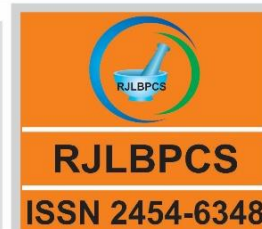


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Original Research Article

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**BIOLOGICAL APPROACHES OF *HALODULE PINIFOLIA* (MIKI)
HARTOG ON THE GROWTH AND DEVELOPMENT OF
BENINCASA HISPIDA (THUNB.) COGN.**

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ABSTRACT: In the present investigation the analysis of phytochemical constituents from *Halodule pinifolia* were determined. The seaweed *Halodule pinifolia* were collected from Mandapam coast of Rameshwaram, Tamilnadu, India and the standard methods were followed for extraction of phytochemicals from the seaweed. The secondary metabolites synthesized from seaweeds and the broad spectrum of biological activity. The analysis of proximate content of *Halodule pinifolia* with methanol and hexane solvents was individually determined. Among the two extraction solvents, the methanol with *Halodule pinifolia* showed excellent results when compared with hexane solvent. The proximate contents like moisture (16.14mg/g), Ash (3.35mg/g), insoluble ash (3.14mg/g), fat (3.60mg/g), crude fiber (3.37mg/g) and N₂free extractives (3.43mg/g) recorded from methanol extract of *Halodule pinifolia* and hexane extract showed moisture (8.03mg/g) Ash (6.73mg/g), insoluble ash (3.92mg/g), fat (4.02mg/g), crude fiber (4.12mg/g) and N-free extractives (2.23mg/g) recorded in the seaweed whereas qualitatively and quantitatively phytochemicals like alkaloids, saponins, flavonoids, tannin, phenol, steroid, terpenoids, protein, anthroquinone and reducing sugar were analysed in both solvents of methanol and hexane of *Halodule pinifolia* extracts. The effect of *Halodule pinifolia* seaweed organic fertilizer with different concentration like 200 to 800mg/g treated in each pot experiments of *Benincasa hispida*. The maximum concentration 800mg/g seaweed liquid showed excellent performance for the growth and development of *Benincasa hispida*.

KEYWORDS: *Halodula pinifolia*, *Benincasa hispida*, Phytochemiclas, Organic farming.

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1. INTRODUCTION

Seagrass is an angiosperm that lives in marine or brackish environment. It represents a unique flora adapted to rigorous salinity, immersion, occasional desiccation, anchorage on the seabed and hydrophilic pollination. Habitats of seagrasses are known to be highly productive and play an important ecological role as nursery grounds for fish and crustaceans such as shrimps, as food source and shelter for many organisms and in recycling of nutrients [1]. The seaweeds are scientifically recognized as macro algae literally meaning large algae. Algae are relatively simple photosynthetic plants with unicellular reproductive structure from non vascular filamentous plants. Seaweeds contain macro, micro nutrients and other nutrients including minerals to promote soil fertility for sustainable agricultural practice. These nutrients are depending upon the phytochemical constituents and developing all the facility of soil system. The seaweed of the *Halodule pinifolia* can make it good source of physiological, biochemical and enzymatic processes through nitrogen assimilation and mineralization activity. The chemical fertilizer application with crops is a necessary addition for good yield of crops to inherent low fertility status of the soils. The development of strategy of seaweeds as organic fertilizer in the present century must be thought increased productivity of the land under cultivation, reduced costs of production and higher input use efficiency with the soil. Biofertilizers offer an economically attractive and ecologically sound means for reducing the requirement of the chemical fertilizers. Biofertilizers are helpful as bio-control agents and they prevent many plant pathogens. So, there is a need to find out alternative to bulky green marine and organic fertilizers. Thus seaweeds of biofertilizers can serve as an alternative of bulky organic matter and NPK fertilizers up to certain extent. Janarthanan and Senthilkumar [2] studied the qualitative and quantitative analysis of phytochemical of selected seaweeds *Acanthopora spicifera* and *Sargassum nidhtic* with five different solvents for extraction. The estimation of phytochemicals like alkaloids, terpenoids, steroids, tannin, saponins, flavonoids, polyphenols and glycosides were tested with different solvents like petroleum ether, methanol, acetone and water for both the seaweeds.

2. MATERIALS AND METHODS

Collection of sample

The fresh and dried seaweed materials of *Halodule pinifolia* were collected from the Mandapam coast of Rameshwaram, Ramnadu district, Tamil Nadu.

Preparation of plant powder

The collected sea weed of *Halodule pinifolia* samples were air-dried and powdered. Exposure of direct sunlight avoided to prevent the loss of active components. These powdered materials were used for further analysis.

Analysis of proximate content

The proximate content of ash, moisture, insoluble ash, crude fiber, fat and N-free extractives

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Preparation of leaf extract

Fresh leaves of *Halodule pinifolia* were washed with sterilized water then crushed with Pestle and Mortar by Giriya [4].

Qualitative and quantitative phytochemical analysis

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Brain and Turner [5], Harborne [6]. Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration reaction to identify the major natural chemical groups such as alkaloids, flavonoids, tannins, reducing sugars, amino acids and saponin. General reactions in these analyses revealed the presence or absence of these compounds in crude extract were tested.

Pot culture experiments

The effect of *Halodule pinifolia* seaweed powder formulated in 200, 300, 400, 500, 600, 700 and 800mg/g treated in each pot experiments of *Benincasa hispida* and after the 30th days, the plant growth and development were measured and tabulated [7].

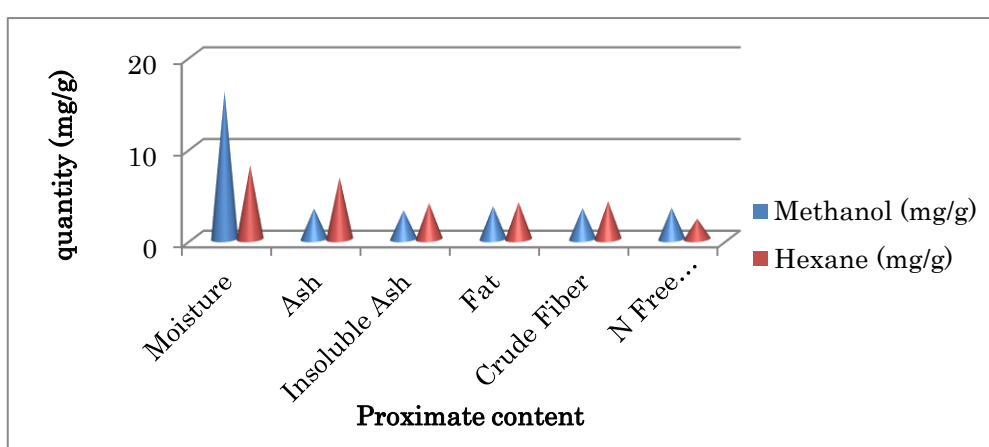
3. RESULTS AND DISCUSSION

In the current investigation stated that the proximate content has showed excellent quantity from methanolic extract of seaweed than the hexane extract of *H. pinifolia*. The maximum qualitative phytochemicals of *H. pinifolia* with methanolic extract were analysed than the other extract. The quantitative phytochemicals like alkaloids, anthroquinone, flavonoids, phenols, proteins, reducing sugar, saponin, steroids, terpenoids, tannin and triterpenoids was 0.69, 0.87, 0.58, 0.18, 3.67, 0.63, 0.71, 0.18, 0.73, 0.23 and 0.73 mg/g recorded respectively (Table-1, Fig-1). Earlier study by Anwariyah [8] reported flavonoids have been found in seagrasses *C. rotundata*. Further screening of seagrass of *H. uninervis* showed the result of assayed of saponin compounds from seagrass of *H. uninervis* have not been found, due to the extracts not form foam. Saponin compounds also have been found in methanol extract of *H. uninervis* seagrasses. Saponin is a strong active compounds and cause foam when homogenized with water. Saponins are glycosides triterpena and sterols that have been detected in more than 90 tribes plants [9,10]. This supports the earlier reports like Ergeneet al., [11] who revealed the presence of tannins, saponins, proteins, resins, reducing sugar, acidic compounds, alkaloids, cardiac glycosides and terpenoids in the phytochemical analysis of *C. rotundata*. The phytochemical compounds viz., glycoside, saponins, tannins, flavonoids, terpenoides and alkaloids have antimicrobial activity [12]. The preliminary phytochemical studies of the active fraction of root extracts of *C. serrulata* had variety of phytochemical constituents, namely alkaloids, carboxylic acid, coumarins, flavonoids, phenols, saponins, xanthoprotein, protein, steroids, tannins and sugar [13, 14]. The seaweed extract of *H. pinifolia* using hexane as solvents

showed the presence of alkaloids, anthroquinone, flavonoids, phenol, protein, reducing sugar, saponin, steroids, terpenoids, tannin and triterpenoids was 0.71, 0.76, 0.57, 0.47, 4.37, 0.46, 0.59, 0.85, 0.75, 0.86 and 0.86 mg/g recorded respectively (Table 2 and 3). The qualitative phytochemical analysis showed most of the phytoconstituents with methanol extraction compared to acetone extract. The presence of sugar somehow facilitated the growth of microorganisms and hence antagonizing the antibacterial activity of active compounds of the extracts [15]. The seaweed *Thalassia testudinum* inhibits the growth of *S. aggregatum* due to the presence of flavones and glycosides [16]. Seaweeds are large plants growing in the sea, especially various marine algae like the rockweeds, kelps, sea lettuce and pulses. Dried or fresh seaweeds and liquid extracts have been increasingly employed by all plants as fertilizers. Seaweeds extracts, are now commercially available in the market. The effect of seaweed extract is used as a foliar spray, application to soil and for soaking of seeds before sowing. It enhanced the rate of germination of seeds, increases uptake of plant nutrients and give resistance to frost and fungal diseases. Seaweed extract is effective for ripening of fruits, increasing shelf life of the product, improves the quality of produce and serves as an excellent soil conditioner [17]. Ashwiniet *al.*, [18] reported the studies on pharmacognostical and biochemical constituents of selected seaweeds and their effects of liquid fertilizers on growth of crop plants. The effect of seaweeds of liquid fertilizer (SLF) of three seaweeds was tested at different concentrations on the growth and developments of different crop plants were treated. The forty percentage of seaweed liquid fertilizer enhances the growth and yield parameters when compared with other concentrations. Bokil *et al.*, [19] studied that the seaweed extracts are bioactive at low concentration (diluted as 1:1000 or more). Liquid extracts obtained from seaweeds are successfully used as foliar sprays for several crops by Crouch and Staden [20]. Sivasankari, *et al.*, [21] observed that the growth enhancing potential of seaweeds might be attributed to the presence of macro and micronutrients were reported. Temple [22] reported the increase in the harvest of bean by seaweed extract by foliar application with high yield was increased by 25% observed respectively. The growth and development was determined by Principal Component Analysis (PCA) of morphometric characters that have relevance to other characters. Based on the analysis of PCA program, obtained a major component that is able to retain most of the information which was measured using the total diversity by using a bit of the main components only [23]. Effect of *H. pinifolia* at higher concentration of 800mg/g per pot treated showed maximum morphometric measurement observed and tabulated (Table 4). Many studies have shown changes in germination and growth of target seedlings, but few have evidenced the physiology and method of action of allelochemicals [24,25]. Delays in seed germination of any species can have important biological implications, because this will affect the establishment of seedlings in natural conditions [26,27) and their chances of competing for resources with neighboring species [28].

Table 1: Proximate analysis of *Halodulepinifolia*

Proximate content	Methanol (mg/g)	Hexane (mg/g)
Moisture	16.14	8.03
Ash	3.35	6.73
Insoluble Ash	3.14	3.92
Fat	3.60	4.02
Crude Fiber	3.37	4.12
N Free Extractives (NFE)	3.43	2.23

**Fig 1: Proximate analysis of *Halodulepinifolia*****Table 2: Qualitative phytochemical analysis of *Halodulapinifolia***

Name of the phytochemicals	Methanol	Hexane
Alkaloid	++	+
Anthroquinone	+	+
Flavonoids	+	++
Phenol	+	+
Protein	++	+
Reducing sugar	++	+
Saponin	+	++
Steroid	+	+
Tannin	++	++
Terpenoids	+	+
Triterpenoids	+	+

++ (Strongly present), + (present), - (absent)

Table 3: Quantitative phytochemical analysis of *Halodulapinifolia*

Name of the phytochemicals	Quantity (mg/g)	
	Methanol	Hexane
Alkaloids	0.69	0.71
Anthroquinone	0.87	0.76
Flavonoids	0.58	0.57
Phenol	0.18	0.47
Protein	1.67	1.32
Reducing sugar	0.63	0.46
Saponin	0.71	0.59
Steroids	0.18	0.85
Terpenoids	0.73	0.75
Tannin	0.23	0.86
Triterpenoids	0.73	0.86

Table 4: The effect of seaweed *H.pinifolia* on the morphometric analysis of *Benincasa hispida*

S.No	Growth parameters	Growth measurement (30days)							
		C	200 mg/g	300 mg/g	400 mg/g	500 mg/g	600 mg/g	700 mg/g	800 mg/g
1	Shoot length (cm)	3.4	4.0	5.6	6.7	9.3	14.3	15.0	18.3
2	Root length (cm)	4.3	4.5	5.3	7.5	8.7	13.5	11.7	12.3
3	Plant height (cm)	7.3	8.5	8.7	8.8	6.3	12.3	27.0	30.4
4	Leaf length (cm)	3.7	4.6	5.3	6.4	8.3	13.2	12.0	12.3
5	Leaf width (cm)	3.2	3.8	4.3	5.5	6.3	12.3	15.0	9.3
6	Leaf area (cm)	2.6	3.7	4.0	5.0	5.9	6.7	7.6	8.3
7	No.of leaves	6	8	8.0	9	10	13	7.3	13
8	Shoot girth (cm)	0.1	0.4	1.3	1.7	2.6	4.5	5	7
9	No.of branches	-	-	-	-	-	-	-	-
10	No.of hairy roots	12	13	13	13	13	13	13	15

C-control, 200 to 800mg/g different concentration of *H.pinifolia* extract

4. CONCLUSION

The effect of *H.pinifolia* on the growth and development has increased on the basis of quantity of seaweed fertilizer in the pot experiments. The seaweed fertilizer has maximum effect for the plant growth regulator because it contains more proximate content, phytochemicals and growth hormone for development of *Benincasa hispida*. The organic fertilizer can sustain plant growth under modern farming. However, it may be suggested that developing countries showed their attention on the use

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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