Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(4):2188-2192

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Histochemical studies on *Laurencia obtusa* (Hudson) Lamourux

Johnson M*, Babu A, Janakiraman N, Renola Joy Jeba Ethal T and Sivaraman A

Centre for Plant Biotechnology, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India

---

ABSTRACT

The present study was aimed to know the occurrence and distribution of chemical constituents of Laurencia obtusa (Hudson) Lamourux using histochemical analysis. Histochemical tests were made on the fresh sections of the thallus treated with the following reagents to identify the presence or absence of metabolites like phenol, lignin, chitin, suberin and tannin. The stained sections were observed under Motic trinocular microscope and photographed at different magnifications and at different views. Phenols, polyphenols, tannin and lignin are profusely present in the outer layer of the thallus. Phenol shows the presence small quantity only in outer layer of the thallus. Polyphenols, tannin and lignin and abundantly present in the middle part of the thallus. Polyphenol and tannin shows minimal quantity and lignin was abundantly present in central part of the thallus. Hence this study offers a base of using L. obtusa as herbal alternative for the synthesis of active compounds.

Key words: *Laurencia obtusa*, histochemistry, microscope.

INTRODUCTION

Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Seaweeds are one of the important marine living resources and are excellent source of Vitamins (A, B, B_{12}, C, D& E), riboflavin, niacin, pantothanic acid and folic acid as well as minerals such as Ca, P, Na and K. Seaweed dietary fibres perform varied range of functions such as antioxidant, antimutagenic, anticoagulant, antitumor etc [1]. For evaluation of therapeutic efficiency of herbal drugs certain special and routine histological and histochemical techniques are of great use in development and use the understanding mechanisms inherited in bioresources in response to various biotic and abiotic environmental factors and the potential and useful functions of these mechanisms. This offers ample opportunities to find the solutions for the taxonomical and pharmacognostical problems. The evaluation of a crude drug is an essential part of pharmacognosy. The individual drug plant would undergo many procedures. One of them is microscopic assessment usually for powdered drugs.

To date, over 2400 natural products have been isolated from seaweeds (mainly from the divisions Rhodophyta, Phaeophyta, and Chlorophyta), the majority of which come from subtropical and tropical populations [2,3]. As an example of Rhodophyta's broad chemical variety, members of the genus *Laurencia* produce an amazing array of complex terpenoids and acetogenins, possibly making it the world's most chemically complex genus. Red algae of the genus *Laurencia* J. V. Lamourux (Rhodomelaceae) are found in tropical and subtropical regions throughout the world and are an extremely rich source of secondary metabolites with diverse structural features, mainly

halogenated sesquiterpenes and C15-acetogenins [4,5]. It is suggested that these metabolites function as a chemical defense against herbivores [6], fouling organisms, and pathogens [7]. These compounds have been shown to possess some interesting pharmacological activities, such as antitumor [8], antibacterial [9], antifungal [10], and antiviral activities [11]. Marine organisms are a promising source of antileishmanial compounds [12-14]. However, only a few studies have been carried out to evaluate the anti-leishmanial properties of seaweed natural products [15,16]. Though information on the anatomy and phytochemistry of different species of *Laurencia* are available, there is no specific investigation conducted specifically on the histochemical studies of seaweeds. With this background, the present study was aimed to know the occurrence and distribution of chemical constituents viz., phenols, polyphenols, lignin, chitin, suberin and tannins of *Laurencia obtusa* (Hudson) Lamourux using histochemical analysis.

EXPERIMENTAL SECTION

Collection of materials

Laurencia obtusa (Hudson) Lamourux was collected by handpicking at Rasthacaud coastal waters (Gulf of Mannar Coast, Lat N $08^0$08'308'' E$77^0$32'80"). The collected samples were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The collected samples were then thoroughly washed with tap water followed by distilled water.

Histochemical analysis

Fresh free hand sections were made from the thallus of *L. obtusa*. Histochemical tests were made on the fresh sections of the thallus treated with the following reagents to identify the presence or absence of metabolites like phenol, lignin, chitin, suberin and tannin. Lugol's Iodine to detect tannins [17], 10% sodium nitrite, 20% urea and 10% acetic acid were used to detect phenols [18] and Fast Blue BB for polyphenols [19]. Detection of lignin, chitin and suberin were done by Lugol's Iodine [20]. The stained sections were observed under Motic trinocular microscope (Japan). They were photographed at different magnifications and at different views. The results were registered on photomicrograph. Based on the photographs taken, anatomical description and localization of tested chemicals were done.

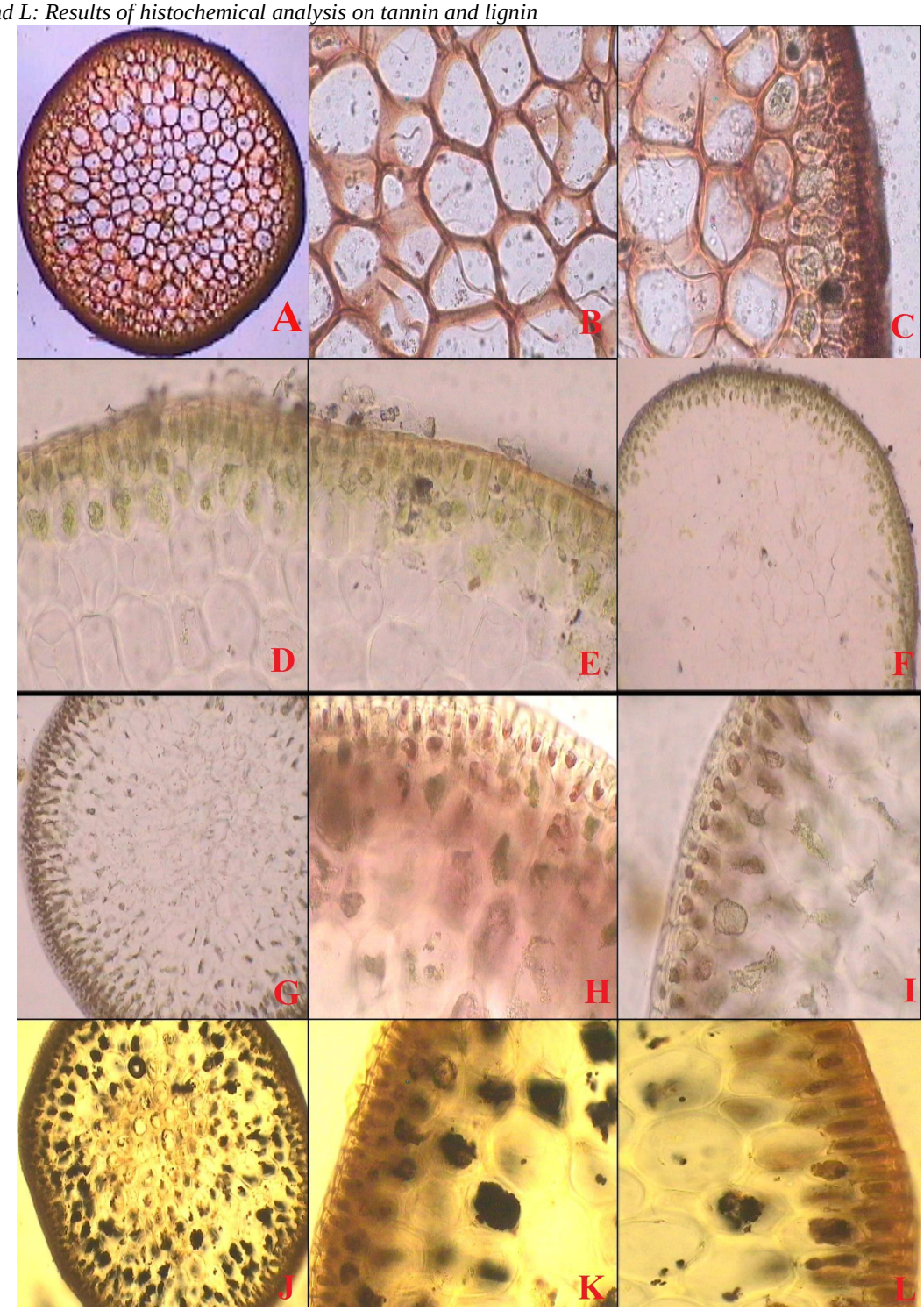
RESULTS AND DISCUSSION

In transverse section, the outline of the thallus is circular. The cells of outer layer are with thick outer wall. Usually it is smooth without bearing any appendages. Middle part of thallus in transverse section is as a continuous ring and it ranges from 2-5. The middle part of thallus gives mechanical support to the thallus. The central part of the thallus is filled with thin walled parenchymatous cells (Fig. 1 A-C). The occurrence and distribution of various metabolites (phenols, polyphenols, lignin and tannins) in *L. obtusa* were illustrated (Table 1; Fig. 1 D-L). Phenols, polyphenols, tannin and lignin are profusely present in the outer layer of the thallus. Phenol shows the presence small quantity only in outer layer of the cells. Polyphenols, tannin and lignin and abundantly present in the middle part of the thallus. Polyphenol and tannin shows minimal quantity and lignin was abundantly present in central part of the thallus.

The importance of histochemistry in solving critical biosystematic problems is as popular as the use of cytological, biochemical and molecular markers. The application of histochemical characters in taxonomic problems is now a common practice for the identification and characterization of taxon. The present study results confirmed the presence of phenols, polyphenols, lignin and tannin in different parts of the *L. obtusa* thallus. Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties [21-23]. In the present study also the phenolics presence was confirmed by the qualitative analysis in the crude extracts of *L. obtusa*. Reports have revealed that phenolic compounds are one of the most effective antioxidants in brown algae [24]. The results of the present study are promising as algal phenolic compounds are effective antioxidants in delaying oil rancidity, therefore the seaweed extracts could have potential in food applications [25].

Fig. 1: Histochemical studies on *Laurencia obtusa* (Hudson) Lamourux

A: T. S. of Thallus – Entire view
B and C: A portion enlarged at different magnifications showing outer, middle and central part of thallus
D, E and F: Results of histochemical analysis on phenol
G, H and I: Results of histochemical analysis on polyphenol
J, K and L: Results of histochemical analysis on tannin and lignin



Tannins are defined as naturally occurring plant polyphenolic compounds and are widespread among terrestrial and marine plants [26]. Vegetable tannins are secondary plant metabolites subdivided into condensed and hydrolysable compounds. Hydrolyzable tannins are gallic or egallic acid which easily hydrolyze in acidic media, and condensed tannins are polymeric flavonoids [27]. In contrast to terrestrial tannins, phlorotannins are tannin compounds, which have been found only in marine algae; Phlorotannins are formed by the polymerization of phloroglucinol (1, 3, 5-trihydroxybenzene) monomer units and synthesized in the acetate-malonate pathway in marine alga [26]. Phlorotannin purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton [28]. Phlorotannins from brown algae have up to eight interconnected rings. They are therefore more potent free radical scavenger than other polyphenols derived from terrestrial plants, including green tea catechins, which only have three to four rings [29]. Many tannin-containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering; They are also medicinally used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote. Tannins has been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications [30,31]. The present study results confirm the tannin presence in *L. obtusa*. It suggests that the selected seaweeds can be used as antiviral, antibacterial, antiparasitic agents and exercise to treat the diseases like ulcer, gonnorrhoea, leucorrhoea after clinical screening.

Lignin is present in all vascular plants, but not in bryophytes. The original function of lignin was restricted to water transport. However, it is present in red algae, which seems to suggest that the common ancestor of plants and red algae also synthesised lignin. This would suggest that its original function was structural; it plays this role in the red alga Calliarthron, where it supports joints between calcified segments [32]. Lignin is found in the cell walls of plants. Lignins impart strength to cell walls, facilitate water transport, and impede the degradation of wall polysaccharides, thus acting as a major line of defense against pathogens, insects, and other herbivores [33]. Several studies refer to the possible involvement of phenolics and lignin in resisting haustorial penetration within the host tissue [34]. Phenyl propanoids and derivatives such as coumarins, lignin, suberin, cutin, and tannin represent structural material for plant stability [35] and can be involved in the resistance of the host as defence compounds against broomrape [34]. Recently, a number of studies have been reported on the histochemistry and phytochemistry of medicinal plants across the world [36-42]. In the present investigation, we reported histochemical studies on seaweeds *L. obtusa* and confirmed the presence of phenolics, polyphenols, tannins and lignin, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Hence this study offers a base of using *L. obtusa* as herbal alternative for the synthesis of active compounds.

REFERENCES

[1] VK Dhargalkar, P Neelam. *Science and Culture* **2005**; 71: 60-66.
[2] MHG Munro, JW Blunt. Marine chemical Group, University of Canterbury, Christchurch, New Zealand, **1999**;
[3] DJ Faulkner. *J. Natural Product* **2001**; 18: 1-49.
[4] JW Blunt, BR Copp, WP Hu, MHG Munro, PT Northcote, MR Prinsep. *Nat prod Rep* **2007**; 24: 31-86.
[5] ML Souto, CP Manriquez, M Norte, JJ Fernandez. *Tetrahedron* **2002**; 58: 8119-8125.
[6] RC Pereira, BAP Da Gama, VL Teixeira, Y Yoneshigue-Valentin. *Braz. J. Biol*. **2003**; 63(4): 665-672.
[7] GM Konig, AD Wright. *J Nat prod* **1997**; 60: 967-970.
[8] KA Mohammed, CF Houssain, L Zhang, RK Bruick, YD Zhou. *J Nat prod* **2004**; 67: 2002-2007.
[9] CS Vairappan, T Kawamato, H Miwa, M Suzuki. *Planta Med* **2004**; 70: 1087-1090.
[10] JL Morales, ZO Cantillo-Ciau, I Sanchez-Molina, GJ Mena-Region. *Pharm Biol* **2006**; 44: 632-635.
[11] S Sakemi, T Higa, CW Jefford, G Bernardinelli. *Tentrahedrom Lett* **1986**; 27: 4287-4290.
[12] CA Gray, SP Lira, M Silva, EF Pimenta, OH Thiemann, G Oliva, E Hajdu, RJ Andersen, RG Berlinuck. *J org chem* **2006**; 71: 8685-8690.
[13] KV Rao, MS Donia, J Peng, DA Garcia-Palomero, A Martinez, M Medina, BL Franzblau Tekwani, SI Khan, S Wahyuono, KL Willet, MT Hamann, B Manzamine. *J Nat prod* **2006**; 69: 1034-1040.
[14] A Dube, N Singh, A Saxena, V Lakshmi. *Parasitol Res* **2007**; 101: 317-324.
[15] Y Freile-Pelegrin, D Robledo, MJ Chan-Bacab, BO Ortega-Morales. *Fitoterapia* **2008**; 79: 374-377.
[16] G Genovese, L Tendone, MT Hamann, M Morabito. *Marine Drugs* **2009**; 7: 361-366.
[17] ET Haridass, N Suresh Kumar. Some techniques in the study of insect-host plant interactions. In *Dynamics of Insect Plant interactions*. Ananthakrishnan, TN, Entomology Research Institute, Loyola College, Madras.
[18] RM Reeve. *Stain Technol.* **1951**; 26: 91-96.

[19] PB Gahan. Academic Press, Florida, **1984**;
[20] CJ Chamberlin. Univ. Chicago Press, Chicago, **1924**;
[21] XJ Duan, WW Zhang, XM Li, BG Wang. *Food Chemistry* **2006**; 95: 37-43.
[22] T Kuda, T Kunii, H Goto, T Suzuki, T Yano. *Food Chemistry* **2007**; 103: 900-905.
[23] BG Wang, WW Zhang, XJ Duan, XM Li. *Food Chemistry* **2009**; 113: 1101-1105.
[24] T Nagai, T Yukimoto. *Food Chemistry* **2003**; 81: 327-332.
[25] XJ Yan, Y Chuda, M Suzuki, T Nagata. *Biosci. Biotechnol. Biochem* **1999**; 63: 605-607.
[26] PG Waterman, S Mole. Analysis of Phenolic Plant Metabolites. In *Methods in Ecology*. Blackwell Scientifc Publications, Oxford, UK, **1994**;
[27] J Huang, Y Liu, X Wang. *Journal of Hazardous Materials* **2008**; 160: 382-387.
[28] GN Ahn, KN Kim, SH Cha, CB Song, J Lee, MS Heo, IK Yeo, NH Lee, YH Jee, JS Kim, MS Heu, YJ Jeon. *European Food Research and Technology* **2007**; 226: 71-79.
[29] RAS Hemat. Fat and muscle dysfunction. In *Hemat RAS*, Andropathy. Dublin, Ireland: Urotext, **2007**; pp. 83-85.
[30] L Lu, SW Liu, SB Jiang, SG Wu. *Acta Pharmacologica Sinica* **2004**; 25(2): 213-218.
[31] H Kolodziej, AF Kiderlen. *Phytochemistry* **2005**; 66(17): 2056-2071.
[32] PT Martone, JM Estevez, F Lu, K Ruel, MW Denny, C Somerville, J Ralph. *Current biology* **2009**; 19(2): 169-175.
[33] RR Sederoff, JJ MacKay, J Ralph, RD Hatfield. *Curr Opin Plant Biol* **1999**; 2: 145-152.
[34] J Jorrin, E De Ruck, K Serghini, A Perez De Luuque, J Munoz-Garcia, L Garcia-Torres, M Castejon. Biochemical aspects of the parasitism of sunflower by *Orobanche*. In Oreno, MT, **1996**;
[35] HW Heldt. Phenylpropanoids. Oxford University Press. Oxford, UK, **1997**; pp. 377-393.
[36] FN Mbagwu, CIN Unamba, CI Onuoha, IO Ezeibekwe. *Research Journal of Biological Sciences* **2009**; 4(3): 254-257..
[37] HO Edeoga, G Omosun, GGE Osuagwu, OO Emezue. *Asian Journal of Plant Sciences* **2007**; 6(4): 688-691.
[38] EA Hassan, SS El-Akkad, SM Moustafa, ME El-Awadi. *International Journal of Agriculture & Biology* **2004**; 6(3):430-434.
[39] R Senthamarai, AM Ismail, T Shri Vijaya Kiurbha, P Balasubramanian. *Journal of Chemical and Pharmaceutical Research* **2012**; 4(3):1457-1464.
[40] T Shri Vijaya Kirubha, R Senthamarai, P Mariya, Praveen Mani. *Journal of Chemical and Pharmaceutical Research* **2012**; 4(3):1465-1469.
[41] M. Balasubramanian. *Journal of Chemical and Pharmaceutical Research* **2012**; 4(3):1686-1695.
[42] S Suganya, R Bharathidasan, G Senthilkumar, P Madhanraj A Panneerselvam. *Journal of Chemical and Pharmaceutical Research* **2012**; 4(3):1846-1850.