Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(4): 165-168
Received: 10-09-2015
Accepted: 13-10-2015

Babu A
Centre for Plant Biotechnology, PG and Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India.

Johnson M
Centre for Plant Biotechnology, PG and Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India.

Patric Raja D
Centre for Plant Biotechnology, PG and Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India.

Correspondence:
Johnson M
Centre for Plant Biotechnology, PG and Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India.

Identification of green seaweeds *Caulerpa scalpelliformis* (R.Br.) Weber- van- Bosse and *Caulerpa corynephora* Montagne using TLC profile

Babu A, Johnson M, Patric Raja D

Abstract
The present study was aimed to produce the identification, chemical marker for the two seaweeds viz., *Caulerpa scalpelliformis* (R.Br.) Weber- van- Bosse and *Caulerpa corynephora* Montagne using steroid and phenolic TLC profile. Phenolics and steroid profile was detected using the specific mobile phase and spraying agents. The appearance of blue colour and bluish green colour spot in the TLC chromatogram indicated the presence of steroids and phenolics in the crude extracts of *C. scalpelliformis* and *C. corynephora* respectively. The ethanolic extracts showed the maximum number of spots. Ethanolic extract of *C. scalpelliformis* and *C. corynephora* showed ten and nine steroidal and phenolics bands respectively with different R_f values, Ethanolic extract of *C. scalpelliformis* and *C. corynephora* showed ten and nine steroidal bands respectively with different R_f values. The TLC profile developed for the identification of *C. scalpelliformis* and *C. corynephora* is simple, precise, specific, accurate, rapid and cost effective and also act as a biochemical marker for these medicinally important seaweeds in the pharmaceutical industry.

Keywords: TLC, Seaweed, Phenolics, Steroid.

1. Introduction
In the plant kingdom, the seaweeds showed unique characteristic features by morphological, anatomical and biochemical. They are not possesing the true leaves, stems and roots or vascular systems as similar to higher plants. Identification of seaweeds by using morphological character is the difficult task. Due to that, seaweeds are an untapped resource compared to flowering plants eventhough they offer substantial potential for the extraction of natural chemical constituents. Among the three major groups, Chlorophyceae is showing more nutritious value and possesses a range of metabolites with various biological properties [1]. In Asia, the seaweeds are extensively used as food salad for centuries [2], but in western countries they are cultivated and used in the industries for the commercial production of valuable chemicals, ingredients, metabolites, pharmaceutical products, cosmetics and fertilizers [3]. The primary and secondary metabolites of seaweeds possess some important medicinal properties viz., antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations [4]. Due to their versatile application the chromatographic methods TLC and HPTLC are commonly employed for the identification medicinal plants or plant derived products in the pharmaceutical industries [5]. With this background the present study was aimed to differentiate the green seaweeds *Caulerpa scalpelliformis* (R.Br.) Weber-van-Bosse and *Caulerpa corynephora* Montagne using TLC.

2. Materials and Methods
2.1. Collection of Materials
Caulerpa scalpelliformis (R.Br.) Weber- van- Bosse and *Caulerpa corynephora* Montagne were collected by handpicking from the coast of Rasthacaud, Kanyakumari District, Tamil Nadu, India (Lat N 08º08'308" E77º32'80"). The collected seaweeds were cleaned well with sea water to remove all the extraneous matters such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The collected seaweeds were then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in the shade. The shade dried seaweeds were ground to a fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use.

2.2. Preparation of extracts

The dried and powered seaweed materials (30 g) were extracted successively with 180 ml of hexane, petroleum ether, chloroform and ethanol by using a Soxhlet extractor for 8 hrs at a temperature not exceeding the boiling point of the solvent. The aqueous extract was prepared by directly boiling the powder with distilled water. The extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuum at the 40 ºC using Rotary evaporator. The residues obtained were stored in a freezer -20 ºC until further tests.

2.3. TLC analysis

TLC was carried out on 10 × 20 cm silica gel plates (Merck, Germany). The phenolic and steroidal compounds present in various extracts of *Caulerpa scalpelliformis* and *Caulerpa corynephora* were tentatively detected by TLC. The mobile phase used for phenolics was chloroform and methanol at 9:1 ratio. After spraying with the solution composed of folin-ciocalteu reagent, the appearance of a blue colour spot in the TLC chromatogram indicated the presence of phenolic compounds. The mobile phase used for steroids was benzene and methanol at 9:1 ratio. After spraying with the solution composed of 5% alcohol sulphuric acid, the appearance of a bluish green colour spot in the TLC chromatogram indicated the presence of steroidal compounds.

3. Results and Discussion

TLC separation of steroids and phenolics present in various extracts (Hexane, petroleum ether, chloroform, ethanol and aqueous) of *Caulerpa scalpelliformis* and *Caulerpa corynephora* were tabulated in Table 1 and 2. Ethanolic extract of *C. scalpelliformis* showed ten steroidal bands and *C. corynephora* showed nine bands with different R_f values ranged from 0.05 to 0.85 (Table 1). Among these four bands with the R_f values 0.14, 0.30, 0.37 and 0.78 were observed in *C. scalpelliformis* and *C. corynephora.*

Chloroform extract of *C. scalpelliformis* showed seven steroidal bands and *C. corynephora* showed eight bands with different R_f values ranged from 0.05 to 0.90 (Table 1). Among these five steroidal bands with the R_f values 0.14, 0.25, 0.51, 0.75, 0.90 were showed their existence in *C. scalpelliformis* and *C. corynephora.* The bands with the R_f values 0.05 and 0.09 were expressed their restricted presence in *C. scalpelliformis,* and the steroidal bands with the Rf values 0.20, 0.68 and 0.78 were showed their unique presence in *C. corynephora.*

Petroleum ether extract of *C. scalpelliformis* showed four steroidal bands and *C. corynephora* showed five bands with different R_f values viz., 0.14, 0.37, 0.48, 0.55 and 0.85. The bands with the R_f value 0.85 was expressed its restricted presence in *C. corynephora.* All the other bands were showed their occurrence in *C. scalpelliformis* and *C. corynephora* (Table 1).

Table 1: Steroidal profiles of ethanolic extract of *C. scalpelliformis* and *C. corynephora* using TLC

Rf values	*Caulerpa scalpelliformis*					*Caulerpa corynephora*				
	H	**P**	**C**	**E**	**A**	**H**	**P**	**C**	**E**	**A**
0.05	-	-	+	+	-	-	-	-	-	-
0.09	-	-	+	-	-	-	-	-	-	-
0.11	-	-	-	+	-	-	-	-	-	-
0.14	-	+	+	+	-	-	+	+	+	-
0.20	-	-	-	+	-	+	-	+	-	-
0.25	-	-	+	+	-	-	-	+	-	-
0.27	+	-	-	-	-	+	-	-	-	-
0.30	-	-	-	+	-	-	-	-	+	-
0.37	-	+	-	+	-	-	+	-	+	-
0.43	-	-	-	-	-	-	-	-	+	-
0.48	+	+	-	-	-	+	+	-	-	-
0.49	-	-	-	-	-	-	-	-	+	-
0.51	-	-	+	-	-	-	-	+	-	-
0.55	-	+	-	+	-	-	+	-	-	-
0.60	+	-	-	-	-	+	-	-	+	-
0.68	+	-	-	-	-	+	-	+	-	-
0.70	-	-	-	-	-	-	-	-	+	-
0.75	-	-	+	-	-	-	-	+	-	-
0.78	-	-	-	+	-	-	-	+	+	-
0.85	-	-	-	+	-	-	+	-	-	-
0.90	-	-	+	-	-	+	-	+	+	-

H-Hexane, P- Petroleum ether, C-Chloroform, E- Ethanol, A-Aqueous

Hexane extract of *C. scalpelliformis* showed four steroidal bands and *C. corynephora* showed five bands with different R_f values viz., 0.20, 0.27, 0.48, 0.60 and 0.90. Among these 0.20, 0.27, 0.48 and 0.60 were commonly present in *C. scalpelliformis* and *C. corynephora* (Table 1). The band with R_f value 0.90 was expressed only in *C. corynephora.* Aqueous extracts failed to illustrate spot which showed the absence of steroidal compounds in *C. scalpelliformis and C. corynephora* (Table 1).

Ethanolic extract of *C. scalpelliformis* showed ten phenolics bands and *C. corynephora* showed nine bands with different R_f values viz., 0.02, 0.05, 0.12, 0.38, 0.45, 0.53, 0.56, 0.65, 0.73, 0.78, 0.80 and 0.88. Among these seven bands with the R_f values 0.05, 0.12, 0.38, 0.45, 0.56, 0.80 and 0.88 were showed

their common occurrence in *C. scalpelliformis* and *C. corynephora*. The bands with the R_f values 0.02, 0.65 and 0.78 were expressed their restricted presence in *C. scalpelliformis* (Table 2). Chloroform extract showed seven phenolics bands in *C. scalpelliformis* and *C. corynephora* respectively (Table 2). Among these four phenolics bands with the R_f values 0.12, 0.20, 0.32 and 0.65 were showed their common existence in *C. scalpelliformis* and *C. corynephora*. The bands with the R_f values 0.02, 0.45 and 0.73 were expressed their restricted presence in *C. scalpelliformis,* and the phenolics bands with the R_f values 0.20, 0.56 and 0.78 were showed their unique presence in *C. corynephora*.

Petroleum ether extract of *C. scalpelliformis* showed three phenolics bands and *C. corynephora* displayed five bands with different R_f values viz., 0.42, 0.56, 0.58 0.73 and 0.80 (Table 2). The bands with the R_f value 0.42 and 0.80 were showed their restricted presence in *C. corynephora*. All the other bands showed their common occurrence in *C. scalpelliformis* and *C. corynephora*. Hexane extract of *C. scalpelliformis* showed five phenolics bands and *C. corynephora* demonstrated six bands with different R_f values viz., 0.02, 0.25, 0.32, 0.56, 0.58, 0.69 and 0.73. Among these, the bands with R_f Values 0.56 and 0.73 were showed their common existence in *C. scalpelliformis* and *C. corynephora*. The bands with the R_f value 0.08, 0.25 and 0.69 were showed their restricted presence in *C. scalpelliformis*. The bands with the R_f value 0.02, 0.32, 0.35 and 0.58 were showed their restricted presence in *C. corynephora* (Table 2). Aqueous extracts failed to illustrate bands which showed the absence of phenolics compounds in *C. scalpelliformis* and *C. corynephora* (Table2).

Table 2: Phenolics profiles of different extract of *C. scalpelliformis* and *C. corynephora* using TLC

Rf values	*Caulerpa scalpelliformis*					*Caulerpa corynephora*				
	H	**P**	**C**	**E**	**A**	**H**	**P**	**C**	**E**	**A**
0.02	-	-	+	+	-	+	-	-	-	-
0.05	-	-	-	+	-	-	-	-	+	-
0.08	+	-	-	-	-	-	-	-	-	-
0.12	-	-	+	+	-	-	-	+	+	-
0.20	-	-	+	-	-	-	-	+	-	-
0.25	+	-	-	-	-	-	-	-	-	-
0.32	-	-	+	-	-	+	-	+	-	-
0.35	-	-	-	-	-	+	-	-	-	-
0.38	-	-	-	+	-	-	-	-	+	-
0.42	-	-	-	-	-	-	+	-	-	-
0.45	-	-	+	+	-	-	-	-	+	-
0.53	-	-	-	-	-	-	-	-	+	-
0.56	+	+	-	+	-	+	+	+	+	-
0.58	-	+	-	-	-	+	+	-	-	-
0.65	-	-	+	+	-	-	-	+	-	-
0.69	+	-	-	-	-	-	-	-	-	-
0.73	+	+	+	-	-	+	+	-	+	-
0.78	-	-	-	+	-	-	-	+	-	-
0.80	-	-	-	+	-	-	+	-	+	-
0.85	-	-	-	-	-	-	-	+	-	-
0.88	-	-	-	+	-	-	-	-	+	-

H-Hexane, P- Petroleum ether, C-Chloroform, E- Ethanol, A-Aqueous

Seaweeds are extensively used in the drug and pharmaceutical industry due to the existence of rich chemical constituents [6]. of which phenolics are one of the most ubiquitous groups of secondary metabolites found throughout the plant kingdom [7]. It includes a range of compound types that include structures such as simple aromatic phenols, hydroxy and substituted benzoic acids and aldehydes, hydroxy and substituted cinnamic acids, coumarins, tannins and perhaps a few of the flavonoids [8]. Similarly, plants produce a variety of sterols. The plant sterols are membrane constituents as well as the precursors for plant hormones and other secondary metabolites, i.e., substances interfering with pathogens and insects [9-12].

For the pharmacological as well as pathological discovery of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. The TLC profiles of hexane, petroleum ether, chloroform and ethanolic extracts of C. *scalpelliformis* and *C. corynephora* confirmed the existence of sterols and phenolic compounds. The TLC banding profile (R_f values) clearly differentiated the two green seaweeds and supplemented to the morphological classification. In addition the variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Compound showing high R_f value in the less polar solvent system have low polarity and with less R_f value have high polarity. The TLC profile developed for the identification of *C. scalpelliformis* and *C. corynephora* is simple, precise, specific, accurate, rapid and cost effective. These TLC profile may be used effectively for the identification seaweeds and its derived products. The different extracts of *C. scalpelliformis* and *C. corynephora* TLC chromatographic fingerprints could be useful for the quality assessment and also act as a biochemical marker for these medicinally important seaweeds in the pharmaceutical industry.

4. Conflict of Interest Statement

We declare that we have no conflict of interest.

5. References

1. Rodr´ıguez-Bernaldo AFS, Frecha P, Vidal A, L´opez HJ. Antioxidant compounds in edible brwn seaweeds. European Food Research and Technology 2010; 231(3):495-498.
2. Darcy-Vrillon B. Nutritional aspects of the developing use of marine macroalgae for the human food industry, International Journal of Food Science and Nutrition. 1993; 44:S23-S35.
3. Lewis JG, Stanley NF, Guist GC. In Lembi CA, Waaland JR (eds.), Algae and Human A. airs, Cambridge University Press, New York, 1988, 205-236.
4. Fayaz M, Namitha KK, Chidambara Murthy KN, Mahadeva Swamy M, Sarada R, Salma Khanam Subbarao PV *et al*. Chemical composition, iron bioavailability and antioxidant activity of *Kappaphycus alvarezii* (Doty), J Agric. Food Chem. 2005; 53:792-797.
5. Szepesi G. Some aspect of the validation of planar chromatographic methods used in pharmaceutical analysis. I. General principles and practical approaches, J Planar Chromatogr. 1993; 6:187-189.

6. Balandrin MJ, Klocke JA. Medicinal, aromatic and industrial materials from plants, In Bajaj YPS. (ed.) Biotechnology in Agriculture and Forestry. Medicinal and Aromatic Plant, Springer-Verlag, Berlin, Heidelberg, 1998; 4:1-36.
7. Boudet A. Evolution and current status of research in phenolic compounds. Phytochemistry 2007; 68:2722-2735.
8. Zeng RS, Mallik AU, Luo SM. Allelopathy in sustainable agriculture and forestry Springer Science Business Media, LLC: New York, USA, 2008.
9. Hartmann MA. Plant sterols and the membrane environment, Trends in Plant Science 1998; 3:170-175.
10. Lindsey K, Pullen ML, Topping JF. Importance of plant sterols in pattern formation and hormone signalling, Trends in Plant Science 2003; 8:521-525.
11. Schaller H. The role of sterols in plants growth, Progess in Lipid Research 2003; 42:163-175.
12. Hartmann MA. Sterol metabolism and functions in higher plants; In Daum G. (ed) Lipid metabolism and membrane biogenesis, Berlin, Heidelberg, Germany Springer Verlag 2004; 6:183-211.