

Phytochemical analysis, antibacterial and anti-diabetic activity of three different seaweed extracts

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Abstract

Aim: Marine seaweed contains potential phytochemical compounds that could be able to cure different health issues in humans naturally. Hence the research was aimed to investigate the antibacterial and *in vitro* anti-diabetic activity of three different seaweed extracts.

Methods: Phytochemical compounds like phenol, flavonoids, tannins and alkaloids were quantitatively identified. Antibacterial activity of the seaweed extracts were determined against five different test bacteria. *In vitro* anti-diabetic activity of the extracts were investigated based on its inhibition effect on the alpha-amylase and alpha-glucosidase activity.

Findings: Phytochemical analysis showed more phenolic and flavonoid content in green and brown seaweed extracts and lesser content in red seaweed extract. Antibacterial activity of green and brown seaweed extracts showed good inhibitory zones against all test bacteria ranging from 22.9 ± 0.57 mm to 24.9 ± 0.57 mm (green seaweed extract) and 17.9 ± 0.57 mm to 21.6 ± 0.75 mm (brown seaweed extract) respectively. *In vitro* anti-diabetic activity of seaweed on the inhibition of enzymes showed promising results. Among the three types, green seaweed extracts showed maximum inhibition of α -Amylase and α -glucosidase activity of 86.6 ± 0.76 % and 82.9 ± 0.57 % respectively.

Conclusion: Findings showed that the phytochemicals present in the methanolic extract of seaweeds attribute positively for different pharmacological properties like antibacterial, anti-oxidant and anti-diabetic activity. Optimizing the production factors would be able to commercialize the seaweeds in drug forms and could be used for the treatment of various diseases in humans.

Keywords: Antibacterial activity; Anti-diabetic activity; α -Amylase; α -Glucosidase; Phytochemicals

1. Introduction

Natural products and their derivatives represent more than 50% of all the drugs in clinical use of the world are known to possess antioxidant potential. Thus, potential antioxidant and anti-diabetic properties of plant extracts or isolated products of plant origin can possibly be explored for developing the anti-diabetic drugs [1]. Novel natural antioxidants in the diets of animals and humans, such as seaweeds, are the current research needs. Seaweeds are marine, photosynthetic algae abounding in all oceans. Seaweeds have been suggested as a promising source of bioactive substance that might have pharmaceutical application [2]. Three main seaweed phyla are identified: Chlorophyta, Rhodophyta, and Phaeophyta [3]. Green, red, and brown algae have compounds with antioxidants [4], anti-inflammatory, anti-diabetic [5], and anticancer activities [6].

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Significant phytochemical compounds that attributes for antibacterial, antioxidant and anti-diabetic properties present in these types of different seaweeds. Seaweeds are recognized as a gorgeous origin of natural and bioactive compounds such as polyphenols, vitamins, polysaccharides, peptides, and fatty acids with various functional properties and structures that supply the living organisms with various health benefits [7].

The pharmaceutical, medicinal and nutritional applications of the tested seaweeds were documented by assessing the cytotoxicity, antioxidant, anti-diabetic, anti-inflammatory, and anticancer effects of these seaweed extracts. Strong antioxidant activities were detected with seaweed extracts [8]. Bioactive compounds from marine brown algae are potential as an antitumor, antifungal, antiviral, antioxidant, antihypertensive and anti-diabetic [9]. The polyphenol content of marine algae has pharmacological effects as antioxidants, antibiotics, anti-inflammatory, hypo-allergenic, antibacterial, and antidiabetic [10].

Marine algae also have a high content of antioxidants and can be used to stave off free radicals that arise due to the condition of hyperglycemia in diabetic people. It's source of dietary fiber especially soluble fiber such as alginates which can influence satiety and glucose intake from food [11]. Nwosu et al., [2] stated that phenol extract of seaweed *Palmaria palmate* and *Ascophyllum nodosum* have potential as an anti-diabetic agent by inhibiting α -amylase and α -glucosidase activity.

Based on these properties of marine algae, following objectives were framed in the present research, i) to collect and extract significant phytochemical compounds from three different seaweeds, ii) to analyse the antibacterial and antioxidant activity of seaweed extracts and iii) to determine the *in vitro* anti-diabetic activity of seaweed extracts.

2. Materials and methods

2.1. Collection of three types of marine seaweed

The seaweeds (Green seaweed, Brown seaweed and Red seaweed) were collected fresh from Tuticorin (Lat 8 45' N; Long 78 10'E), South east coast of India. The seaweed samples were transported to the laboratory in sterile polythene bags immediately after collection for further analysis.

2.2. Phytochemical analysis of seaweeds

2.2.1. Total phenolic content

The total phenolic content was demonstrated according to the method of Taga et al. [12]. Folin–Ciocalteu reagents (100 μ L, 50% v/v) were added to sodium carbonate (2 mL, 2% w/v) then were mixed well with the extract of seaweeds (100 μ L). This was followed by incubation at room temperature for 30 min, and then the absorbance of the blue color solution was assessed at 750 nm against a blank (100 μ L solvent was used instead of algal extract). The standard curve was done using various concentrations of gallic acid. The total phenolic content was expressed as mg/g crude extract.

2.2.2. Total flavonoid content

For flavonoids estimation, an aluminum chloride colorimetric assay was performed [13]. Algal extract was mixed with ethanol (95%, 0.5 mL), aluminum chloride (0.1 mL, 10%), potassium acetate (0.1 mL, 1 M) and distilled-deionized water (2.8 mL). The mixture was incubated for 30 min at room temperature, and then the absorbance was detected at 415 nm. An amount of aluminum chloride (10%) was used with the same amount of distilled-deionized water for blank samples. The calibration curve was performed using quercetin as a standard flavonoid. The total content of flavonoid was described as mg/g crude extract.

2.2.3. Total tannin content

The total tannin content was assessed in accordance with the method described by European Community [14]. Two hundred microliters of the extract were added to 1 mL of distilled-deionized water, 200 μ L of ferric ammonium citrate (3.5 g/L) freshly prepared, and 200 μ L of ammoniac (20%). The absorbance was estimated at 525 nm after 10 min of the incubation period. For the blank sample, 200 μ L solvent was used rather than the algal extract. The standard curve was measured using tannic acid, and the results were expressed as mg/g crude extract.

2.2.4. Total alkaloid content

Total alkaloids were developed quantitatively according to Harbone, 1973 [5]. One gram of the algal extract was mixed with 70% ethanol and glacial acetic acid (4: 1). The reaction mixture was allowed to stand for 6 hours at least and then

was filtered. By a drop-wise adding of concentrated ammonia solution, alkaloids in the supernatant were precipitated. The precipitated alkaloids were filtered on a pre-weighed filter paper (Whatman No.1) and then dried in an oven at 70 °C to a constant weight. The content of alkaloids was measured and expressed as mg/g dry. wt. of the seaweed samples.

2.3. Antibacterial activity of seaweeds

To determine the antibacterial activity of seaweed extracts against the pyogenic organisms, modified Kirby-Bauer method was carried out. The antibacterial activity was evaluated against the five test organisms (O1 - *Escherichia coli*, O2 - *Staphylococcus aureus*, O3 - *Staphylococcus epidermidis*, O4 - *Klebsiella pneumoniae*, O5 - *Enterobacter* sp) by disc diffusion method. Sterile Nutrient Agar plates were prepared and allowed to solidify. About 0.1% inoculum suspensions of each of the bacterial cultures were swabbed with the sterile cotton swab three times by turning the plate at 60° angle between each streaking. Under sterile conditions, film size of 20 mm in diameter was cut and placed on the agar surface of each Nutrient Agar (NA) plates. Filter paper disc impregnated with standard antibiotic solution (Gentamicin – 4µg/ml) was also placed on the same plate to compare the efficacy of seaweed extracts. All the plates were incubated at 37°C for 24 – 48 h. The antibacterial activity was evaluated in terms of zone of inhibition around the wells of each extract in all the inoculated NA plates. The inhibition clear zones were measured and recorded in millimetre.

2.4. In vitro Anti-diabetic activity [16]

2.4.1. Inhibition of α -Amylase Activity

Inhibitory activity of α -amylase was decided by calculating changes in 3,5-dinitrosalicylate acid into nitro-aminosalicylate utilizing spectrophotometry. A volume of test solution was made from 25 mL of sample extract at differing concentrations and 25 mL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 13 U/mL-1 of α -amylase. The test solution was mixed using vortex mixer and incubated at 37 °C for 10 min. After that, 25 mL of soluble starch 1% in 0.02 M sodium phosphate buffer was added into the test solution and it was incubated at 37°C for 10 min. Then, it was further handled with the addition of 50 mL 96mM 3, 5-dinitrosalicylate (DNS) and incubated for 5 min in water bath. The solution was cooled at room temperature and the absorbance of the solution was recorded at a wavelength of 550 nm. Absorbance values of sample were obtained and used to calculate the percentage (%) inhibition of the enzyme.

$$\% \text{ Inhibition} = K - (S1 - S0) / K \times 100$$

Where,

K= Absorbance of control-blank

S1= Absorbance of sample with enzyme

S0= Absorbance of sample without enzyme

2.4.2. Inhibition of α -Glucosidase Activity

Test solution consists of 50 mL 0.1M phosphate buffer (KH₂PO₄) pH 7; about 25 mL substrate 0.5 mM p-nitrophenyl- α -D-glucopyranoside (PNP-G), 10 mL of sample at various concentrations and 25 mL of 0.2 U/mL-1 α -glucosidase. The test solution was combined and incubated at 37 °C for 30 min. The reaction was finished by the addition of 100 mL of 0.2 M Na₂CO₃. Inhibition of enzyme activity was analyzed by the amount of p-nitrophenyl formed by measurement of the absorbance utilizing a microplate reader at a wavelength of 405 nm. Absorbance values of sample were obtained and used to calculate the percentage (%) inhibition of the enzyme.

$$\% \text{ Inhibition} = K - (S1 - S0) / K \times 100$$

Where,

K= Absorbance of control-blank

S1= Absorbance of sample with enzyme

S0= Absorbance of sample without enzyme

3. Results and discussion

3.1. Phytochemical analysis of seaweeds

Phytochemical analysis of the seaweed extracts revealed that a considerable amount of phenolics, flavonoids, saponin and alkaloids were found significantly present (Table-1). Among the three seaweeds, green and brown seaweed extracts showed more phytochemical compounds than red seaweed extracts.

Table 1 Phytochemical analysis of seaweed extracts

S. No.	Seaweed extracts	Phytochemicals			
		Phenolics (mg gallic acid equivalents/g crude extract)	Flavanoids (mg quercetin equivalents/g crude extract)	Tannins (mg tannic acid equivalents/g crude extract)	Alkaloids (mg/g DW)
1	Green seaweed	124.6 ± 0.76	163.3 ± 1.05	21.9 ± 0.57	24.3 ± 1.05
2	Brown seaweed	118.3 ± 1.05	126.6 ± 0.76	17.3 ± 1.05	12.9 ± 0.57
3	Red seaweed	48.6 ± 0.76	53.6 ± 0.76	8.9 ± 0.57	4.6 ± 0.76

3.1.1. Total phenolic content

Maximum value of phenolics was recorded for the methanol extract of green seaweeds (124.6 ± 0.76 mg gallic acid equivalents/g crude extract). While the minimum value was shown with the methanol extract of red seaweed (48.6 ± 0.76 mg gallic acid equivalents/g crude extract) (Table-1). This was found supportive with the work done by Dang et al. [17]. Their results showed that the great total phenolic content levels were detected in the seaweed extracts of *Sargassum* sp and *Padina* sp (48.13 and 158.82 mg gallic acid equivalents/g extract) respectively. Oueslati et al., [18] highlighted that phenolics found in marine algae were recorded to show therapeutic and medicinal properties as anti-inflammatory and antimicrobial properties.

3.1.2. Total flavonoid content

Williams et al., [19] reported that flavonoids are attributed for potential antioxidant activity, scavenging wide range of ROS, inhibiting lipid peroxidation, and hence found to contain pharmacological activities against many diseases. In our present research maximum flavonoid contents were recorded for the methanol extract of green seaweed (163.3 ± 1.05 mg quercetin equivalents/g crude extract); followed by 126.6 ± 0.76 mg for brown seaweed (Table-1). However, the lowest value was detected with the methanol extract of red seaweed (53.6 ± 0.76 mg). The obtained results for brown and red seaweed extracts were found supportive with the work done by El-Shenody et al. [1]. During their research, brown algae extract (*Dictyota dichotoma*) showed maximum total flavonoid contents, and red algae extract (*Laurencia obtusa*) revealed lowest total flavonoid contents.

3.1.3. Total tannin content

Kolodziej and Kiderlen, [20] reported that therapeutically, tannins have been used as antiulcer, antibacterial, antiviral, and antioxidant agents; also used to treat inflammation and burns. In the present research, green seaweed and brown seaweed extracts showed good tannin content of 21.9 ± 0.57 mg and 17.3 ± 1.05 mg tannic acid equivalents/g crude extract respectively. Red seaweed extract showed only 8.9 ± 0.57 mg tannic acid equivalents/g crude extract (Table-1). This was found consistent with the work done by Fauzi et al., [21], who stated that brown algae, *Padina* sp contains a greater total tannin content than other seaweed extracts.

3.1.4. Total alkaloid content

Alkaloids are heterocyclic nitrogen compounds, which naturally exist in marine organisms, plants, animals, and microbes. (Guyen et al., [22]. The highest alkaloid content in the collected seaweeds was shown with the methanol extract of green seaweed and brown seaweeds (24.3 ± 1.05 mg and 12.9 ± 0.57 mg/g dwt.), respectively. And the methanol extract of red seaweed recorded the lowest alkaloid content of about 4.6 ± 0.76 mg/g dwt (Table-1). The obtained results were found contradictory with the result obtained for Kumbhar et al., [23]. During their study, alkaloids in red seaweeds were found remarkable and higher than brown seaweeds.

3.2. Antibacterial activity of seaweed extracts

Antibacterial activity of the seaweed extracts showed significant inhibitory zones against all test bacteria. During the analysis, following observations were found evident. Maximum inhibitory zones of 24.3 ± 1.05 mm was found evident for the seaweed extracts against *Escherichia coli*. Followed, inhibitory zones of 22.9 ± 0.57 mm was obtained for *Staphylococcus aureus*. *Staphylococcus epidermidis* exhibited inhibitory zones of 23.6 ± 0.75 mm, *Klebsiella pneumoniae* showed inhibitory zone of 24.3 ± 1.05 mm; and *Enterobacter* 4sp expressed 24.9 ± 0.57 mm of inhibitory zones. The obtained values were found significant to retard or prevent the growth of test bacteria using different seaweed extracts (Table-2; Fig. 1).

Table 2 Antibacterial activity of seaweed extracts

S. No	Test Bacteria	Zone of inhibition (mm)		
		Green seaweed extracts	Brown seaweed extracts	Red seaweed extracts
1	<i>Escherichia coli</i>	24.3 ± 1.05	21.6 ± 0.75	0
2	<i>Staphylococcus aureus</i>	22.9 ± 0.57	18.9 ± 0.57	0
3	<i>Staphylococcus epidermidis</i>	23.6 ± 0.75	19.6 ± 0.75	0
4	<i>Klebsiella pneumoniae</i>	24.3 ± 1.05	17.9 ± 0.57	0
5	<i>Enterobacter</i> sp	24.9 ± 0.57	18.6 ± 0.75	0

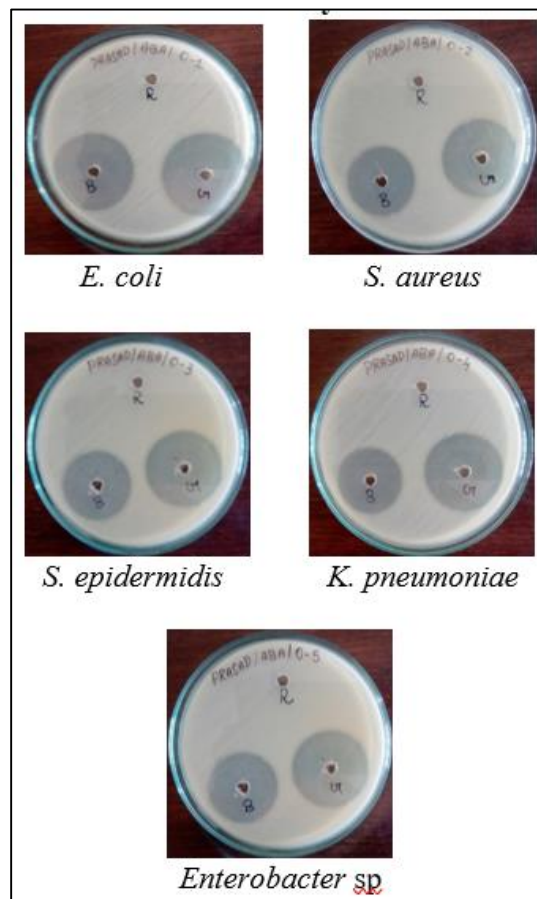


Figure 1 Antibacterial activity of seaweed extracts

3.3. *In vitro* Anti-diabetic activity

Alpha-amylase and alpha-glucosidase are enzymes that are closely associated with diabetes mellitus. Phenols are one of the bioactive components that can inhibit the action of α -amylase and α -glucosidase [24]. Polyphenols can inhibit the enzyme in the breakdown of carbohydrates into glucose. Based on the principle inhibitory effect of phenolic content in the seaweeds explained, similar enzyme inhibiting standard called acarbose was used in the present study. The principle of both phenolic content and acarbose inhibition is by generating delays and disaccharide carbohydrate hydrolysis and absorption of glucose and inhibiting the metabolism of sucrose into glucose and fructose [25]. Based on this principle, the inhibition of α -amylase and α -glucosidase by the seaweed extracts were measured and the values were expressed in percentage. In Table-3, the inhibitory effects of all three seaweeds on the enzymes were presented.

Table 3 *In vitro* Anti-diabetic activity

S. No.	Seaweed extracts	Enzyme Inhibition (%)	
		α -Amylase activity	α -Glucosidase activity
1	Green seaweed	86.6 \pm 0.76%	82.9 \pm 0.57%
2	Brown seaweed	81.9 \pm 0.57%	78.6 \pm 0.76%
3	Red seaweed	28.6 \pm 0.76%	21.9 \pm 0.57%
4	Acarbose (standard)	92.9 \pm 0.57%	89.3 \pm 1.05%

3.4. Inhibition of α -Amylase activity

The α -amylase inhibition by the seaweed extracts showed promising results in the present research. During the phytochemical analysis it was recorded that both green and brown seaweed extracts showed highest phenol content of about 124.6 \pm 0.76 mg and 118.3 \pm 1.05 mg gallic acid equivalents/g crude extract. Hence, these two seaweed extracts showed higher inhibitory activity of about 86.6 \pm 0.76 % and 81.9 \pm 0.57 % respectively. As the red seaweed showed 48.6 \pm 0.76mg gallic acid equivalents/g of phenol content during phytochemical analysis, the α -amylase inhibition percentage was recorded as 28.6 \pm 0.76 % (Table-3). Standard acarbose showed inhibitory percentage of 92.9 \pm 0.57 %.

According to Kunyanga et al., [26], phenolic compounds were able to bind to the active site of α -amylase. The content of phenol has an inhibitory effect on α -amylase through bond hydroxylation and ring substitution on β . Bioactive components group of phenolics such as anthocyanin, flavonoids, gallic acid, vanillic acid and, quercetin have been reported to have inhibitory activity against the activity of α -amylase.

3.5. Inhibition of α -Glucosidase activity

The α -glucosidase inhibition by both green and brown seaweed extracts also showed good inhibition percentage during the analysis. Green seaweed extracts inhibited the α -glucosidase activity upto 82.9 \pm 0.57 %; followed by brown seaweed also expressed good inhibitory effect on the enzyme of about 78.6 \pm 0.76 %. Comparatively red seaweed inhibited lesser α -glucosidase activity of about 21.9 \pm 0.57 % (Table-3). Standard acarbose showed inhibitory percentage of 89.3 \pm 1.05 %.

According to Zhang et al., [27], the ability of polyphenols to inhibit α -glucosidase in the digestive tract and activation of glucose uptake lowered blood glucose. Wilson et al., [28] reported that phytochemicals of seaweeds like, polyphenols, flavonoids, anthocyanin, and phenolic acids, significantly suppress the elevated blood glucose and reduce the rate of digestion of sucrose and glucose absorption in the intestine. Earlier in our study, the phytochemical analysis showed the higher content of these phytochemicals (phenols, flavonoids, tannins and alkaloids) and hence the enzyme inhibition by the seaweed extracts were found supportive to the statement of researchers.

4. Conclusion

Different types of seaweeds were reported to contain different types of phytochemicals that attributes for antibacterial activity, anti-oxidant activity, anti-diabetic activity and other properties. These marine phytochemicals could able to cure different health issues in humans naturally. Phytochemical compounds like phenol, flavonoids, tannins and alkaloids were quantitatively identified. Phytochemical analysis showed more phenolic and flavonoid content in green and brown seaweed extracts and lesser content in red seaweed extract. Antibacterial activity of the seaweed extracts were determined against five different test bacteria using well diffusion method. Green and brown seaweed extracts

showed good inhibitory zones against all test bacteria ranging from 22.9 ± 0.57 mm to 24.9 ± 0.57 mm and 17.9 ± 0.57 mm to 21.6 ± 0.75 mm respectively. *In vitro* anti-diabetic activity of the extracts were investigated based on its inhibition effect on the alpha-amylase and alpha-glucosidase activity. Among the three types, green seaweed extracts showed maximum inhibition of α -Amylase and α -glucosidase activity of 86.6 ± 0.76 % and 82.9 ± 0.57 % respectively. The present findings revealed that the phytochemicals present in the methanolic extract of seaweeds attribute positively for different pharmacological properties like antibacterial, anti-oxidant and anti-diabetic activity. Optimizing the production factors would able to commercialize the seaweeds in drug forms and could be used for the treatment of various diseases in humans.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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Author's short biography



Dr. Venkata Nagendra Prasad. B. hailed from Kanigiri, Prakasam District, Andhra Pradesh is a renowned senior educationist with more than 20 years of experience in school educational field. He had published 3 other publications in different journals. His worth giving guidance, motivation and consultation has led many students and teachers to achieve success in different fields. Currently he is working as Principal for Vedanta-Malco School, Mettur Dam, Salem District, Tamil Nadu. He is specialized and imparting his services in fields of educational counselling, consultation, child psychology and Teacher's training.