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ASSESSMENT OF PHYTOCHEMICAL PROFILING IN SELECTED SEAGRASSES

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ABSTRACT

Seaweeds are primarily found in the intertidal zone and tropical waters of the ocean. The objective of this investigation focused on a specific group of seaweeds observed along the Chorwad coast in Gujarat. The phytochemical analysis involves six species representing three algal divisions: *Caulerpa taxifolia* and *Caulerpa scalpelliformis* from the Chlorophyta division, *Halymenia venusta* and *Tricleocarpa fragilis* from the Rhodophyta division, and *Spatoglossum asperum* and *Scytosiphon lomentaria* from the Phaeophyta division. The dried algal mass was finely crushed into a powder, and three solvents methanol, chloroform, and acetone were used to extract phytochemicals. The preliminary qualitative screening indicated the presence of various secondary metabolites. The results showed various phytochemicals among the algae species. This research provides important findings on the phytochemical makeup of algae, which could have significant implications for the development of new pharmaceuticals and functional food items.

Keywords: Seaweeds, Phytochemical Screening, Secondary metabolites, Solvent

INTRODUCTION

Early scientists in phytochemical studies significantly expanded the exploration of plant metabolites to investigate bioactive compounds with notable pharmacological effects. In recent years, there has been growing interest in exploring the plant kingdom as a promising source of new drugs and strategies, focusing on plant extracts rich in biologically active compounds rather than specific classes or groups of compounds (Rout et al., 2020). Phytochemical research involving marine resources includes authenticating, extracting, and characterizing isolated compounds, as well as qualitatively, quantitatively, and pharmacologically assessing their activities (Willam, 2009).

Seaweeds are macro marine algae that thrive in tropical and subtropical shallow waters of seas and estuaries, encompassing over 200 species. They are categorized based on pigmentation and chemical composition into Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae). Seaweeds are non-toxic and contain numerous organic compounds, numbering in the hundreds (Antonisamy et al., 2012). The Western Coast of India exhibits abundant growth of seaweeds, representing a diverse array of potential reservoirs for biochemical compounds that could be valuable sources for drug discovery in the future. Over 2400 marine natural products have been isolated from seaweeds (Domettila et al., 2013). These natural products, categorized as secondary metabolites, demonstrate a wide range of biological activities. Research findings indicate that seaweeds contain antibacterial properties (Sahoo et al., 2001).

The main algal divisions observed along the coast of Chorwad include Rhodophyta, Phaeophyta, and Chlorophyta. Seaweeds found in Saurashtra region are utilized extensively for therapeutic and commercial purposes, especially in food manufacturing and as primary sources for various industries. Numerous studies have highlighted the medicinal properties of seaweeds, particularly in treating cardiac disorders, blood purification, and as antimicrobial agents. The significant role of phytochemicals, specifically secondary metabolites, contributes to these medicinal properties. These six seaweed species were examined for secondary metabolites such as proteins, terpenoids, flavonoids, alkaloids, carbohydrates, saponins, coumarins, quinines, and tannins.

MATERIALS AND METHODS

The algae were collected from their natural habitat in the Chorwad coastal region of Gujarat, India. They were carefully cleaned to remove any debris, sand, or impurities by rinsing them with distilled water. After cleaning, the algae were air-dried to remove moisture over a period of two weeks, and then ground into a fine powder using a mechanical grinder. The powdered algae were stored in an airtight container in a cool, dry place and used for analysis.

Extraction of shade dried plant material

The phytochemicals were extracted using a Soxhlet apparatus. Algal powder was mixed with solvents of increasing polarity (chloroform, methanol, and acetone) in a ratio of 1:5 (w/v). The mixture was placed in the Soxhlet apparatus and extracted at 50°C for 24 hours. The extraction was filtered using Whatman No. 1 filter paper. After completion of the extraction, the solvent was evaporated under vacuum, leaving behind a crude residue, which was then stored in a freezer at -20°C for preliminary phytochemical analysis (Becerro et al., 1988; Chovatia et al., 2022; Murugan and Santhanaramasamy, 2003; Kanjana et al., 2011; Krishnaveni et al., 2012).

The chloroform, methanol, and acetone extracts from selected algae were subjected to phytochemical analysis to screen for the presence or absence of active secondary metabolites including alkaloids, phenols, flavonoids, anthraquinones, tannins, saponins, coumarins, carbohydrates, proteins, quinones, and terpenoids. General reactions conducted during these analyses indicated whether these compounds were present or absent in the algal extracts using following procedures.

The phytochemicals of the extracts were determined qualitatively as stated by (Trease and Evans, 1989; Sadasivam and Manickam, 1996).

Test for Alkaloids: 1ml of 1% HCl was added to 3ml of extract in a test tube and was treated with few drops of Meyer's reagent. A creamy white precipitate indicted the presence of alkaloids.

Test for Phenols: Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Test for Flavonoids: A few drops of 1% NH_3 solution was added to the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

Test for Tannins: To 0.5 ml of extract solution, 1 ml of distilled water and 1-2 drops of ferric chloride solution were added and observed for brownish green or a blue-black coloration.

Test for Saponins: 5 ml of extract was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins.

Test for Coumarins: For coumarins identification, 1ml of extract, 1ml of 10% NaOH was added. Formation of yellow colour indicates presence of coumarins.

Test for Carbohydrates: Mix 2ml of plant extract, 1ml of molisch's reagent and few drops of conc. Sulphuric acid were added. Purple or reddish color indicates the presence of carbohydrates.

Test for Proteins: To 2ml of extract 1ml of 40% NaOH solution and 2 drops of 1% $CuSo_4$

solution was added. A violet color indicates presence of peptide linkage molecule.

Test for Quinones: For quinines identification, 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinines.

Test for Terpenoids: 5 ml of extract was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish-brown coloration was formed for the presence of terpenoids.

RESULTS AND DISCUSSION

In this study, preliminary phytochemical screening was conducted on extracts from six algae species: *Caulerpa taxifolia*, *Caulerpa scalpelliformis, Halymenia venusta,* and *Tricleocarpa fragilis*, as well as two additional species, *Spatoglossum asperum* and *Scytosiphon lomentaria*. The screening involved testing for the presence of 10 different chemical compounds including alkaloids, phenols, flavonoids, tannins, saponins, coumarins, carbohydrates, proteins, quinones, and terpenoids using chloroform, methanol, and acetone as solvents. The compositions of these compounds varied considerably among the different algae species. The results of the phytochemical evaluations for each extract are presented in Tables 1, 2, and 3.

Among the three solvents used, the chloroform extract of Caulerpa taxifolia exhibited the presence of the highest number of compounds (eight). The methanol extract showed seven compounds, while the acetone extract contained only four compounds. For *Caulerpa scalpelliformis*, the acetone extract revealed the presence of seven compounds, followed by six compounds in the methanol extract, and four compounds in the chloroform extract. In the methanol extract of *Halymenia venusta*, seven compounds were detected, followed by four compounds in the chloroform extract, and five compounds in the acetone extract. *Tricleocarpa fragilis* showed the presence of six components in the chloroform extract, eight components in the acetone extract, and five components in the methanol extract. In the chloroform extract of *Spatoglossum asperum*, eight compounds were present, followed by five compounds in the methanol extract, and six compounds in the acetone extract. *Scytosiphon lomentaria* exhibited the presence of four components in the chloroform extract, eight components in the acetone extract, and six components in the methanol extract.

According to the results, different solvents yield different results for each algal species. Chloroform solvent shows the highest levels of phytochemicals in *Caulerpa taxifolia* and *Spatoglossum asperum,* whereas *Halymenia venusta* shows maximum phytochemicals in methanol solvent. Acetone solvent provides the best results for *Caulerpa scalpelliformis*, *Tricleocarpa fragilis*, and *Scytosiphon lomentaria*.

The medicinal seaweeds are abundant in various secondary metabolites such as alkaloids,

glycosides, flavonoids, saponins, tannins, steroids, and related bioactive compounds (Indra et al., 2016). These substances hold significant medicinal value and have found extensive application in the drug and pharmaceutical industries (Eluvakkal et al., 2010, Chovatia and Bhuva, 2022). Our current study findings align closely with this research observations. Several drugs containing tannins are utilized in medicine for their astringent properties. Tannins are utilized medicinally for their healing properties in conditions such as inflammation, leucorrhoea, gonorrhoea, burns, piles, and as antidotes (Kolodziej and Kiderlen, 2005). Alkaloids display a diverse range of pharmacological properties, encompassing anticancer, cholinomimetic vasodilatory, antiarrhythmic, analgesic, antibacterial, and antihyperglycemic activities (Kasim et al., 2010). Flavonoids have demonstrated anti-inflammatory, anti-cancer, and antioxidant properties. Phenols have been linked to various potential health benefits for humans (Jeeva et al., 2012). Quinones regulates DNA and RNA replication and mitochondrial oxidative pathways. They also generate peroxide, superoxide, and hydroxyl radicals within cells through cytotoxic activity (Solanki et al., 2008).

Table: 1 Phytochemicals Screening of Algae using Chloroform solvent

Sr. no.	Phytochemicals	Algae					
		Caulerpa taxifolia	***Caulerpa scalpelliformis***	***Halymenia venusta***	***Tricleocarpa fragilis***	***Spatoglossum asperum***	***Scytosiphon lomentaria***
1	Alkaloids	+	+	+	+	+	+
2	Phenols	+	–	–	–	+	+
3	Flavonoids	+	–	–	+	+	–
4	Tannins	+	+	–	+	+	+
5	Saponins	+	–	–	–	+	–
6	Coumarins	–	–	–	–	–	–
7	Carbohydrates	+	+	+	+	+	+
8	Proteins	+	–	–	+	+	–
9	Quinines	–	–	+	–	–	–
10	Terpenoids	+	+	+	+	+	–

***Abbreviation: – = Absent, + = Present**

Table: 2 Phytochemicals Screening of Algae using Methanol solvent

Sr. no.	Phytochemicals	Algae					
		Caulerpa taxifolia	***Caulerpa scalpelliformis***	***Halymenia venusta***	***Tricleocarpa fragilis***	***Spatoglossum asperum***	***Scytosiphon lomentaria***
1	Alkaloids	+	+	–	+	+	+
2	Phenols	+	+	+	+	+	+
3	Flavonoids	+	+	+	–	+	–
4	Tannins	+	+	+	+	+	+
5	Saponins	+	–	–	–	–	+
6	Coumarins	–	–	–	–	–	–
7	Carbohydrates	–	+	+	+	–	+
8	Proteins	+	–	+	–	–	–
9	Quinines	–	–	+	+	–	–
10	Terpenoids	+	+	+	–	+	+

***Abbreviation: – = Absent, + = Present**

Table: 3 Phytochemicals Screening of Algae using Acetone solvent

Sr. no.	Phytochemicals	Algae					
		Caulerpa taxifolia	***Caulerpa scalpelliformis***	***Halymenia venusta***	***Tricleocarpa fragilis***	***Spatoglossum asperum***	***Scytosiphon lomentaria***
1	Alkaloids	+	+	+	+	+	+
2	Phenols	+	+	–	+	+	+
3	Flavonoids	+	+	+	+	–	+
4	Tannins	–	+	+	+	+	+
5	Saponins	–	–	–	–	–	–
6	Coumarins	–	–	–	–	–	–
7	Carbohydrates	–	+	+	+	+	+
8	Proteins	+	+	+	+	+	+
9	Quinines	–	–	–	+	–	+
10	Terpenoids	–	+	–	+	+	+

***Abbreviation: – = Absent, + = Present**

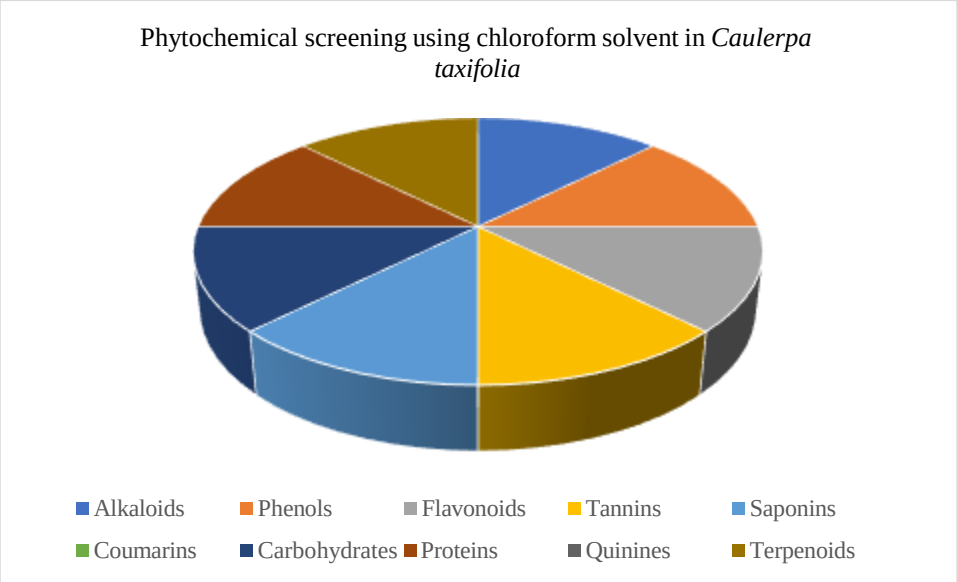

Figure 1 showing analysis of phytochemicals using chloroform solvent in *Caulerpa taxifolia*

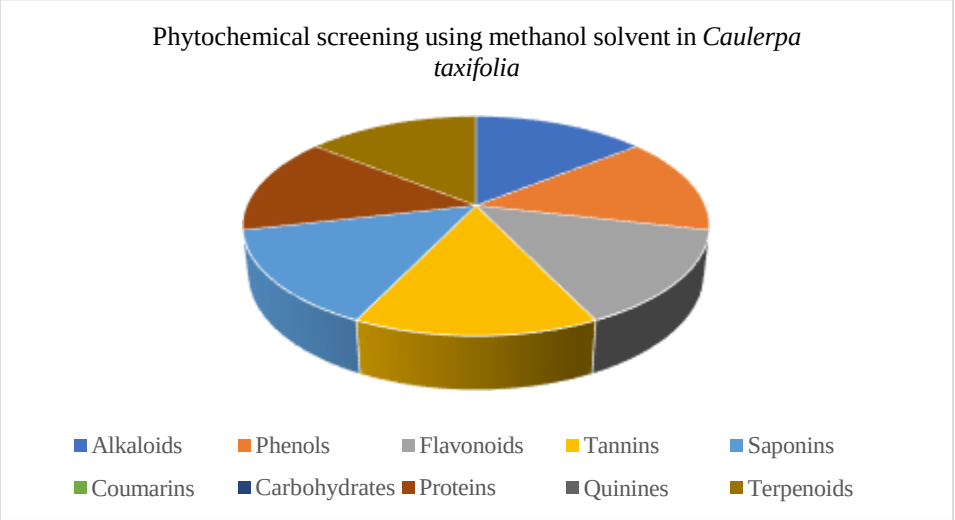

Figure 2 showing analysis of phytochemicals using methanol solvent in *Caulerpa taxifolia*

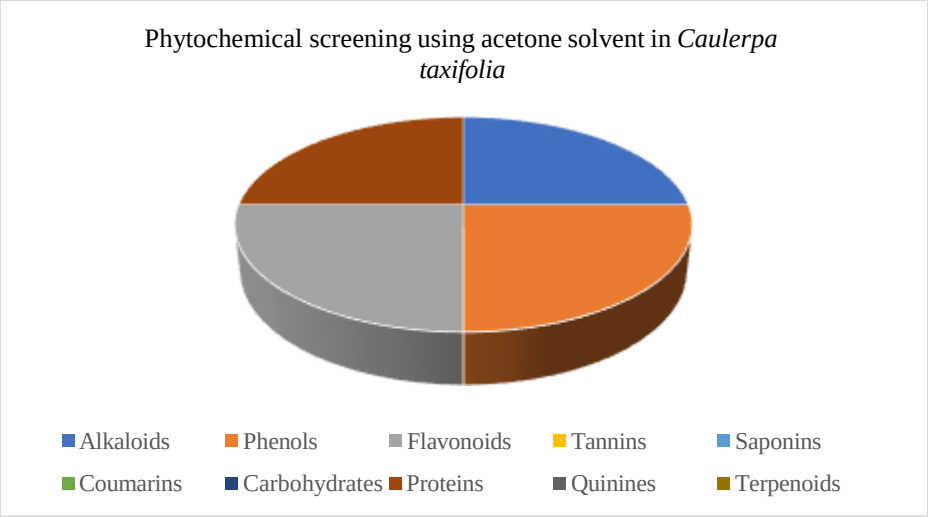

Figure 3 showing analysis of phytochemicals using acetone solvent in *Caulerpa taxifolia*

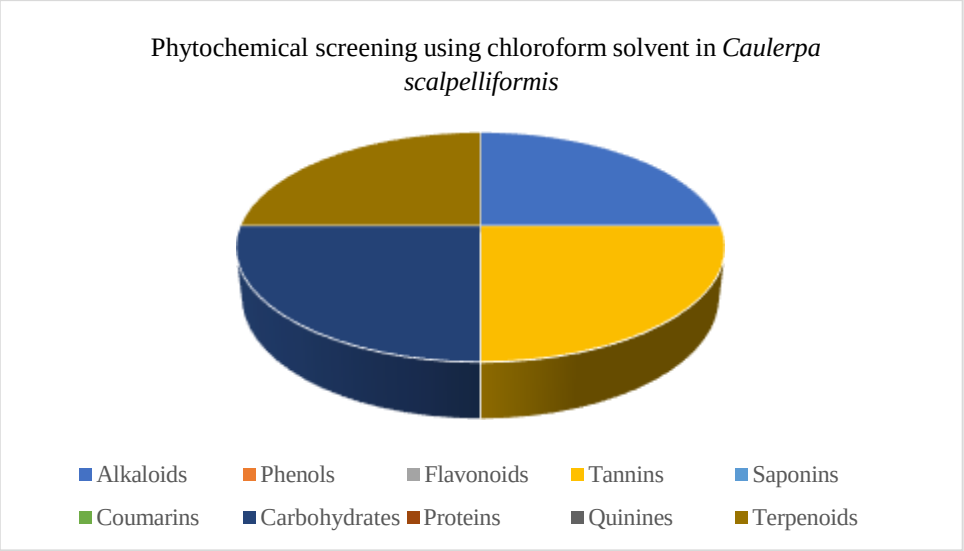

Figure 4 showing analysis of phytochemicals using chloroform solvent in *Caulerpa scalpelliformis*

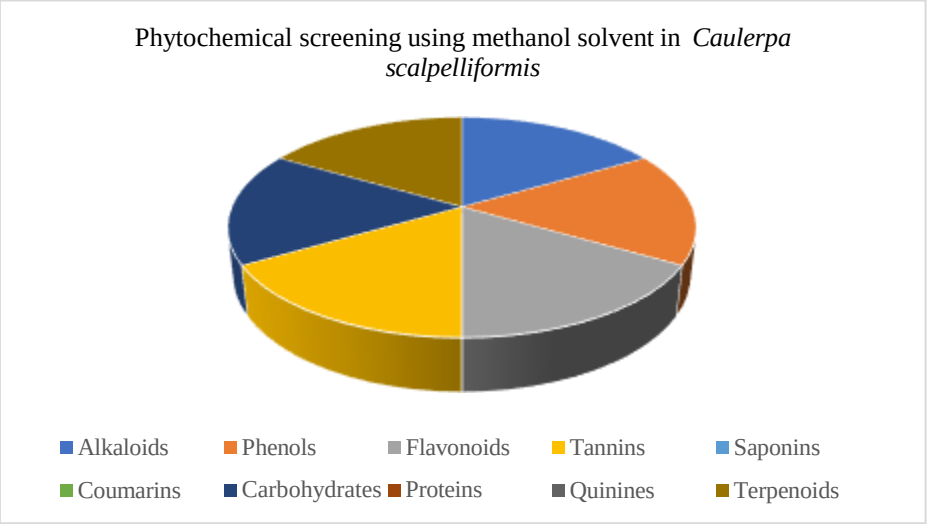

Figure 5 showing analysis of phytochemicals using methanol solvent in *Caulerpa scalpelliformis*

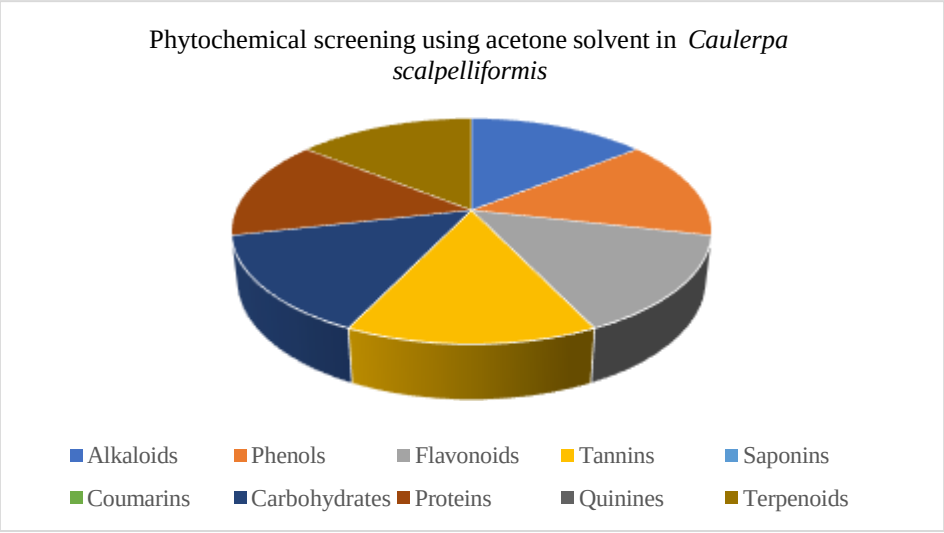

Figure 6 showing analysis of phytochemicals using acetone solvent in *Caulerpa scalpelliformis*

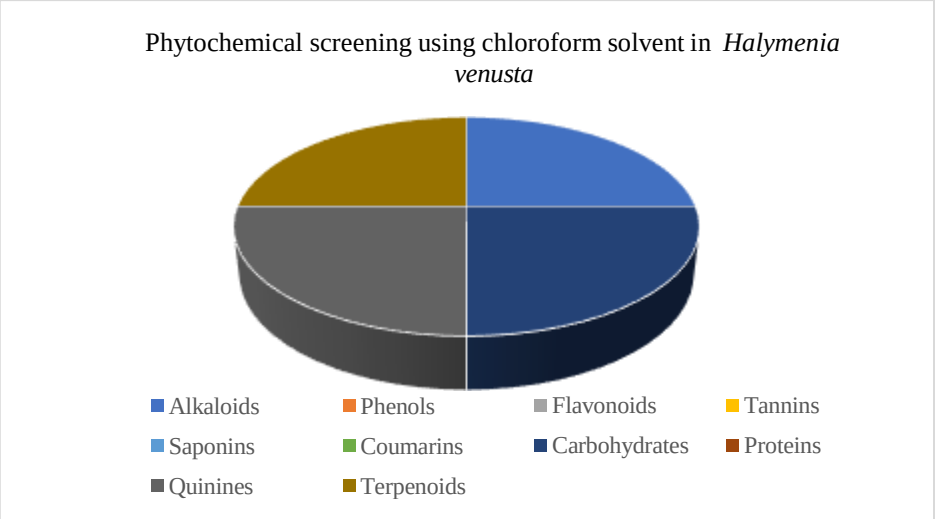

Figure 7 showing analysis of phytochemicals using chloroform solvent in *Halymenia venusta*

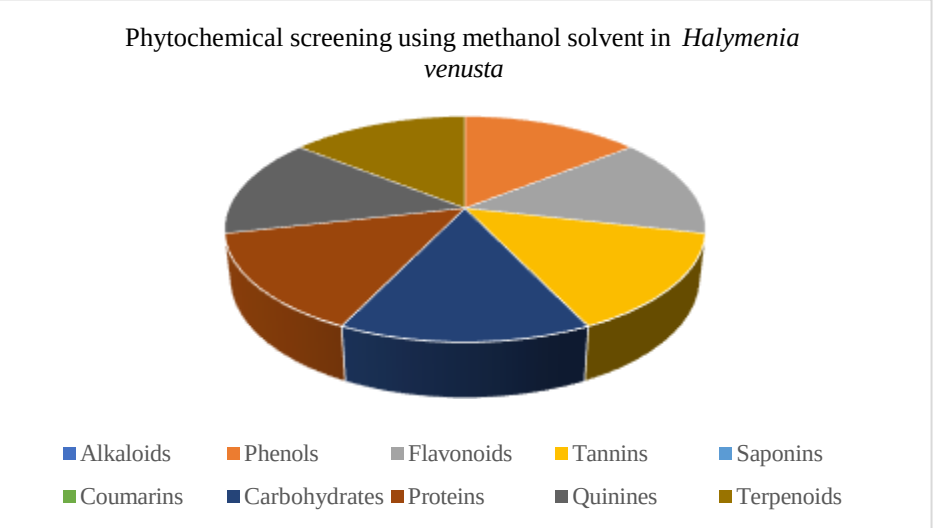

Figure 8 showing analysis of phytochemicals using methanol solvent in *Halymenia venusta*

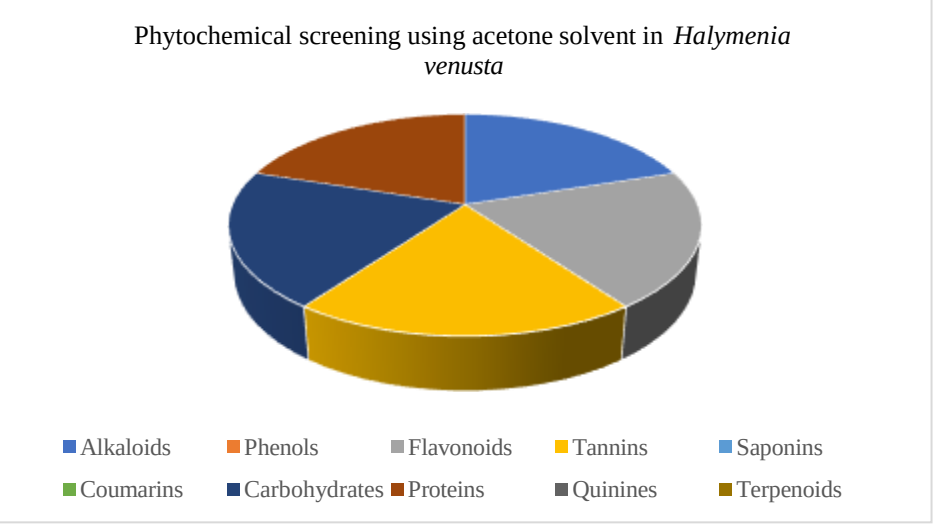

Figure 9 showing analysis of phytochemicals using acetone solvent in *Halymenia venusta*

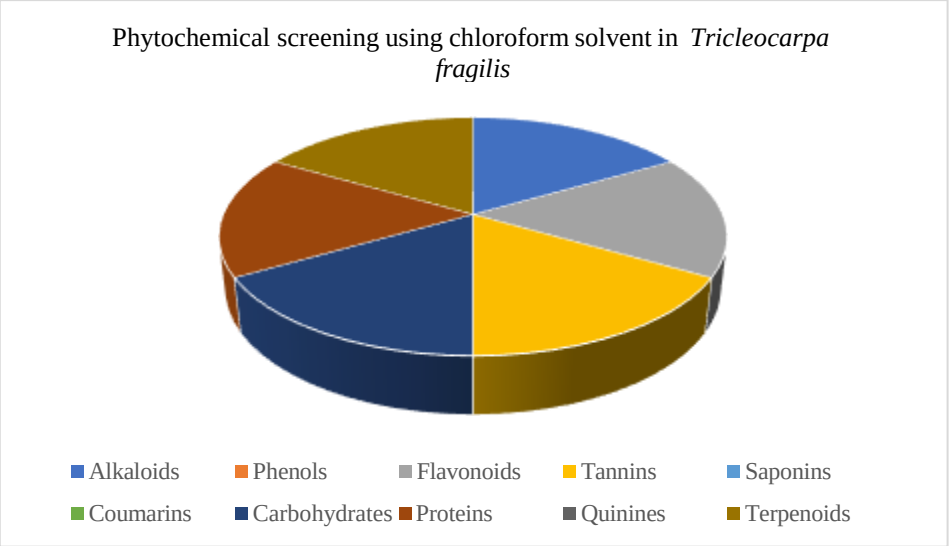

Figure 10 showing analysis of phytochemicals using chloroform solvent in *Tricleocarpa fragilis*

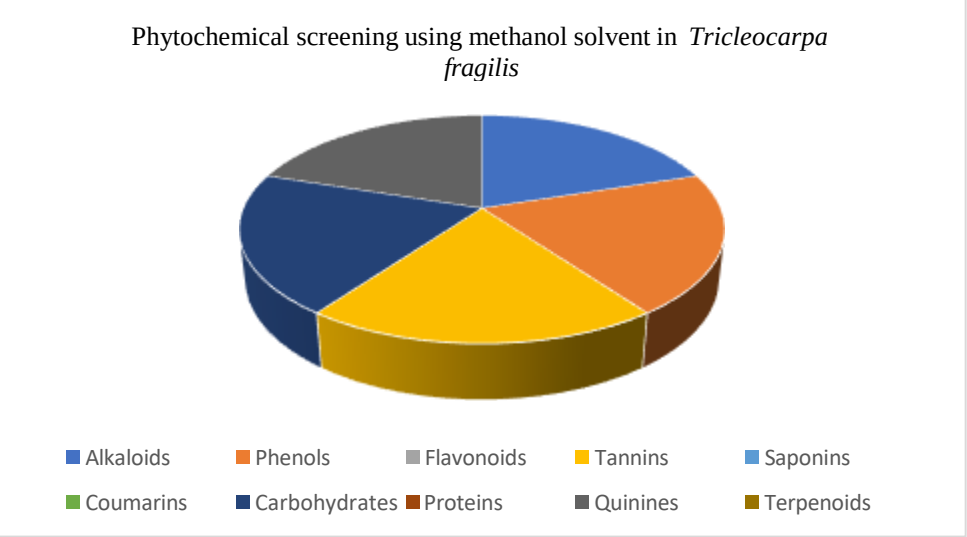

Figure 11 showing analysis of phytochemicals using methanol solvent in *Tricleocarpa fragilis*

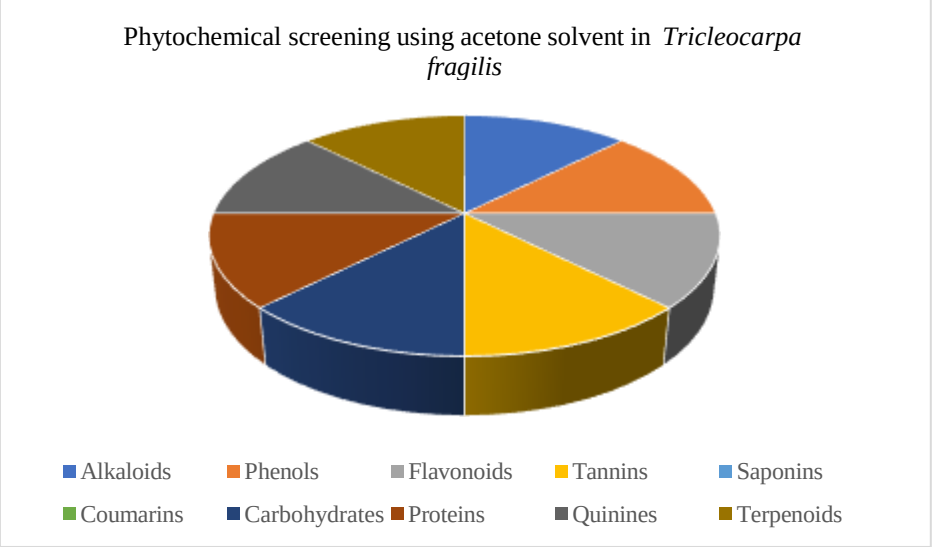

Figure 12 showing analysis of phytochemicals using acetone solvent in *Tricleocarpa fragilis*

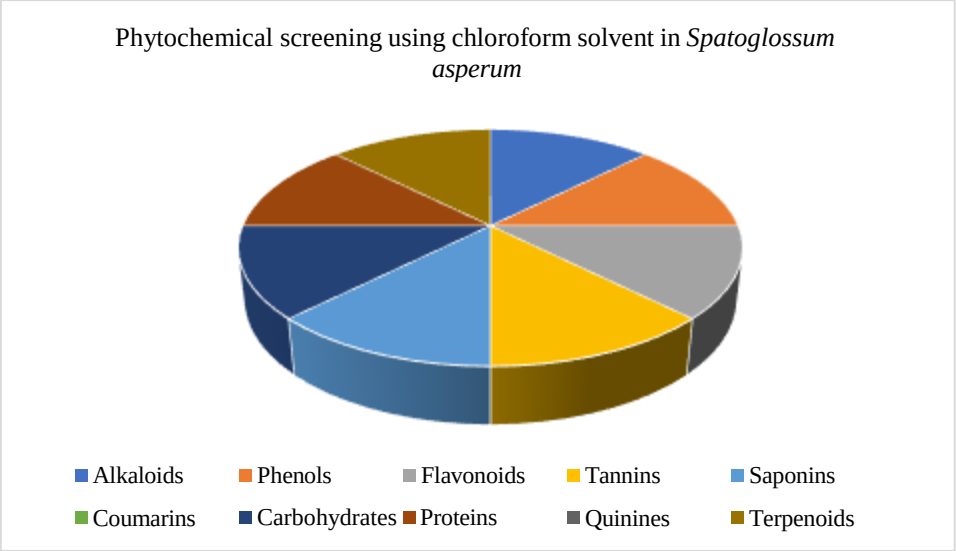

Figure 13 showing analysis of phytochemicals using chloroform solvent in *Spatoglossum asperum*

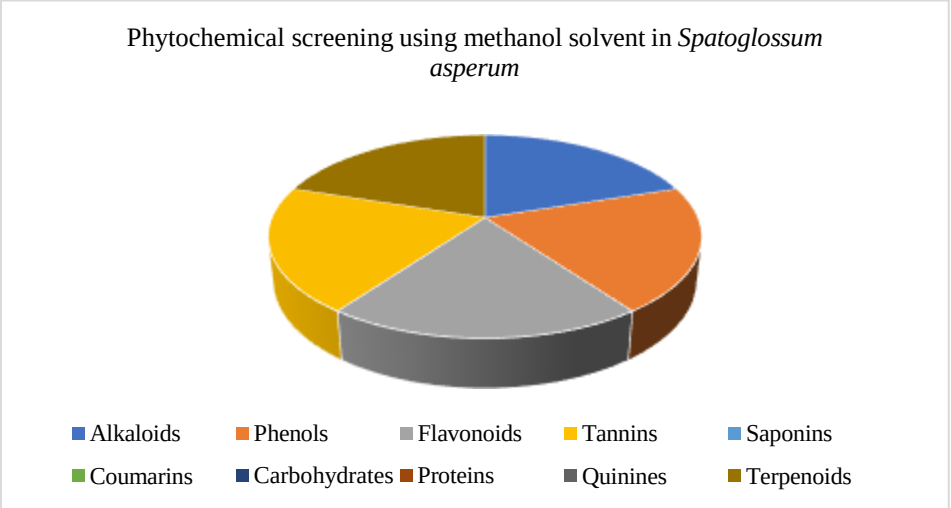

Figure 14 showing analysis of phytochemicals using methanol solvent in *Spatoglossum asperum*

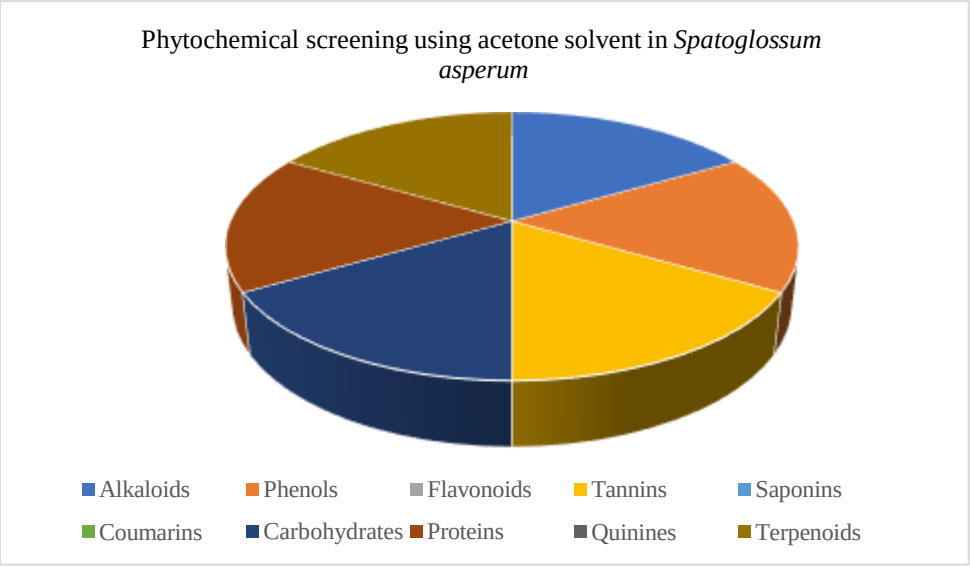

Figure 15 showing analysis of phytochemicals using acetone solvent in *Spatoglossum asperum*

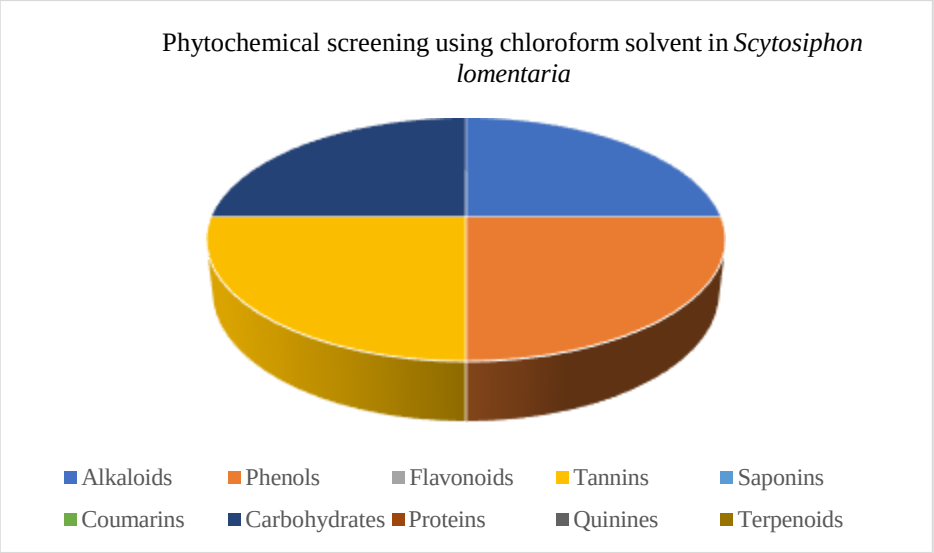

Figure 16 showing analysis of phytochemicals using chloroform solvent in *Scytosiphon lomentaria*

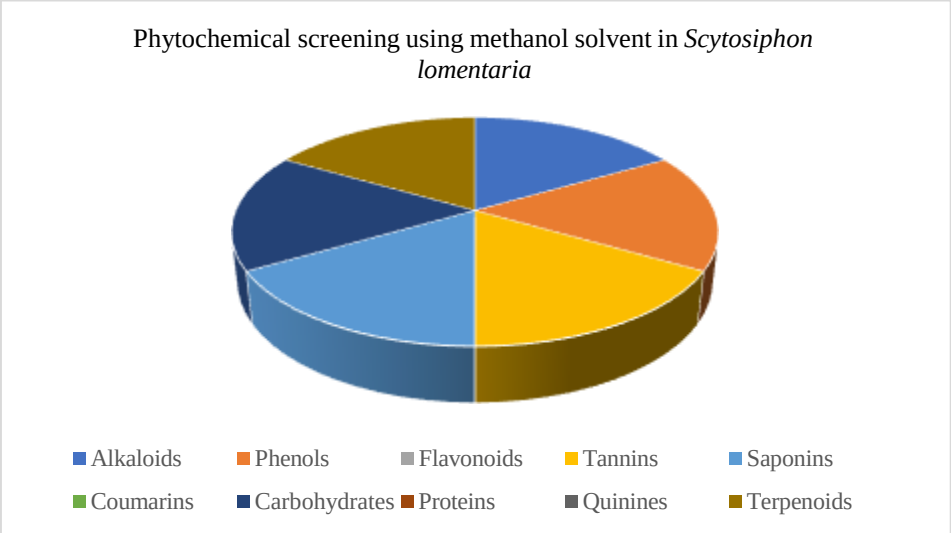

Figure 17 showing analysis of phytochemicals using methanol solvent in *Scytosiphon lomentaria*

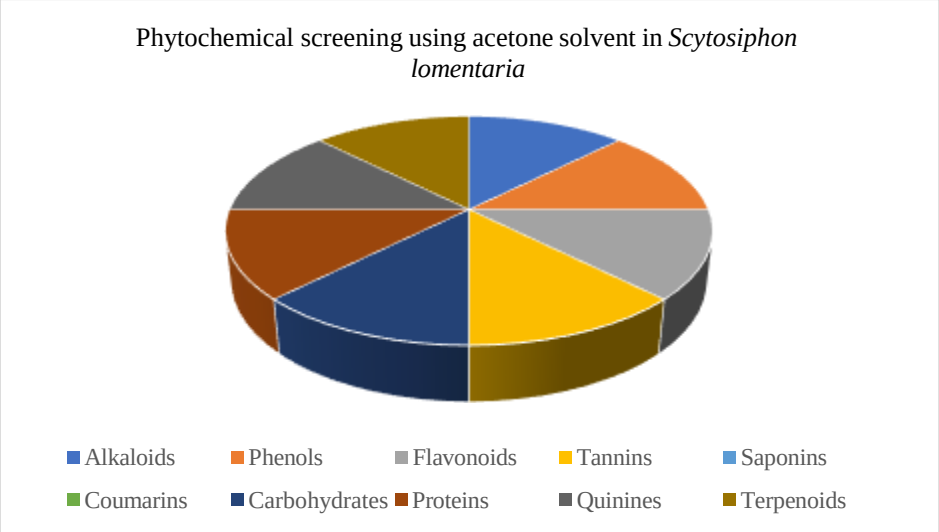

Figure 18 showing analysis of phytochemicals using acetone solvent in *Scytosiphon lomentaria*

PLATES

Plate 1: ***Caulerpa taxifolia***

Plate 2: ***Caulerpa scalpelliformis***

Plate 3: ***Halymenia venusta***

Plate 4: ***Tricleocarpa fragilis***

Plate 5: ***Spatoglossum asperum***

Plate 6: ***Scytosiphon lomentaria***

CONCLUSION

This study aimed to conduct preliminary phytochemical screening of various extracts from six different seaweeds, revealing the presence of alkaloids, flavonoids, phenolic compounds, tannins, quinones, coumarins, carbohydrates, and proteins in the extracts. The screenings indicated the presence of active compounds with therapeutic effects. Consequently, these seaweeds could be harvested and effectively utilized in product development for human benefit.

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