed marked cytotoxic activity of crotomembranafuran, β-sitosterol-3-D-glucoside and DL-threitol against human prostate (PC-3) cells (Bayor, 2008).

In looking at the possible usefulness of *C. membranaceus* in the treatment of infections associated with measles, we isolated more of the compounds and investigated the antimicrobial activity of these compounds and the extract on selected microorganisms, for which we now report.

MATERIALS AND METHODS

General experimental procedures

^{13}C (150 MHz) – the spectrometer used was 600 MHz. Electrospray mass spectrometry (ESIMS) was conducted in methanol and DMSO on a Micromass QUATTRO Ultima (with the sample infused through a syringe pump). Electron-impact mass spectra (EIMS) and accurate mass determinations were conducted on a Micromass Autospec M Spectrometer at the Advanced Chemical and

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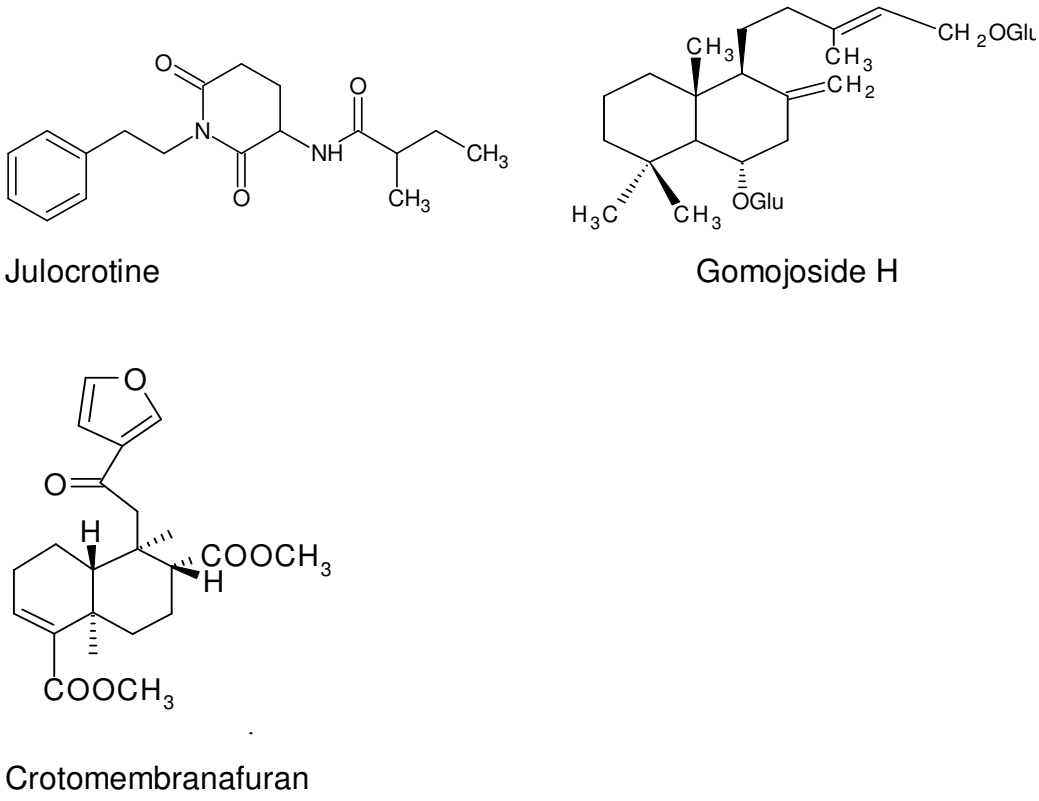

Figure 1. Some compounds obtained from *C. membranaceus.*

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Plant material

C. membranaceus root was collected in October, 2003 at Mampong-Akwapem, Ghana, and authenticated at the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akwapem, Ghana, where a voucher specimen (CSRPM/011/03) has been deposited. The material was dried in the shade and comminuted into suitable particle sized powder for extraction.

Extraction and isolation

Powdered *C. membranaceus* root (500 g) was extracted with methanol (2.5 L) using a Soxhlet extractor and the extract obtained was concentrated to a syrupy mass under reduced pressure before drying completely over silica gel in a vacuum chamber at room temperature, thus producing a dark brown organic extract (15 g). The above was repeated and the combined dried extracts (30 g) were stored in a refrigerator until used further.

The methanolic extract (25 g) was fractionated by column chromatography over silica gel 60 G (50 g, average particle size 5 - 40 µm) (Merck) and sequentially eluted with hexane, ethyl acetate and methanol, which were then concentrated under reduced pressure at 45°C to obtain hexane (2.6 g), ethyl acetate (14.4 g), and methanol (7.8 g) fractions.

Column chromatography of the ethyl acetate fraction, eluting with hexane containing increasing amounts of ethyl acetate yielded eight fractions (F_A-F_H). Fraction F_A (1.50 g) was re-chromatographed over silica gel eluted with hexane-ethyl acetate (4:1) and a sub-fraction F_{A2} further purified by repetitive chromatography on silica gel with hexane-ethyl acetate (4:2) to afford the new furano-clerodane diterpenoid [12-oxo-15,16-epoxy-3,13(16),14-clerodatrien-17,18-dioicacid dimethyl ester] (crotomembranafuran) (51 mg) (Bayor, 2008).

Further column chromatography of the other fractions on silica gel led to the isolation of the other compounds, thus: F_B (2.02 g)-julocrotine (20 mg) (Aboagye et al., 2000), F_C (2.07 g) - (β -sitosterol) (20.3 mg) (Lee et al., 2004; Nguyen et al., 2004) and β-sitosterol-3-D-glucoside (40 mg) (Kadowaki et al., 2003), F_D (1.56 g) - gomojoside H (9 mg) (Iwagawa et al., 1992), F_G (1.951 g) and F_H (2.07 g) - DL-threitol (132 mg) (Kitajima et al., 1999) (Figure 1).

Antimicrobial assays

The antimicrobial activities of the *Croton membranaceus* root extract and the compounds; crotomembranafuran, julocrotine, β-sitosterol, β-sitosterol-3-D-glucoside, gomojoside H and DL-threitol were assessed against; *S. aureus* (NCTC 10788), *B. subtilis* (NCTC 10073), *E. coli* (NCTC 9002), *P. aeruginosa* (NCTC 10662), *A. niger* (ATCC 6275) and *C. albicans* (ATCC 90028); by the standard 96-micro-well dilution technique (Eloff, 1998) and the Kirby-Bauer disk-diffusion method (Jones et al., 2001) using Mueller-Hinton agar. The test organisms were from the stock kept at the Microbiology Section of the Department of Pharmaceutics, KNUST, Kumasi, Ghana. For positive controls; gentamicin (Pharm-Intas, India) (10 µg/ml) was used for *B. subtilis*, *P. aeruginosa* and *S. aureus*; ampicillin (Glaxo-SmithKline, UK) (10 µg/ml) for *E. coli;* and ketoconazole (Janssen-Cilag, Belgium) (10 µg/ml) for *C. albicans* and *A. niger*.

Table 1. Zones of growth inhibition exhibited by *Croton membranaceus* root extract (Kirby-Bauer agar-diffusion method).

Test organism	Concentration of extract (mg/ml)				Positive controls	MIC (mg/ml)
	2.5	5.0	10.0	20.0	10 µg/ml	
	Zone of inhibition (Mean ± SD) (mm)					
S. aureus	3.5 ± 0.3	5.0 ± 0.5	7.5 ± 1.0	8.0 ± 1.5	25.6 ± 0.5	0.53
B. subtilis	2.0 ± 0.5	4.7 ± 0.6	8.0 ± 0.8	9.0 ± 1.3	24.6 ± 0.5	1.31
E. coli	4.0 ± 1.5	7.3 ± 0.2	10.5 ± 0.7	11.0 ± 1.6	24.2 ± 1.3	0.68
P. aeruginosa	1.0 ± 0.6	2.5 ± 0.3	3.5 ± 0.9	5.0 ± 1.2	24.1 ± 1.1	1.43
A. niger	3.7 ± 1.0	5.0 ± 0.7	8.0 ± 1.1	10.0 ± 1.5	18.6 ± 0.4	0.86
C. albicans	2.5 ± 0.8	4.5 ± 0.6	6.0 ± 1.3	7.5 ± 2.2	29.2 ± 0.3	0.82

Results are mean ± SD (n = 6).

Table 2. Inhibition of growth of the organisms exhibited by *Croton membranaceus* root extracts (broth tube dilution technique).

Test organism	Concentration of the extract (mg/ml)					Positive controls	MIC (mg/ml)
	1.10	1.20	1.30	1.40	1.50	10 µg/ml	(Mean ± SD)
S. aureus	+++	+++	-----	-----	-----	-----	1.30 ± 0.39
B. subtilis	+++	+++	+++	-----	-----	-----	1.40 ± 0.05
E. coli	+++	+++	+++	-----	-----	-----	1.40 ± 0.36
P. aeruginosa	+++	+++	+++	-----	-----	-----	1.40 ± 0.01
A. niger	+++	+++	+++	+++	-----	-----	1.50 ± 0.32
C. albicans	+++	+++	+++	+++	-----	-----	1.50 ± 0.34

Key: +++ Growth; ----- No growth. Results are mean ± SD (n = 6).

RESULTS AND DISCUSSION

Generally, the extract showed significant ($p < 0.01$) antibacterial and antifungal activities against the test organisms used with MICs as; *S. aureus* (0.53 mg/ml), *B. subtilis* (1.31 mg/ml), *E. coli* (0.68 mg/ml), *P. aeruginosa* (1.43 mg/ml), *A. niger* (0.86 mg/ml) and *C. albicans* (0.82 mg/ml) [Tables 1 and 2]. The results were consistent with reports of antimicrobial activity associated with some other species of *Croton*, such as; *Croton urucurana* Baill (Peres et al., 1997, Gurgel et al., 2005), *Croton zambesicus* (Abo et al., 1999), *Croton cajucara* Benth (Alviano et al., 2005) and *Croton sonderianus* (McChesney et al., 1991).

Out of the six compounds tested, gomojoside H showed significant ($p < 0.01$) antimicrobial activity against; *S. aureus, B. subtilis and P. aeruginosa* giving minimum inhibitory concentrations (MICs) as; 7.52 µg/ml, 9.28 µg/ml and 9.81 µg/ml respectively. The rest [crotomembranafuran, julocrotine, β-sitosterol, β-sitosterol-3-D-glucoside and DL-threitol] had no significant antimicrobial activity with MICs above 200 µg/ml (Table 3). The activities of gomojoside H were similar to the effects of gentamicin on these organisms. Although gomojoside H had earlier been obtained from *Viburnum suspensum* (Caprifoliaceae) (Iwagawa et al., 1992), this is the first report of its antimicrobial activity.

Unfortunately, julocrotine which is a glutarimide alkaloid did not show significant antimicrobial activity, though several glutarimide antibiotics including streptimidone and its synthetic analogues have been widely reported (Akhrem et al., 1979; Becker and Rickards, 1982; Buravskaya and Lakhvich, 1996; Buravskaya and Lakhvich, 1998; Kim et al., 1999; Kondo et al., 2000).

The lack of antimicrobial activity shown by β-sitosterol in these investigations were also consistent with reports of no antimicrobial activity associated with β-sitosterol, obtained from several plant sources (Chandramu et al., 2003; Hess et al., 1995; Cota et al., 2003) including *C. urucurana* Baillon (Peres et al., 1997).

Interestingly however, there are numerous reports of β-sitosterol together with its 3-D glucopyranoside found in plants, whose extracts have shown significant antimicrobial activity (Ramesh et al., 2004; Venkatesan et al., 2005) including some species of *Croton* (Abo et al., 1999). There are also reports of improved outcome with the use of β-sitosterol and its glucoside as adjuvants in the treatment of pulmonary tuberculosis (Donald et al., 1997).

The results therefore showed that, gomojoside H has considerable antibacterial activity especially, against *S. aureus, B. subtilis and P. aeruginosa*, and its presence could explain, at least in part, the antimicrobial activity exhibited by the *C. membranaceus* root extract, and

Table 3. Zones of growth inhibition [Mean ± SD, mm] exhibited by the test compounds (Kirby - Bauer disk - diffusion method).

Compound	Concentration (µg/ml)	Sa	Bs	Ec	Pa	An	Ca
PC	10	25.1 ± 0.7	24.4 ± 0.5	24.6 ± 0.9	26.0 ± 0.7	18.3 ± 0.2	29.1 ± 0.3
Crotomembra-nafuran	200	----	----	----	----	----	----
Julocrotine	200	----	----	----	----	----	----
β-sitosterol	200	----	----	----	----	----	----
β-sitosterol-3-D-glucoside	200	----	----	----	----	----	----
DL-threitol	200	----	----	----	----	----	----
Gomojoside H	20	5.2 ± 0.3	4.0 ± 0.5	----	3.5 ± 0.6	----	----
	40	9.5 ± 0.5	8.3 ± 0.9	----	7.4 ± 1.0	----	----
	80	12.2 ± 1.5	10.6 ± 1.0	----	11.0 ± 0.8	----	----
	160	16.8 ± 1.1	15.5 ± 1.4	----	14.2 ± 1.3	----	----
	200	18.0 ± 0.8	17.4 ± 1.8	----	14.2 ± 1.5	----	----
MIC (µg/ml)		7.52	9.28		9.81		

Key: PC - positive control; Sa - *S. aureus*; Bs - *B. subtilis*; Ec - *E. coli*; Pa - *P. aeruginosa*; An - *A. niger*; Ca - *C. albicans*. Results are mean ± SD (n = 6).

supports its usefulness in treating secondary bacterial infections associated with measles.

AKNOWLEDGEMENT

The authors wish to thank the staff of the Spectroscopy Unit of the University of Newcastle, Newcastle upon Tyne for generating the spectroscopic data on the compounds.

REFERENCES

Abo KA, Ogunleye VO, Ashidi JS (1999). Antimicrobial potential of Spondias mombin, *Croton zambesicus* and *Zygotritonia crocea*. Phytother. Res. 13: 494-497.

Aboagye FA, Sam GH, Massiot G, Lavaud C (2000). Julocrotine, a glutarimide alkaloid from *Croton membranaceus*. Fitoterapia, 71: 461-462.

Akhrem AA, Lakhvich FA, Khripach VA and Klebanovich IB (1979). Synthesis of Glutarimide Antibiotics and Their Analogs through 1,3-Cycloaddition of 3-Glutarimidylacetonitrile Oxide to 2-Cyclohex-enones. Khimiya Geterotsiklicheskikh Soedinenii 12: 230-234.

Alviano WS, Mendonca RR, Alviano DS, Bizzo HR, Souto-Padron T, Rodrigues ML, Bolognese AM, Alviano CS, Souza MMG (2005). Antimicrobial activity of Croton cajucara Benth linalool-rich essential oil on artificial biofilms and planktonic microorganisms. Oral Micro. Immun., 20: 101-105.

Bayor MT (2008). The anticancer and other bioactivity investigations on the extract and some compounds of Croton membranaceus (Euphorbiaceae). PhD Thesis. Department of Pharmaceutical Chemistry. Kumasi, Ghana, Kwame Nkrumah University of Science and Technology.

Bayor MT, Ayim JSK, Phillips RM, Shnyder SD, Wright CW (2007). The evaluation of selected Ghanaian medicinal plants for cytotoxic activities. J. Sci. Tech. 27: 16-22.

Becker AM, Rickards RW (1982). The Identity of Protomycin with the Glutarimide Antibiotic Streptimidone. Austr. J. Chem. 35: 1405-1410.

Buravskaya TN, Lakhvich FA (1996). Synthesis of glutarimide antibiotic analogies with conjugated enaminodiketone fragment. Z. Org. Khim. 32: 1010-1015.

Buravskaya TN, Lakhvich FA (1998). Synthesis of pyrazole-containing analogs of glutarimide antibiotics. Z. Org. Khim. 34: 277-279.

Chandramu C, Manohar RD, Krupadanam DGL, Dashavantha RV (2003). Isolation, characterization and biological activity of betulinic acid and ursolic acid from Vitex negundo L. Phytother. Res. 17: 129-134.

Cota BB, De Oliveira AB, De Souza J D, Braga FC (2003). Antimicrobial activity and constituents of Coccoloba acrostichoides. Fitoter. 74: 729-731.

Donald PR, Lamprecht JH, Freestone M, Albrecht CF, Bouic PJD, Kotze D, Van Jaarsveld PP (1997). A randomised placebo-controlled trial of the efficacy of beta-sitosterol and its glucoside as adjuvants in the treat-ment of pulmonary tuberculosis. Int. J. Tuberculosis Lung Dis. 1: 518-522.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitor concentration of plant extracts for bacteria. Planta Medi. 64: 711-713.

Gurgel LA, Sidrim JJC, Martins DT, Cechinel V, Rao VS (2005). In vitro antifungal activity of dragon's blood from *Croton urucurana* against dermatophytes. J. Ethnopharmacol. 97: 409-412.

Hess SC, Brum RL, Honda NK, Cruz AB, Moretto E, Cruz RB, Messana I, Ferrari F, Cechinel V, Yunes RA (1995). Antibacterial Activity and Phytochemical Analysis of *Vochysia divergens* (Vochysiaceae). J. Ethnopharmacol. 47: 97-100.

Iwagawa T, Yaguchi S, Hase T, Okubo T, Kim M (1992). Gomojosides, labdane diterpenoids from *Vibernum suspensum*. Phytochem. 31: 1311-1315.

Jones RN, Ballow CH, Biedenbach DJ (2001).Multi-laboratory assessment of the linezolid spectrum of activity using the Kirby-Bauer disk iffusion method: Report of the Zyvox Antimicrobial Potency Study (ZAPS) in the United States. Diagn. Microbiol. Infect. Dis. 40(1-2): 59-66.

Kadowaki E, Yoshida Y, Baba N, Nakajima S (2003). Beta-Sitosteryl-D-glucoside from the Olive Tree (*Olea europaea* LINNE; Oleaceae) as a Feeding Stimulant towards the Olive Weevil (*Dyscerus perforatus*). Scientific Reports of the Faculty of Agriculture, Okayama University. 92: 1-4.

Kim BS, Moon SS, Hwang BK (1999). Isolation, antifungal activity, and structure elucidation of the glutarimide antibiotic, streptimidone, produced by *Micromonospora coerulea*. J. Agric. Food Chem., 47: 3372-3380.

Kitajima J, Ishikawa T, Tanaka Y, Ida Y (1999). Chemical Pharmacol. Bull. 47: 988-992.

Kondo H, Oritani T, Kiyota H (2000). Synthesis and antifungal activity of the four stereoisomers of streptimidone, a glutarimide antibiotic from *Streptomyces rimosus* forma *paromomycinus*. Eur. J. Org. Chem. 61: 3459-3462.

Lee CK, Lu CK, Kuo YH, Chen JZ, Sun GZ (2004). New prenylated flavones from the roots of *Ficus beecheyana*. J. Chin. Chem. Soc. 51: 437-441.

McChesney JD, Clark AM, Silveira ER (1991). Antimicrobial Diterpenes of *Croton sonderianus* . 1. Hardwickic and 3,4-Secotrachylobanoic

Acids. J. Nat. Prod. 54: 1625-1633.
Mshana NR, Abbiw DK, Addae-Mensah I, Adjanouhoun E, Ahyi MRA, Ekpere JA, Enow-Orock EG, Gbile ZO, Noamesi GK, Odei MA, Odunlami H, Oteng-Yeboah AA, Sarpong K, Sofowora A. Tackie AN (2000). Traditional medicine and pharmacopoeia: contribution to the revision of ethnobotanical and floristic studies in Ghana. Organization of African Unity/Scientific, Technical and Research Commission (OAU/STRC).
Nguyen AT, Malonne H, Duez R, Vanhaelen-Fastre R, Vanhaelen M, Fontaine J (2004). Cytotoxic constituents from *Plumbago zeylanica*. Fitoterapia, 75: 500-504.
Peres M, Dellemonache F, Cruz AB, Pizzolatti MG, Yunes RA (1997). Chemical composition and antimicrobial activity of *Croton urucurana* Baillon (Euphorbiaceae). J. Ethnopharmacol.56: 223-226.
Ramesh N, Viswanathan MB, Selvi VT, Lakshmanaperumalsamy P (2004). Antimicrobial and phytochemical studies on the leaves of *Phyllanthus singampattiana* (Sebastine and AN Henry) Kumari & Chandrabose from India. Med. Chem. Res. 13: 348-360.
Venkatesan M, Viswanathan MB, Ramesh N, Lakshmanaperumalsamy P (2005). Antibacterial and phytochemical studies on the stem of *Suregada angustifolia* from Peninsular India. Pharm. Biol. 43: 201-204.