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

M. Johnson*, E. Petchiammal

Department of Botany, Centre for Plant Biotechnology, St. Xavier’s College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India

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 intended 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, 2.580, 3.777, and 4.879 (Figure 1c). The presence of phenol and steroid compounds in the extracts of S. swartzii was confirmed by the preliminary phytochemical analysis.

**Table 1: Fluorescence analysis of S. swartzii**

<table>
<thead>
<tr>
<th>Light source</th>
<th>50% H₂SO₄</th>
<th>1 N HCl</th>
<th>Acetone</th>
<th>NaOH</th>
<th>Nitric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescent</td>
<td>Blackish</td>
<td>Green</td>
<td>Green</td>
<td>Brown</td>
<td>Yellowish Brown</td>
</tr>
<tr>
<td>UV</td>
<td>Blackish</td>
<td>Brownish</td>
<td>-</td>
<td>Light</td>
<td>Yellow Brown</td>
</tr>
</tbody>
</table>

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...
chloroform extracts of \textit{S. swartzii}. Harborne (1994) noted the antimicrobial potentials of plant-derived alkaloids. The presence alkaloid was validated only in the acetone extracts of \textit{S. swartzii}.

Saponins possess various biological properties viz., antimicrobial, anti-inflammatory, antifeedent, and hemolytic effects (Harborne, 1994). In the present investigation, saponins showed its presence in acetone, chloroform, and benzene extracts of \textit{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 \textit{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; Mehrutra 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 \textit{S. swartzii}. These profiles may be used to distinguish the \textit{S. swartzii} from other seaweeds and adulterants.

**CONCLUSION**

The present phytochemical study also confirmed the occurrence of phenols, alkaloids, steroids, saponins, and flavonoids in \textit{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.

**REFERENCES**


