Original Article

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Phytochemical characterization of *Sargassum swartzii* (Turner) C. Agardh using thin-layer chromatography, Fourier transform infrared, and high-performance liquid chromatography

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ABSTRACT

The present study was aimed to investigate the phytochemical properties of *Sargassum swartzii* (Turner) C. Agardh. The standard method was employed to detect the metabolites presence or absence in the tested extracts of *S. swartzii*. To confirm the occurrence of the functional constituents in the crude extracts of *S. swartzii,* spectroscopic UV-Vis and Fourier transform infrared (FTIR) analysis, chromatographic thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) analysis were performed. The preliminary phytochemical analysis of *S. swartzii* displayed the existence of alkaloids, steroids, phenolic groups, saponins, and flavonoids. TLC profile of *S. swartzii* chloroform extract illustrated three phenolic bands with different Rf values 0.258, 0.777, and 0.879 and only one distinct steroid band was observed in the chloroform extract of *S. swartzii* with the Rf value 0.724. Methanolic extracts of *S. swartzii* showed five compounds (peaks) with different retention time 1.960, 2.143, 2.303, 2.610, and 10.80 min. The HPLC chromatogram of *S. swartzii* chloroform extracts displayed six compounds (Peaks) with different retention time 1.983, 2.143, 2.280, 2.623, 3.500, and 13.147 min. The results of FTIR analysis validated the occurrence of functional groups such as alkynes, phosphorus compounds, unsaturated oximes, alcohols or phenols, and halogen compounds in *S. swartzii*. The results of the present study confirmed that *S. swartzii* may be pool of natural metabolites further isolation may be bring out the novel biopotential compound with various biological activities.

KEY WORDS: Fourier transform infrared, high-performance liquid chromatography, phytochemical, *Sargassum swartzii*, thin-layer chromatography

INTRODUCTION

Seaweeds, the marine, and macroalgae are used as food supplements, source of vitamins, as a food additive (Subramian and Uma, 1996). Recently, phycologist and phytochemist are focusing their interest on the discovery of natural phytochemicals (Dastmalachi and Dorman, 2007). Nearly 2400 seaweeds secondary metabolites (SSM) are described, and many of the SSM are used as a natural source of medicine (Al-Fadhli *et al*., 2006; El-Baroty *et al*., 2007). The available literature suggests that consumption of seaweeds with a high content of phytochemical decreases the risk of chronic diseases (Bhasker and Miyashita, 2005). Seaweeds offer a promising source of bioactive compounds in the field of medical and biochemical applications (Leary *et al*., 2009). The seaweed compounds have demonstrated various biological properties (Thirumaran *et al*., 2006; Kim and Lee, 2008; Venkateswarlu *et al*., 2007; Yang *et al*., 2006).

Sargassum is a large genus, showed their common presence in tropics, and they are economically and medicinally important. In India, nearly 38 species are showed their existence (Mallikharjuna *et al*., 2007). *Sargassum* can be used as fertilizers, food additives, and animal feed (Chandini *et al*., 2008; Dawczynski *et al*., 2007). *Sargassum* has been studied, and they showed promising antibacterial, antipyretic, analgesic and anti-inflammatory, cytotoxicity, and antitumor activity (Kim and Lee, 2008; Kang *et al*., 2008; Hoang *et al*., 2007). Ethyl acetate

extracts of *Sargassum swartzii* showed the antibacterial and anti-larvicidal activity (Khanavi *et al*., 2011). Thus, point out that *Sargassum* is a good source for the phytochemical investigation to identify the occurrence of biomolecules. With this knowledge, the present study was indented to determine the phytochemical profiles of *S. swartzii* (Turner) C. Agardh using thin-layer chromatography (TLC), Fourier transform infrared (FTIR), and high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Collection of Materials

S. swartzii (Turner) C. Agardh was collected from the coast of Rasthacaud (Kanyakumari District, Tamil Nadu, India, Lat N 08°08′308″ E77°32′80 ″). The collected seaweeds were shade dried for 15 days and grounded to fine powder using a conventional mechanical grinder. For cold extraction, 10 g powdered materials were incubated with 60 ml of various solvents individually for 72 h under dark condition. The solution was filtered through Whatman No. 41 filter paper, and the filtrate was used for phytochemical analysis.

Fluorescence Analysis

As per the procedure described in the Indian Pharmacopoeia, the powdered materials were treated with various reagents and examined under a visible and UV light. The changes in color were recorded.

Preliminary Phytochemical Analysis

According to the method described by Harborne (1998), the phytochemical analysis was performed to detect the presence or absence of steroids, phenolic compounds, saponins, tannins, flavonoids, and anthraquinones in the extracts of *S. swartzii*.

TLC Analysis

To know the phenolic and steroid profile of *S. swartzii,* TLC was carried out. The occurrence of blue spot and a bluish green spot in the TLC chromatogram indicated the presence of phenol and steroid compounds in the extracts of *S. swartzii*.

HPLC Analysis

As per the procedure described by Gavidia *et al.* (2007), Mizukoshi *et al*. (1993), and Sharanabasappa *et al*. (2007), the HPLC analysis was performed. The 0.1% v/v methanol (solvent A) and water (solvent B) was employed as the mobile phase. The sample injection volume was 20 µl while the wavelength of the UV-Vis detector was set at 254 nm. 20 µl of samples were loaded, and 15 min run was performed for the HPLC analysis. The retention time with different peak values were observed for methanolic and chloroform extracts of *S. swartzii*.

FTIR Analysis

To know the occurrence of functional groups in the crude powder of *S. swartzii,* the FTIR analysis was performed using the Perkin Elmer spectrophotometer system (Janakiraman *et al*., 2011). The peak values of the FTIR were recorded, and their corresponding functional groups were identified.

RESULTS

The existence of steroids, alkaloids, phenolic groups, saponins, flavonoids, and anthraquinone in the extracts of *S. swartzii* was confirmed by the preliminary phytochemical analysis. Anthraquinone failed to show its occurrence in the tested extracts of *S. swartzii*. Aqueous extract of *S. swartzii* failed to show the metabolites occurrence. The steroid showed its occurrence in methanolic, benzene, chloroform, and petroleum ether extracts of *S. swartzii*. Saponin demonstrated its existence in the acetone, benzene and chloroform extracts of *S. swartzii*. Flavonoids showed its presence in the methanolic, benzene, and chloroform extracts of *S. swartzii*. Alkaloids displayed its occurrence only in acetone extracts of *S. swartzii*. Phenol showed its existence only in the methanolic extracts of *S. swartzii*.

Fluorescence Analysis

The fluorescence analyses of the seaweed extracts of *S. swartzii* is recorded in Table 1.

TLC Analysis

TLC profile of *S. swartzii* chloroform extract showed three phenolic bands with different Rf values 0.258, 0.777, and 0.879 (Figure 1a), and only one distinct steroid band was observed in the chloroform extract of *S. swartzii* with the Rf value 0.724 (Figure 1b).

HPLC Analysis

Methanolic extracts of *S. swartzii* showed five compounds (peaks) with varied retention time viz., 1.960, 2.143,

Table 1: Fluorescence analysis of *S. swartzii*

Light source	50% H_2SO_4	1 N HCl	Acetone	NaOH	Nitric acid
Fluorescent	Blackish Brown	Green	Green	Brown	Yellowish Brown
UV	Blackish Brown	Brownish Green	-	Light Brown	Yellow

S. swartzii: Sargassum swartzii

2.303, 2.610, and 10.80 min (Figure 2). The HPLC chromatogram of methanolic extracts of *S. swartzii* displayed a single prominent peak with a retention time of 2.610 min, and few other moderate peaks were also observed (1.960, 2.143, 2.303, and 10.803 min) in the methanolic extracts of *S. swartzii* (Figure 2). The HPLC chromatogram of *S. swartzii* chloroform extracts displayed six compounds with varied retention time 1.983, 2.143, 2.280, 2.623, 3.500, and 13.147 min. The HPLC profile of chloroform extracts of *S. swartzii* demonstrated a single prominent peak at a retention time of 2.623 min and few other moderate peaks at 1.983, 2.143, 2.280, 3.500, and 13.147 min (Figure 3).

FTIR Analysis

The results of FTIR analysis confirmed the occurrence of alkynes, phosphorus compounds, unsaturated oximes, alcohols or phenols, and halogen compounds in *S. swartzii* (Figure 4).

DISCUSSION

The research on secondary metabolites of seaweeds confirmed the various biological properties of the seaweeds (Smith, 2004; Blunt *et al.*, 2007). Recent findings evidenced that seaweeds contain antibacterial (Tuney *et al.*, 2006), antiviral (Serkedjieva, 2004), antifungal (Tang *et al.*, 2002), and larvicidal potentials (Thangam and Kathiresan, 1991). Previous results on the phytochemical analysis of seaweeds confirmed the presence of primary and secondary metabolites with a varied degree in the seaweed extracts. Similar to previous observations, in the present study also, we observed the varied degree of metabolites occurrence in the tested extracts of *S. swartzii*.

Kuda *et al.*, (2007) and Wang *et al.*, (2009) noted that phenolic compounds are the good source of antioxidant and found in the plants commonly. Algal phenolic compounds are valuable antioxidants (Nagai and Yukimoto, 2003). Methanolic extracts of *S. swartzii* showed the phenolics existence and confirmed their antioxidant properties of *S. swartzii*. The results suggest that methanolic extracts of *S. swartzii* may have potential applications in nutraceutical industries. Ndhlala et al. (2007) listed out the various types of flavonoids and Thangam and Kathiresan (1991) pointed out the importance of flavonoids. They serve as a defense mechanism to protect the plant from biotic and abiotic stress. In the present study, the occurrence of flavonoids was noted in methanolic, benzene, and

Figure 1: (a-b) Thin-layer chromatography profile of *Sargassum swartzii* chloroform extract - phenolics and steroids

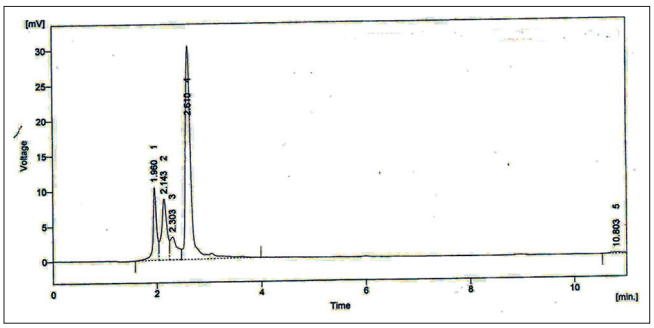

Figure 2: High-performance liquid chromatography of the methanolic extracts of *Sargassum swartzii*

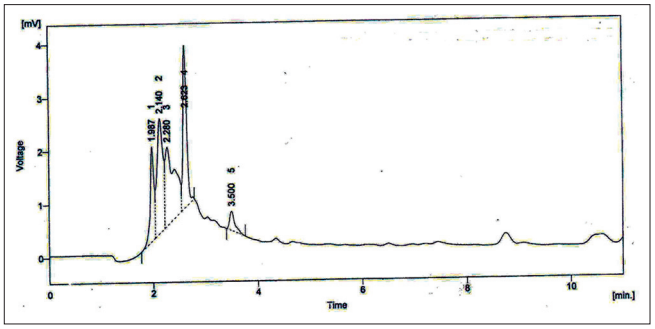

Figure 3: High-performance liquid chromatography of the chloroform extracts of *Sargassum swartzii*

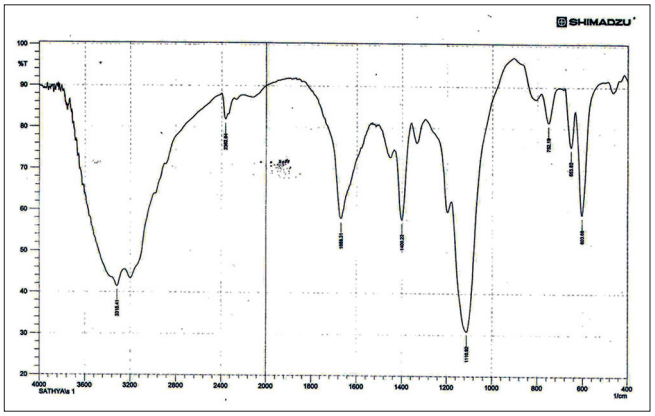

Figure 4: Fourier transform infrared spectrum of crude powder of *Sargassum swartzii*

chloroform extracts of *S. swartzii*. Harbome (1994) noted the antimicrobial potentials of plant-derived alkaloids. The presence alkaloid was validated only in the acetone extracts of *S. swartzii*.

Saponins possess various biological properties viz., antimicrobial, anti-inflammatory, antifeedent, and hemolytic effects (Harbome, 1994). In the present investigation, saponins showed its presence in acetone, chloroform, and benzene extracts of *S. swartzii*. Steroids may act as potential precursor and intermediater for the biosynthesis of secondary metabolites in plants (Cowan, 1999). In the present study, steroids were present in methanolic extracts of *S. swartzii*.

In the pharmaceutical industries distinguishing, the drug from adulterants is very important. The biologists and botanist are depended on the physico-chemical properties of drugs for proper evaluation (Harborne, 1998). The fluorescence, spectroscopic, and chromatographic analysis employed to distinguish the drugs from adulterants (Herl *et al*., 2006; Pimenta *et al*., 2006). Most of the researchers were characterized the plant-derived drugs using TLC (Joshi *et al*., 2011; Mehrotra *et al*., 2011). They employed the Rf values and phytoprofile as a marker to differentiate the medicinal source from other adulterant species. These phytoprofiles are used in the pharmaceutical industries and identified the medicinal sources. In the present study also, we developed the TLC, HPLC, and FTIR profile for *S. swartzii*. These profiles may be used to distinguish the *S. swartzii* from other seaweeds and adulterants.

CONCLUSION

The present phytochemical study also confirmed the occurrence of phenols, alkaloids, steroids, saponins, and flavonoids in *S. swartzii*. In addition, fluorescence, TLC, HPLC, and FTIR profiles can be used as biochemical markers in the pharmaceutical industries to identify the authentic mother plants and differentiate from its adulterants.

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