Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (10):113-117
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Chemical Constituents of *Laurenciapapillosa* (C.Ag.) Greville

Consolacion Y. Ragasa[1,2,*], Faith Marie Lagua[1], Maria Carmen S. Tan[1] and Chien-Chang Shen[3]

[1]*Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines,*
[2]*Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines*
[3]*National Research Institute of Chinese Medicine, Ministry of Health and Welfare, 155-1, Li-Nong St., Sec. 2, Taipei 112, Taiwan*

ABSTRACT

*Chemical investigation of the dichloromethane extract of Laurenciapapillosa(C.Ag) Greville led to the isolation of zeinoxanthin (**1**), β-carotene (**2**), chlorophyll a (**3**),cholesterol (**4**), monogalactosyl diacylglycerol (**5**), trilinolein (**6**), anda mixture of linolenic acid (**7a**), linoleic acid (**7b**). The structures of **1**-**7**were identified by comparison of their NMR data with those reported in the literature.*

Keywords: zeinoxanthin, β-carotene, cholesterol, chlorophyll a, monogalactosyl diacylglycerol, trilinolein, linolenic acid, linoleic acid

---

INTRODUCTION

Laurencia papillosa(C.Ag) Grevilleis a popular edible seaweed which is also a source of agaroid [1]. *L. papillosa* exhibited the highest level of gastric protection activity (81%) at 200 mg/kg, comparable to the standard drug ranitidine (90%) and it showed marked wound-healing activity [2]. A number of studies have been previously conducted on the chemical constituents of *L. papillosa*. An earlier study reported that *L. papillosa* yielded 3α,6α-dihydroxy-5β-cholestan-12-one and (*E*)-2-[(*E*)-tridec-2-en-2-yl]heptadec-2-enal which exhibited significant activities against *Candida albicans*, *Aspergillus fumigatus*, and *A. flavus*. 6β-Hydroxycholest-4-en-3-one was also isolated from *L. papillosa*[3]. The isolation of 24-propylidene-cholest-5-en-3β-ol which showed activity against gram negative pathogenic bacteria has also been reported [4]. Furthermore, *P. papillosa* was found to contain cholesterol as the major constituent, together with 22-dehydrocholesterol, brassicasterol, 24-methylene-cholesterol, campesterol, sitosterol, fucosteroland 28-isofucosterol [5]. Another study reported that *L. papillosa* afforded (12*E*)-*cis*-maneonene-E, (12*Z*)-*trans*-maneonene-B, 2,10-dibromo-3-chloro-α-chamigrene and fatty acid aldehydes. (12*E*)-*Cis*-maneonene-E showed high potential as a natural insecticide against flour beetle larvae *Tribolium confusum* and *Culexpipiens* mosquito larvae [6]. Moreover, the isolation of cholesterol, β-sitosterol and lanosterol from *L. papillosa* has been reported [7].

This study was conducted to investigate the chemical constituents of the dichloromethane extract of *L. papillosa*, a popular edible seaweed in the Northern Philippines. We report herein the isolation of zeinoxanthin (**1**), β-carotene (**2**), chlorophyll a (**3**),cholesterol (**4**), monogalactosyl diacylglycerol (**5**), trilinolein (**6**),linolenic acid (**7a**), and linoleic acid (**7b**)from *L. papillosa*.The chemical structures of **1**-**7**are presented in Fig. 1.

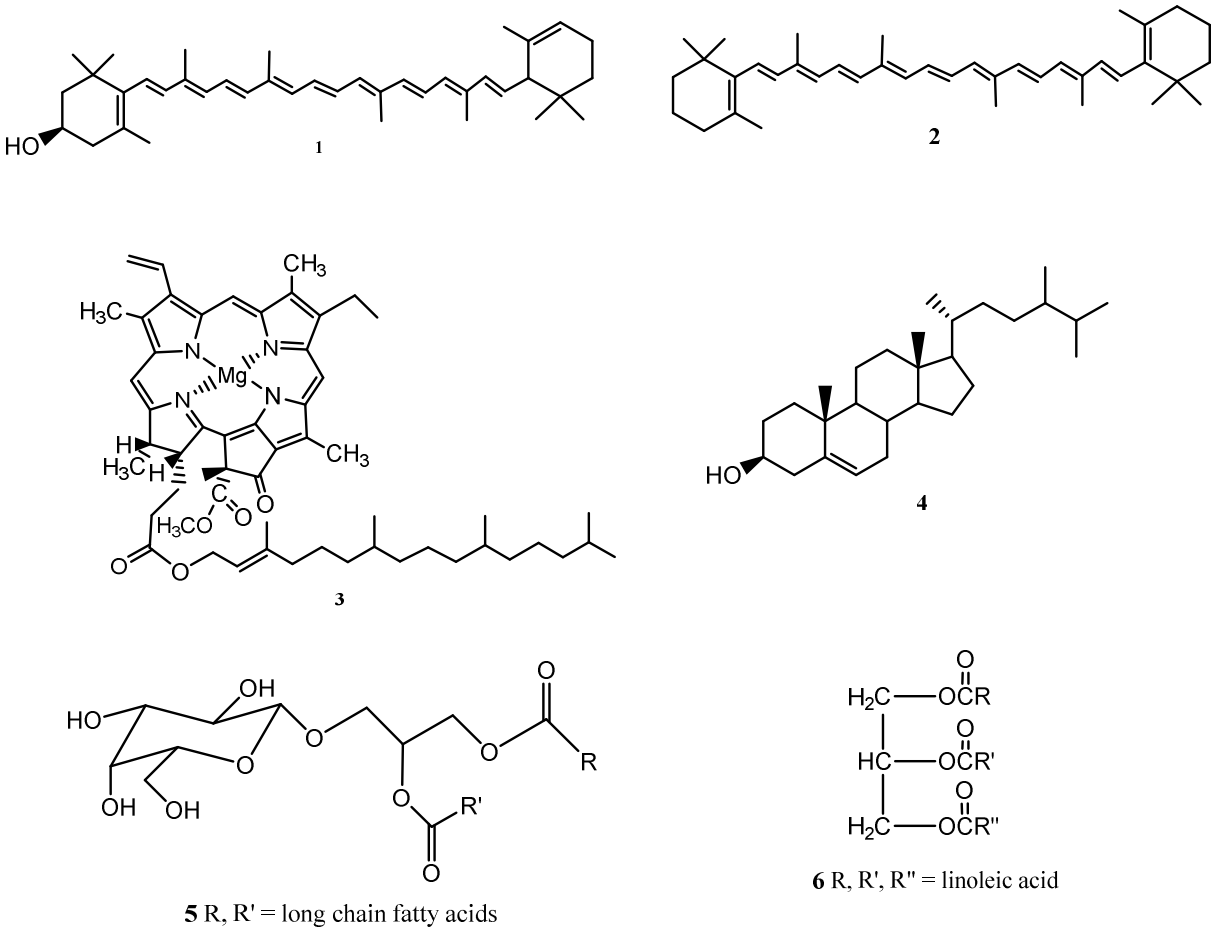

Fig. 1 Chemical structures of zeinoxanthin (1), β-carotene (2), chlorophyll a (3), cholesterol (4),monogalactosyl diacylglycerol (5), trilinolein (6),linolenic acid (7a), and linoleic acid (7b) from*Laurencia papillosa* (C.Ag) Greville

MATERIALS AND METHODS

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. Twenty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f value were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Sample Collection

The seaweed was collected from Sinait, Ilocos Sur, Philippines in April 2016. The sample was authenticated as *Laurencia papillosa (C.Ag) Greville.* by Noe Gapasof the Philippine National Museum.

Isolation

The freeze-dried (282.66 g) *L. papillosa* was cut into small pieces, ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated from the filtrate under vacuum to afford a crude extract (0.9665 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increments by volume as eluents. The CH_2Cl_2 fraction was rechromatographed using petroleum ether to afford **2** (3 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using10% EtOAc in petroleum ether to yield **6** (7 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether to afford **3** (2 mg) and **4** (5 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to afford **1** (2 mg) after washing with petroleum ether, followed by Et_2O. The 60% and 70% acetone in CH_2Cl_2 fractions were combined and rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to afford **7a** and **7b** (5 mg). The 80% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6, v/v) to afford **5** (4 mg) after trituration with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *L. papillosa* yielded **1**-**7**.The NMR spectra of **1** are in accordance with data reported in the literature forzeinoxanthin [8]; **2** for β-carotene [9]; **3** for chlorophyll a [10]; **4**for cholesterol [11]; **5** for monogalactosyl diacylglycerol [12]; **6** for trilinolein [13]; **7a** for linolenic acid [14]; and **7b**for linoleic acid [15].

Although no biological activity tests were conducted on the isolated compounds, a literature search of **2**-**3**, and **5**-**7**revealed that these have diverse bioactivities.

β-Carotene (**2**) dose-dependently induced apoptosis and cell differentiation in cultured leukemia cells, but not in normal cells [16]. Another study reported that β-carotene could reduce damage caused by radiation therapy and decrease local cancer recurrence [17]. It also inhibited angiogenesis by altering the cytokine profile and the activation and nuclear translocation of transcription factors [18].

Chlorophyll a (**3**) and its various derivatives are used in traditional medicine and for therapeutic purposes [19]. Natural chlorophyll and its derivatives have been studied for wound healing [20], anti-inflammatory properties [21], control of calcium oxalate crystals [22], utilization as effective agents in photodynamic cancer therapy [23-25], and chemopreventive effects in humans [26, 27]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided [28].

Monogalactosyl diacylglycerols (**5**)and dinogalactosyl diacylglycerols are the most widespread non-phosphorous polar lipids in nature, constituting about 80% of membrane lipids in plants and more than half of all lipids in algae [29, 30]. These compounds were reported to exhibit a number of biological properties, such as anti-tumor [31,32], anti-viral [33], algicidal [34] and anti-inflammatory [35-38]. Monogalactosyl diacylglycerols were also found to exhibit cytotoxic and anti-inflammatory activity in RAW 264.7 macrophage cells with IC_{50} values of 60.06 and 65.70 µg/mL, respectively [39]. Compound **5** was also reported to exhibit anti-inflammatory activity in human articular cartilage [40]. Itinhibited the growth of human melanoma cells in a dose-dependent manner with an IC_{50} value of 114 µM [12].

Trilinolein (**6**)exhibited protective effects against cardiovascular disorders [41]. It also inhibits ischemiainduced ventricular arrhythmias and it exhibits an anti-oxidant effect [42]. It was also reported to inhibit the growth of human non-small cell lung carcinoma (A549) and induce apoptosis in a dose- and time- dependent manner [43]. Another study reported that triglycerides showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation. Trilinolenin (18:3; µ-3) was toxic only after prolonged incubation [44].

Linoleic acid (**7b**) belongs to the omega-6 fatty acids. It was reported to be a strong anticarcinogen in a number of animal models. It reduces risk of colon and breast cancer [45] and lowers cardiovascular disease risk and inflammations [46].

Omega-3 polyunsaturated fatty acids (n-3 PUFA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA) (**7a**), and their fatty acid ethyl esters, exhibited strong antibacterial activity against various oral pathogens, including Streptococcus mutans, Candida albicans, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, and Porphyromonas gingivalis. They also showed anti-inflammatory effects [47]. Peroxisome proliferator-activated receptor-γ (PPAR-γ) and cyclooxygenase-2 (COX-2) inhibition serve as two signalling pathways for the inhibitory effects of **7a** on the human renal cell carcinoma (RCC) cell proliferation [48]. Another study reported that apoptosis of hepatoma cells was induced by the α-linolenic acidenriched diet which correlated with a decrease in arachidonate content in hepatoma cells and decreased cyclooxygenase-2 expression [49]. γ-Linolenic acid (GLA) and **7a** exhibited greater than 90% cytotoxicity between 500 µM and 1 mM against all but two malignant micro-organ cultures tested in 5-10% serum. GLA and **7a**were cytotoxic to mutant cell growth at concentrations of 2 mM and above in tests using 30-40% serum [50].

CONCLUSION

This study reports on the isolation of carotenes, chlorophyll, sterol and lipids from the dichloromethane extract of *L. papillosa*. The previously reported compounds from *L. papillosa* were mainly sterols and acetogenins. Compounds **2**-**3** and **5**-**7**which were isolated in this study were reported to exhibit anticancer properties.

Acknowledgements
A research grant from the De La Salle University Science Foundation through the University Research Coordination Office is gratefully acknowledged.

REFERENCES

]1] *Laurencia papillosa* - NIO Bioinformatics Centre. Downloaded from www.niobioinformatics. in/seaweed/system_Laurencia%20papillosa.htm on June 14, **2016**.
[2] K. A. Senthil, A. Murugan,*Braz. J. Pharm. Sci*., **2013**, 49(4), 669-678.
[3] W. M. Alarif, S. S. Al-Lihaibi, A. Abdel-Lateff, S. E. Ayyad,*Nat. Prod. Commun*., **2011**, 6(12), 1821-4.
[4] K. Kavita, V. K. Singh, B. Jha, *Microbiol. Res*., **2014**, 169(4), 301–306
[5] B. P. Lisboa, I. Ganschow, J. M. Halket, F. Pinheiro-Joventino. *Comp. Biochem. Physiol. Part B: Comp. Biochem.*, **1982**, 73(2), 257-264.
[6] Z. S. Abou-Elnaga, W. M. Alarif, S. S. Al-lihaibi, *Clean Soil Air Water*, **2011**, 39, 787–794.
[7] A. F. Abdel-Fattah, N. M. Abed, M. Edrees, *Qualitas Plantarum et Materiae Vegetabiles*
1973, 22(2), 171-179.
[8] F. Khachik, A.-N. Chang, A. Gana, E. Mazzola, *J. Nat. Prod*., **2007**, 70, 220-226.
[9] J-MC Cayme; CY Ragasa, Kimika, **2004**, 20(1/2), 5-12.
[10] C. Y. Ragasa, V. A. S. Ng, N. Lazaro-Llanos, M. C. Tan, R. Brkljača, S. Urban,*Der Pharma Chemica*. **2015**, 7(7), 194-198.
[11]^{13}C Attached-Proton-Test (APT) Spectra - Tecmag, Downloaded from www.tecmag.com/pdf/ APT.pdf on June 14, **2016**.
[12] T. I. Imbs, S. P. Ermakova, S. A. Fedoreyev, S. D. Anastyuk, T. N. Zvyagintseva, *Mar. Biotechnol*., **2013**, 15, 606–612.
[13] T. Y. Shiao; M.-S. Shiao, *Bot. Bull. Acad. Sin.*, **1989**, 30, 191-199.
[14]Human Metabolome Database. Linolenic acid. Downloaded from http://www.hmdb.ca/spectra/ nmr_one_d/1471 on Nov. 27, **2013**.
[15] Human Metabolome Database. Linoleic acid. Downloaded from http://www.hmdb.ca/spectra/ nmr_one_d/1471 on Nov. 27, **2013**.
[16] K. R. Upadhyaya, K. S. Radha, H. K. Madhyastha,*J. Biochem. Mol. Biol*., **2007**, 40(6). 1009-1015.
[17] F. Meyer, I. Bairati, E. Jobin, M. Gélinas, A. Fortin, Nabid, B. Têtu,*Nutr. Cancer*, **2007**, 59(1), 29-35.
[18] C. Guruvayoorappan, G. Kuttan,*Integr.Cancer Ther*.,**2007**, 6(3), 258-70.
[19] B. J. Edwards, *Physiother*., **1954**, 40, 177–179.
[20] J. C. Kephart, *Econ. Bot*.,**1955**, 9, 3–18.
[21] D. C. Larato, F. R. Pfao, *Dent. J*., **1970**, 36, 291–293.
[22] R. Tawashi, M. Cousineau, M. Sharkawi, *Invest. Urol*., **1980**, 18, 90–92.
[23] E. D. Sternberg, D. Dolphin, C. Bruckner, *Tetrahedron*, **1998**, 54, 4151–4152.

[24] W. L. Nourse, R. M. Parkhurst, W. A. Skinner, R. T. Jordan, *Biochem. Biophys. Res. Commun*., **1988**, 151, 506–511.
[25] B. W. Henderson, D. A. Bellnier, W. R. Greco, A. Sharma, R. K. Pandry, L. A. Vaughan, *Cancer Res*., **1997**, 57, 4000–4007.
[26] P. A. Egner, J. B. Wang, Y. R. Zhu, B. C. Zhang, Y. Wu, Q. N. Zhang, *Proc. Natl. Acad. Sci*., **2001**, 98(25), 1401–1406.
[27] P. A. Egner, A. Munoz, T. W. Kensler, *Mutat. Res*., **2003**, 52(3), 209–216.
[28] S. J. Hardwick, K. H. Carpenter, N. S. Law, C. Van Der Veen, C. E. Marchant, R. Hird, M. J. Mitchinson, *Free Radic. Res*., **1997**, 26(4), 351–362.
[29] S. V. Khotimchenko, *Chem. Nat. Compd*., **2002**, 38, 223–229.
[30] P. Dormann, C. Benning, *Trends Plant Sci*., **2002**, 7, 112–118.
[31] N. Maeda, Y. Kokai, T. Hada, H. Yoshida, Y. Mizushina, *Exp. Ther. Med*., **2013**, 5, 17–22. [32] N. Maeda, T. Hada, H. Yoshida, Y. Mizushina, *Curr. Med. Chem*., **2007**, 14, 955–967.
[33] L. M. Souza, G. L. Sassaki, M. T. V. Romanos, E. Barreto-Bergter, *Mar. Drugs*, **2012**, 10, 918–931.
[34] S. Hirao, K. Tara, K. Kuwano, J. Tanaka, F. Ishibashi, *Biosci. Biotechnol. Biochem*., **2012**, 76, 372–374.
[35] A. Bruno, C. Rossi, G. Marcolongo, A. di Lena, A. Venzo, C. P. Berrie, D. Corda, *Eur. J.Pharmacol*., **2005**, 7, 159–168.
[36] V. Ulivi, M. Lenti, C. Gentili, G. Marcolongo, R. Cancedda, F. D. Cancedda. *Arthritis Res. Ther*., **2011**, 13: doi:10.1186/ar3367.
[37] A. H. Banskota, R. Stefanova, A. Sperker, R. Melanson, J. A. Osborne, S. J. B. O'Leary, *J. Appl. Phycol*., **2013**, 25, 951–960.
[38] A. H. Banskota, P. Gallant, R. Stefanova, R. Melanson, S. J. B. O'Leary, *Nat. Prod. Res*., **2012**, 27, 1084–1090.
[39] G. Lopes, G. Daletos, P. Proksch, P. B. Andrade, P. Valentão, *Mar. Drugs*, **2014**, 12, 1406-1418.
[40] V. Ulivi, M. Lenti, C. Gentili, G. Marcolongo, R. Cancedda,F. D. Cancedda. *Arthritis Res. Ther*., **2011**, 13:R92.
[41] P. Chan, G. N. Thomas, B. Tomlinson, *Acta Pharmacol. Sin*., **2002**, 23(12), 1157-1162.
[42] P. Chan, P. F. Kao, B. Tomilson, *Acta Cardio Sin*., **2005**, 21, 71-76.
[43] P. Y. Chou, G. J. Huang, C. H. Pan, Y. C. Chien, Y. Y. Chen, C. H. Wu, M. J. Sheu, H. C. Cheng, *Amer. J. Chin. Med*., **2011**, 39(4), 803-15.
[44] S. J. Hardwick, K. H. Carpenter, N. S. Law; C. Van Der Veen, C. E. Marchant, R. Hird, M. J. Mitchinson, *Free Radic. Res*., **1997**, 26 (4), 351-362. [
[45] P. Chan, G. N. Thomas, B. Tomlinson, *Acta Pharmacol. Sin*.,**2002**, 23(12), 1157-1162.
[46] J. Whelan, *Prostaglandins, Leukot. Essent. Fatty Acids*, **2008**, 79(3-5), 165-167.
[47] C. B. Huang, J. L. Ebersole, *Mol. Oral Microbiol*., **2010**, 25(1), 75-80.
[48] L. Yang, J. Yuan, L. Liu. C. Shi, L. Wang. F. Tian. F. Liu, H. Wang, C. Shao, Q. Zhang; Z. Chen, W. Qin. W. Wen, *Oncol. Lett*., **2013**, 197-202.
[49] A. Vecchini, V. Ceccarelli, F. Susta, P. Caligiana, P. Orvietani, L. Binaglia,G. Nocentini, C. Riccardi, G. Calviello, P. Palozza, N. Maggiano, P. Di Nardo, *J. Lipid Res*., **2004**, 45, 308-316.
[50] D. E. Scheim, *Lipids in Health and Disease*, **2009**, 8, 54